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DNA strand-specific repair of (±)-3α,4β-dihydroxy-1α,2α-epoxy-1,2,3,4-tetrahydrobenzo[*c*]phenanthrene adducts in the hamster dihydrofolate reductase gene

(polycyclic aromatic hydrocarbon/CHO cells/adduct quantitation/UvrABC excision nuclease/^{32}P-postlabeling)

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ABSTRACT **We evaluated the formation and removal of (±)-3α,4β-dihydroxy-1α,2α-epoxy-1,2,3,4-tetrahydrobenzo[*c*]phenanthrene (BcPHDE)–DNA adducts in two Chinese hamster ovary (CHO) cell lines. One line of repair-proficient cells (MK42) carries a stable 150-fold amplification of the dihydrofolate reductase (*DHFR*) locus. The other line of repair-deficient cells (UV-5) is diploid for this gene and is defective in excision of bulky DNA lesions. Two methods were used to quantitate adduct levels in treated cells: *Escherichia coli* UvrABC excision nuclease cleavage and ^{32}P-postlabeling. DNA repair was examined in the actively transcribed *DHFR* gene, in an inactive region located 25 kilobases downstream, and in the overall genome. Between 8 and 24 hr after BcPHDE exposure, preferential repair of the *DHFR* gene compared to the noncoding region was apparent in MK42 cells. This gene-specific repair was associated with adduct removal from the *DHFR* transcribed strand. However, UV-5 cells showed no lesion reduction from this strand of the gene. By both quantitation methods, regions accessible to repair in MK42 cells showed a 2-fold reduction in DNA adduct levels by 24 hr. That the decline in adducts reflects genomic repair was demonstrated by the constant damage level remaining in UV-5 cells. Since BcPHDE-induced mutations in *DHFR* apparently arise from adducted purines on the nontranscribed strand, results from the present study support the idea that a consequence of strand-specific repair is strand-biased mutations.**

We have been studying the mechanism of strand-biased mutations induced by polycyclic aromatic hydrocarbons (PAHs) in a model mammalian gene. Greater than 90% of mutations induced by (±)-7β,8α-dihydroxy-9β,10β-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (BPDE) and (±)-3α,4β-dihydroxy-1α,2α-epoxy-1,2,3,4-tetrahydrobenzo[*c*]phenanthrene (BcPHDE) in the dihydrofolate reductase gene (*DHFR*) of Chinese hamster ovary (CHO) cells affected purine targets on the nontranscribed coding strand of DNA (1, 2). A similar strand bias for BPDE-induced mutations in the hypoxanthine phosphoribosyl transferase gene (*HPRT*) of human fibroblasts was described where 77% of the premutagenic guanine bases were also on this strand (3). Assuming that carcinogen modification of DNA *in vivo* occurs with similar frequency on both strands, several possible explanations for the apparent strand selectivity of induced mutations can be considered and tested.

One explanation for the strand bias of mutations is that the selection for dihydrofolate reductase-deficient mutants is very stringent, causing mutations to occur on one strand preferentially (4). We recently devised a reversion assay to address this possibility (5). BcPHDE-induced forward mutations were transversions favored at triple purine sequences where the 5′ base was altered (2). The assay used *DHFR*$^-$ nonsense mutants that carry this target sequence on both strands at each of two stop codons, and all purine → thymine mutations yield amino acid substitutions compatible with dihydrofolate reductase activity. Randomly induced mutations at these sites would produce a 2:1 ratio of substitutions on the nontranscribed versus the transcribed strand. Sequence analysis of 66 independent BcPHDE-induced revertants showed that the combined ratio of mutations affecting purines on the nontranscribed strand was 15:1. Hence, the strand bias here was independent of phenotypic selection.

Analysis of induced mutations in rodent *HPRT* led to the suggestion that strand-biased mutations relate to the polarity of the marker gene with respect to DNA replication (6). Since strand-biased UV-induced mutations were found in the absence of DNA repair on the transcribed strand of *HPRT* (7), the misinsertion rate differences for replicative polymerases α and δ may also contribute to the strand-bias phenomenon. Indeed, the overall fidelity of polymerase α appears significantly poorer for base substitutions compared with other polymerases tested (8).

A third explanation for strand-biased mutations is that DNA repair of carcinogen adducts occurs preferentially in the *DHFR* gene and specifically removes adducts from the transcribed strand. This model predicts that mutations will occur on the nontranscribed strand because adducts persist there. Repair of UV-induced damage was demonstrated to occur rapidly in the CHO *DHFR* gene but inefficiently (15% in 24 hr) in the genome overall (9). This intragenomic DNA repair heterogeneity is thought to reflect aspects of chromatin structure, and repair in *DHFR* was shown to be maximally efficient in the hypomethylated 5′ end of the gene (10, 11). Gene-specific repair of UV-induced pyrimidine dimers correlated with selective repair of the transcribed strand in active loci of eukaryotes and prokaryotes (11, 12). Facilitation of damage recognition on the transcribed strand is mediated by a transcription-repair coupling factor in *Escherichia coli* (13). The human coupling factor is presumably encoded by *ERCC6*, a gene mutated in Cockayne syndrome (14).

In the work presented here, we quantitated the adduct levels in DNA from cells treated with BcPHDE and evaluated whether there was gene-specific and/or strand-specific repair of these lesions. In addition, we examined the overall genome repair of BcPHDE-induced DNA damage. For these exper-

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Abbreviations: BPDE, (±)-7β,8α-dihydroxy-9β,10β-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene; BcPHDE, (±)-3α,4β-dihydroxy-1α,2α-epoxy-1,2,3,4-tetrahydrobenzo[*c*]phenanthrene; PAH, polycyclic aromatic hydrocarbon.

iments, we used CHO cells that are repair-proficient (MK42), as well as cells that are defective in repair of bulky DNA adducts (UV-5) (15, 16). MK42 cells were derived from the hamster K-1 line and carry *DHFR* amplified 150 times. The level of *DHFR* mRNA is proportional to the relative number of amplified gene copies (17). Since these cells are highly resistant to methotrexate (0.2 mM) and have a 200-fold increase in DHFR activity over wild-type parental cells (15), it is reasonable to assume that most of the gene copies are transcribed normally. In both strains, BcPHDE–DNA adducts formed to about maximal levels within 1 hr after treatment. These adducts in MK42 but not UV-5 cells were repaired on the transcribed strand of *DHFR*. Thus, the results are consistent with the model that the induced mutational strand bias is a consequence of transcription-coupled repair.

MATERIALS AND METHODS

Materials. Cell culture media (Ham's F-12 and F-12 deficient in thymidine) supplemented with 10% (vol/vol) fetal bovine serum and antibiotics were as noted (18). Reagents for repair experiments were as described (19, 20). RNA probes were prepared with a Boehringer Mannheim SP6/T7 transcription kit. [γ-^{32}P]ATP was from DuPont and T4 polynucleotide kinase from United States Biochemical. Micrococcal nuclease, spleen phosphodiesterase, and all other biochemicals were from Sigma. Chromatography used polyethyleneimine-cellulose plates (Polygram Cel-300 PEI; Brinkmann). (±)-BcPHDE was a generous gift from R. Harvey (University of Chicago).

BcPHDE Treatment of MK42 and UV-5 Cells. Cells were grown in Ham's F-12 containing 0.3 μM thymidine (18) and prelabeled with [^{3}H]thymidine as described (21). Prior to carcinogen treatment, the medium in 150-mm dishes was removed and replaced with 10 ml of serum-free medium containing bromo- and fluorodeoxyuridine as detailed elsewhere (21). BcPHDE was in dimethyl sulfoxide; negative control cultures received dimethyl sulfoxide without the carcinogen. To evaluate repair, treated cells were incubated at 37°C for varying lengths of time before they were harvested and DNA was isolated. The preparation and CsCl density gradient separation of DNA were as described (21). Carcinogen-exposed cells for ^{32}P-postlabeling were prepared in the same way except that the density and tritium labeling procedures were omitted.

^{32}P-Postlabeling Analysis. DNA (0.17 μg) from BcPHDE-treated cells digested with micrococcal nuclease and spleen phosphodiesterase was labeled with 50 μCi of [γ-^{32}P]ATP and T4 polynucleotide kinase as described (22). Chromatography was performed: D1 was in 1 M sodium phosphate (pH 6.8); D3 was in 2 M litium formate/6 M urea, pH 3.5; D4 was in 0.45 M HCl/0.5 Tris/6 M urea, pH 8.0; and D5 was in 1.7 M sodium phosphate (pH 6.0). Adduct spots were quantitated by a Betagen Betascope 603 blot analyzer scanning densitometer (Betagen, Waltham, MA).

BcPHDE Adducts in *DHFR* Quantitated by *Escherichia coli* UvrABC Excision Nuclease (ABC Excinuclease) Incision. The treatment of carcinogen-modified DNA with ABC excinuclease and the analysis of the incision products by Southern blotting after alkaline agarose gel electrophoresis were as detailed (19, 20). Repair was assayed using double-stranded DNA probes for hybridization to 14-kilobase-pair (kb) *Kpn* I fragments located in the 5′ region of the *DHFR* gene, as well as in the 3′ downstream region (10). After autoradiography, membranes were dehybridized and reprobed for other sequences. RNA probes (23) were generated to detect the transcribed and nontranscribed strand of the 5′ *DHFR* fragment. The RNA probes used plasmid pZ3d8 specifying mRNA and complementary RNA for *DHFR* exons 1 and 2 on a 2.5-kb fragment (24). The ratio of the ABC excinuclease-treated to -untreated DNA band intensities provides the fraction of DNA single strands that are undamaged. Quantitation of the band intensities on autoradiograms was by densitometry, as above. Based on this quantitation, a Poisson analysis was used to determine the lesion frequency (9).

RESULTS

BcPHDE Dose and Time Interval for Initial DNA Damage. ABC excinuclease incises a variety of bulky carcinogen monoadducts (19, 20) in a sequence-independent manner (25). From the proportion of DNA sample incised by the nuclease, the number of adducts per fragment can be determined (19, 20). In general, the efficiency of incision parallels the modification level and is 33–50% of the total lesion number in kilobase-sized fragments (19, 20, 25). The gene-specific repair assay of Bohr *et al.* (9) requires that ABC excinuclease nicking of the damaged DNA sample reduce or eliminate the appearance of a specific restriction fragment from a Southern blot at early time points. The reappearance of this fragment after excinuclease digestion using DNA of treated cells that were incubated for some period of time is indicative of repair. The lowest BcPHDE concentration that met this criterion was 0.5 μM (data not shown). This dose was used in all the experiments described below and is 5-fold higher than that previously used to induce forward mutations in the *DHFR* gene (2). Treatment at this concentration rendered cells attached to dishes at 24 hr but essentially inviable by colony-forming ability (<0.01%).

Repair assays compare the DNA damage level directly after treatment relative to the level remaining after a time interval. Because the half-life of BcPHDE in tissue culture medium is 10 hr and this chemical is about 100-fold more stable than BPDE (26), we first evaluated the time course of initial DNA damage in the overall genome after BcPHDE treatment. Three cultures of MK42 cells were exposed to BcPHDE in serum-free medium for 1 hr. The results of ^{32}P-postlabeling of the DNA to quantitate BcPHDE adducts are shown in Table 1. The first sample of treated cells (treatment condition A) was immediately harvested for DNA. Medium containing the carcinogen was removed from the second sample of cells (condition B); they were washed twice with phosphate-buffered saline before complete medium was added and then collected for DNA after a 3-hr incubation. Upon treatment of the third sample (condition C), medium containing the carcinogen was not removed. Instead, additional medium supplemented with serum was added, and the incubation was continued as for condition B. The data in Table 1 show comparable BcPHDE–DNA adduct levels with all three test conditions. Hence, these adducts form within 1 hr after treatment and do not appreciably accumulate thereafter. This result was confirmed by the adduct quantitations in DNA of carcinogen-treated UV-5 cells (see below). The subsequent experiments measured repair after this initial

Table 1. Time of initial BcPHDE–DNA adduct formation in treated MK42 cells evaluated by ^{32}P-postlabeling

Treatment condition*	Adducts per nucleotide × 10^6		
	Assay 1	Assay 2	Average
A	5.4	2.3	3.85
B	3.2	0.9	2.25
C	4.4	2.0	3.20

*Treatment condition A consisted of harvesting BcPHDE-treated cells for DNA after a 1-hr incubation. Upon the same BcPHDE exposure, cells were either washed to remove the carcinogen (condition B) or not washed (condition C) and incubated in complete medium for an additional 3 hr.

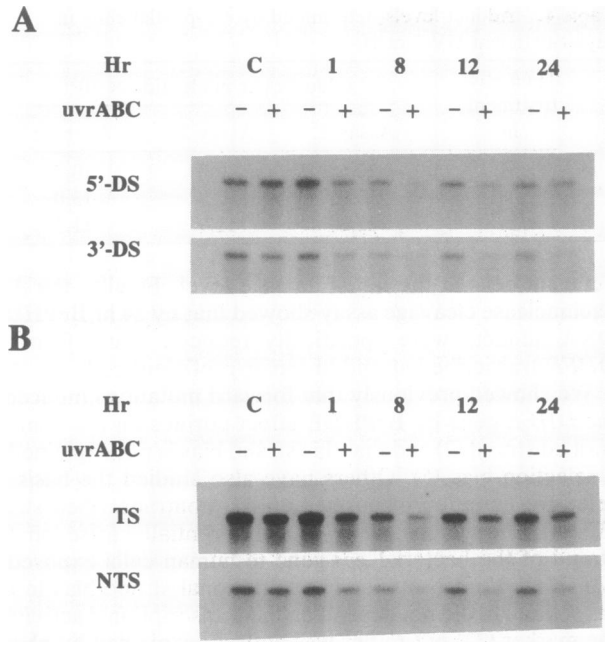

FIG. 1. Autoradiogram of the formation and repair of BcPHDE adducts in a 50-kb region containing the *DHFR* locus. Cells treated with 0.5 μM BcPHDE were incubated for 1, 8, 12, or 24 hr. Untreated control cells are also shown (lanes C). DNA was digested with *Kpn* I. (*A*) The bands are the 14-kb 5′ end of the *DHFR* gene hybridized to the double-stranded probe here designated 5′-DS (pMB5) and downstream noncoding sequences hybridized to a similarly prepared probe designated 3′-DS (a Cs-14 subclone). Preparation of these samples for alkaline electrophoresis, Southern transfer, and hybridization were as detailed (20). (*B*) The same filter shown above in *A* after it was dehybridized and reannealed first with a single-stranded RNA probe for the transcribed (TS) strand and then with a similarly prepared RNA probe for the nontranscribed (NTS) strand.

damage time point. Moreover, the conditions for the repair experiments were those of condition C in Table 1.

Analysis of DNA Adduct Formation and Repair in BcPHDE-Treated Cells. Having established initial damage time, we next performed a repair experiment using repair-proficient MK42 cells. Fig. 1 shows Southern blot analysis of DNA samples from BcPHDE-treated and untreated (lanes C) cells. Duplicates of each sample are shown; they were either incubated with ABC excinuclease or not. Carcinogen-exposed cells were incubated for 1, 8, 12, or 24 hr. The bands depicted on the autoradiographs are DNA fragments comprising the 5′ half of the *DHFR* gene (exons 1–3) (Fig. 1*A*; 5′-DS) or downstream noncoding sequences (Fig. 1*A*; 3′-DS). In Fig. 1*B*, the samples were hybridized with strand-specific RNA probes, which anneal to either the transcribed strand (TS) or the nontranscribed one (NTS). DNA samples for these experiments were obtained from two separate carcinogen treatments of cells, and each Southern blot analysis was performed at least twice. The autoradiographs show that DNA adducts were present at 8 hr in all cases and that they were substantially removed from the *DHFR* 5′ end and from the transcribed strand at 24 hr.

Quantitations of the adduct levels at each time point were made by comparing the ratio of the band intensities for each sample incubated with and without ABC excinuclease. These ratios are derived from densitometry of the hybridization filters (those appearing in Fig. 1 and their duplicates from independent experiments). Data based on this densitometry are presented in Table 2. Adduct levels (expressed as lesions per 10 kb) present after the initial DNA damage were the same for the 5′ end of *DHFR* and the noncoding sequences located about 25 kb downstream. It is significant in terms of explaining the basis for strand-biased mutations that equivalent numbers of adducts formed on both *DHFR* strands. There was preferential gene repair since levels in *DHFR* were reduced 2-fold at 24 hr relative to the downstream flank. Moreover, in these noncoding sequences there was apparently no repair of BcPHDE adducts at 24 hr. The basis for the gene-specific repair was the preferential repair of lesions from the transcribed strand combined with the lack of repair of the nontranscribed strand. The rate of repair of BcPHDE adducts on both strands was plotted from the data in Table 2 and appears in Fig. 2.

To confirm that the reduction in BcPHDE–DNA adducts in MK42 cells was due to DNA repair *per se* and to evaluate whether further modifications form over a period of time in treated cells, we performed a similar analysis using UV-5 cells, which are defective in excision of bulky carcinogen adducts (16). Southern blots prepared in the same manner as for Fig. 1 were hybridized with the transcribed strand-specific RNA probe. Data from the densitometry analysis showed that DNA damage stayed constant in these repair-defective cells: in the 5′ region (transcribed strand) of the *DHFR* gene of UV-5 cells the number of BcPHDE–DNA adducts per 10 kb was 0.65, 0.84, 0.70, and 0.73 after 1, 8, 12, and 24 hr, respectively. Thus, BcPHDE–DNA adduct removal in *DHFR* is due to strand-specific repair. The result also demonstrates that maximal gene-specific adduct formation occurs within 1 hr.

Quantitation of DNA Adduct Levels by ^{32}P-Postlabeling in BcPHDE-Treated Cells. We performed ^{32}P-postlabeling to independently assess the extent of BcPHDE–DNA adduct formation and the rate of repair in the overall genome of treated cells. Samples were prepared from MK42 cells exposed to 0.5 μM BcPHDE and incubated for various times (2, 6, 10, and 24 hr). We followed the procedure of Phillips *et al.* (22), which employs an excess concentration of ATP for the kinase reaction. Use of the P1 enhancement (27) protocol consistently gave 10-fold lower adduct levels, suggesting that some BcPHDE–DNA modifications are sensitive to this nuclease treatment. The ^{32}P-postlabeling data of carcinogen-treated MK42 cells is shown in Table 3. Three assays were performed on each MK42 DNA sample; the mean values are presented as adducts per nucleotide. These modification levels are comparable to recently reported BcPHDE–DNA adduct quantitations (28). However, the ^{32}P-postlabeling procedure may only approximate BcPHDE–DNA modification because it fails to liberate dA nucleotide adducts quan-

Table 2. Formation and removal of BcPHDE adducts in the MK42 *DHFR* gene

Region of *DHFR* gene	Adducts per 10 kb			
	1 hr	8 hr	12 hr	24 hr
5′-Region, both strands	0.58 ± 0.06	0.49 ± 0.04	0.41 ± 0.04	0.27 ± 0.01
Downstream, both strands	0.58 ± 0.02	0.61 ± 0.03	0.61 ± 0.07	0.71 ± 0.05
5′-Region, T-strand	0.53 ± 0.03	0.42 ± 0.03	0.30 ± 0.04	0.27 ± 0.02
5′-Region, NT-strand	0.48 ± 0.02	0.54 ± 0.03	0.55 ± 0.08	0.59 ± 0.02

The values given are the average of two biological experiments; two membranes were probed for each experiment. T-strand, transcribed (coding strand) of the DNA; NT-strand, nontranscribed (template) strand.

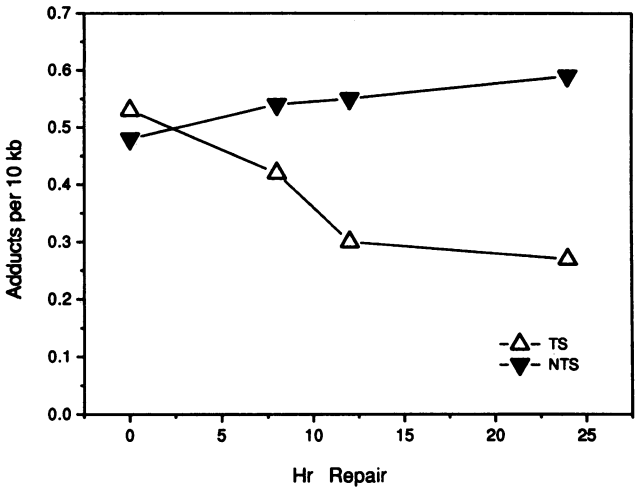

FIG. 2. Formation and removal of BcPHDE adducts in the *DHFR* gene of MK42 cells. Lesion quantitations expressed as adducts per 10 kb on either the transcribed (T) or nontranscribed (NTS) DNA strand were from Table 2 and were plotted versus the incubation time after carcinogen treatment.

titatively (29). The data in Table 3 thus is not directly comparable with the ABC excinuclease quantitations of DNA adducts in Table 2. Results in Table 3 indicate, though, that adduct levels in the genome overall of repair-proficient cells are reduced 2-fold by 24 hr. As expected, this result contrasts with ^{32}P-postlabeling of DNA samples from BcPHDE-treated repair-deficient cells. Table 4 shows that BcPHDE–DNA adducts form and remain at a constant level of about 1×10^{-5} adducts per nucleotide in UV-5 DNA.

DISCUSSION

In murine tumor models, BcPHDE is the most potent PAH yet tested; it is 10-fold more active than BPDE (30). We showed that BcPHDE adducts are rapidly formed in DNA of treated hamster cells. The initial DNA adduct frequencies were about the same in both repair-proficient and -deficient cells. The ^{32}P-postlabeling quantitation of these adducts measured modifications in the genome overall. Calculations of the adducts in the total genome by ^{32}P-postlabeling indicate that there is about a 20-fold lower modification level in the total genome than in the *DHFR* gene. This disparity may reflect preferential gene damage or alternatively reflect the limits of the assay (see *Results*). The repair assay showed preferential *DHFR* gene repair of BcPHDE–DNA adducts with no repair occurring in a downstream region. The *DHFR* 3′ flank was apparently less well repaired at 24 hr than ^{32}P-postlabeling data of the genome overall in repair-proficient cells indicate. The results with BcPHDE are similar to those using hamster cells treated with another PAH, 7-bromomethyl-benz[*a*]anthracene (31), and with an N-substituted aromatic carcinogen, 4-nitroquinoline-1-oxide (24), where adducts were removed at 24 hr from the genome overall by about 55% and 51%, respectively. Both assays used here showed that DNA adducts persist in repair-deficient cells at the initial damage level. Finally, the ABC excinuclease cleavage assay showed that by 24 hr BcPHDE–DNA adducts were specifically reduced 2-fold from the *DHFR*-transcribed strand in repair-proficient cells.

Table 3. Adduct levels determined by ^{32}P-postlabeling in repair-proficient MK42 cells

Time after treatment, hr	Adducts per nucleotide $\times 10^6$, mean ± SD
2	6.70 ± 2.64
6	11.50 ± 2.13
10	5.85 ± 3.22
24	3.63 ± 1.83

Table 4. Adduct levels determined by ^{32}P-postlabeling in repair-deficient UV-5 cells

Time after treatment, hr	Adducts per nucleotide $\times 10^6$		
	Assay 1	Assay 2	Average
1	10.6	10.8	10.7
8	13.0	12.9	13.0
24	11.2	11.9	11.5

We showed previously that forward mutations induced in the *DHFR* gene by BcPHDE affect purines on the nontranscribed strand (2) and that this strand bias is not explained by a selection bias (5). Others have also studied the basis for selective mutation of purines on the nontranscribed strand (32–34). Transitions (G → A) preferentially arise on this strand of the bacterial *gpt* gene in human cells exposed to *N*-methyl-*N*-nitrosourea. The mutational strand bias in this case, though, did not depend on the transcriptional activity of the marker (32) but rather was entirely explained by phenotypic selection (33). Mutations induced by BPDE in the *HPRT* gene are also favored at guanine residues on the nontranscribed strand (3, 34). Recently *HPRT* gene-specific and strand-specific repair of BPDE–DNA adducts was demonstrated in cells synchronized to the G_1 phase of the cell cycle (35). The latter study and the work presented here support the transcription-coupled repair model (11, 12) as the basis for the PAH-induced mutational strand bias.

Since nearly all forward mutations in *DHFR* induced by BcPHDE arise at purines on the nontranscribed strand (2), repair of the transcribed one should be virtually complete by the time treated cells initiate semiconservative replication. Thus, it is noteworthy that 50% of BcPHDE–DNA adducts remained at 24 hr on the transcribed strand of *DHFR* when repair-proficient cells were exposed to the 0.5 μM carcinogen dose (Table 2). If the mutational strand bias is a consequence of repair, it is necessary to posit either that DNA replication is considerably delayed or that the higher BcPHDE dose used for these experiments saturated the cells' repair capacity. For several reasons, we favor the former explanation. First, as the doubling time of CHO cells is relatively brief (14 hr), progression through the cell cycle was delayed in the treated MK42 line, since only half of the population underwent genomic replication after 24 hr. Moreover, transient arrest at either the G_1 or G_2 phase is known to occur after DNA damaging treatment of proliferating eukaryotic cells (36). Last, the *HPRT* repair study (35) showed that entry into S phase of BPDE-treated human cells was significantly retarded (>24 hr). This point can be further pursued by treating our CHO cells with BcPHDE after synchronization to early G_1 (37) and monitoring uptake of ^{3}H[thymidine] to learn when S phase begins.

Studies on strand biased mutations noted above all used model genes in cultured cells. That this same strand preference for induced mutations may occur in human cancer is indicated by the single-base substitutions found in the *P53* gene in lung (38) and liver tumors (39, 40). A frequent and possibly early change in these cancers is G → T transversion arising on the nontranscribed strand of this tumor suppressor gene. Chemical carcinogens are implicated in the etiology of these cancers. Specific environmental exposures lead to particular molecular changes. Since both effects, in turn, will correlate with carcinogenesis and risk assessment (41), establishing an association of induced strand-biased mutations with transcription-coupled repair in cellular oncogenes or

suppressors should be of practical use for molecular epidemiology.

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1. Carothers, A. & Grunberger, D. (1990) *Carcinogenesis* **11,** 189–192.
2. Carothers, A., Urlaub, G., Mucha, J., Harvey, R., Chasin, L. & Grunberger, D. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 5464–5468.
3. Yang, J.-L., Chen, R.-H., Maher, V. & McCormick, J. (1991) *Carcinogenesis* **12,** 71–75.
4. Carothers, A., Steigerwalt, R., Urlaub, G., Chasin, L. & Grunberger, D. (1989) *J. Mol. Biol.* **208,** 417–428.
5. Carothers, A., Mucha, J. & Grunberger, D. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 5749–5753.
6. Vrieling, H., Venema, J., van Rooyen, M.-L., van Hoffen, A., Menichini, P., Zazienicka, M., Simons, J., Mullenders, K. & van Zeeland, A. (1991) *Nucleic Acids Res.* **19,** 2411–2415.
7. Vrieling, H., Zhang, L.-H., van Zeeland, A. & Zazienicka, M. (1992) *Mutat. Res.* **274,** 147–155.
8. Thomas, D., Roberts, J., Sabatino, R., Myers, T., Tan, C.-K., Downey, K., So, A., Bambara, R. & Kunkel, T. (1991) *Biochemistry* **30,** 11751–11759.
9. Bohr, V., Smith, C., Okumoto, D. & Hanawalt, P. (1985) *Cell* **40,** 359–369.
10. Bohr, V., Okumoto, D., Ho, L. & Hanawalt, P. (1986) *J. Biol. Chem.* **261,** 16666–16672.
11. Mellon, I., Spivak, G. & Hanawalt, P. (1987) *Cell* **51,** 241–249.
12. Mellon, I. & Hanawalt, P. (1989) *Nature (London)* **342,** 95–98.
13. Selby, C., Witkin, E. & Sancar, A. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 11574–11578.
14. van Houten, B. & Kow, W. (1992) *New Biol.* **4,** 306–315.
15. Nunberg, J., Kaufman, R., Schimke, R., Urlaub, G. & Chasin, L. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 5553–5556.
16. Thompson, L., Salazar, E., Brookman, K. & Hoy, C. (1983) *Mutat. Res.* **112,** 329–344.
17. Alt, F., Kellems, R., Bertino, J. & Schimke, R. (1978) *J. Biol. Chem.* **253,** 1357–1370.
18. Urlaub, G., McDowell, J. & Chasin, L. (1985) *Somatic Cell Mol. Genet.* **11,** 71–77.
19. Thomas, D., Morton, A., Bohr, V. & Sancar, A. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 3723–3727.
20. Tang, M.-S., Bohr, V., Zhang, X.-s., Pierce, J. & Hanawalt, P. (1989) *J. Biol. Chem.* **264,** 14455–14462.
21. Bohr, V. & Okumoto, D. (1988) in *DNA Repair: A Manual of Research Procedures*, eds. Hanawalt, P. & Friedberg, E. (Dekker, New York), Vol. 3, pp. 347–366.
22. Phillips, D., Glatt, H., Seidel, A., Bochnitachek, W., Oesch, F. & Grover, P. (1986) *Carcinogenesis* **7,** 1739–1743.
23. Melton, D., Krieg, P., Rebagliati, M., Maniatis, T., Zim, K. & Green, M. (1984) *Nucleic Acids Res.* **12,** 7035–7056.
24. Snyderwine, E. & Bohr, V. (1992) *Cancer Res.* **52,** 4183–4189.
25. Thomas, D., Husain, I., Chaney, S., Parigrahi, G. & Walker, I. (1991) *Nucleic Acids Res.* **19,** 365–370.
26. Wood, A., Chang, R., Levin, W., Ryan, D., Thomas, P., Croisey-Delcey, M., Ittah, Y., Yagi, H., Jerina, D. & Conney, A. (1980) *Cancer Res.* **40,** 2876–2883.
27. Reddy, M. & Randerath, K. (1986) *Carcinogenesis* **7,** 1543–1551.
28. Phillips, D., Hewer, A., Seidel, A., Steinbrecher, T., Schrode, R., Oesch, F. & Glatt, H. (1991) *Chem. Biol. Interact.* **80,** 177–186.
29. Cheh, A., Yagi, H. & Jerina, D. (1990) *Chem. Res. Toxicol.* **3,** 545–550.
30. Levin, W., Chang, R., Wood, A., Thakker, D., Yagi, H., Jerina, D. & Conney, A. (1986) *Cancer Res.* **46,** 2257–2261.
31. Thompson, L., Brookman, K. & Mooney, C. (1984) *Somatic Cell Mol. Genet.* **10,** 183–194.
32. Palombo, F., Kohfeldt, E., Calcagnile, A., Nehls, P. & Dogliotti, E. (1992) *J. Mol. Biol.* **223,** 587–594.
33. Palombo, F., Bignami, M. & Dogliotti, E. (1992) *Nucleic Acids Res.* **20,** 1349–1354.
34. Chen, R.-H., Maher, V. & McCormick, J. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 8680–8684.
35. Chen, R.-H., Maher, V., Brouwer, J., van de Putte, P. & McCormick, J. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 5413–5417.
36. Hartwell, L. & Weinert, T. (1989) *Science* **246,** 629–634.
37. Keyomarsi, K., Sandoval, L., Band, V. & Pardee, A. (1991) *Cancer Res.* **51,** 3602–3609.
38. Chiba, I., Takahashi, T., Nau, M., D'Amico, D., Curiel, D., Mitsudomi, T., Buchhagen, D., Carbone, D., Piantadosi, S., Koga, H., Reissman, P., Slamon, D., Holmes, E. & Minna, J. (1990) *Oncogene* **5,** 1603–1610.
39. Hsu, I., Metcalf, R., Sun, T., Welsh, J., Wang, N. & Harris, C. (1991) *Nature (London)* **350,** 427–428.
40. Nigro, J., Baker, S., Preisinger, A., Jessup, J., Hostetter, R., Cleary, K., Bigner, S., Davidson, N., Baylin, S., Devilee, P., Glover, T., Collins, F., Weston, A., Modali, R., Harris, C. & Vogelstein, B. (1989) *Nature (London)* **342,** 705–708.
41. Vogelstein, B. & Kinzler, K. (1992) *Nature (London)* **355,** 209–210.