Repair of Benzo(a)pyrene Diol Epoxide- and UV-induced DNA Damage in Dihydrofolate Reductase and Adenine Phosphoribosyltransferase Genes of CHO Cells*

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Using Uvr proteins we have quantified benzo(a)pyrene diol epoxide (BPDE)-DNA adduct formation and repair at the dihydrofolate reductase (DHFR) and adenine phosphoribosyltransferase (APRT) genes in two Chinese hamster ovary cell lines: B-11 cells, which are 50-fold amplified for DHFR, and AT3-2 cells, which are diploid for DHFR. We have found that: 1) BPDE-DNA adduct formation in different regions of the DHFR gene is proportional to the concentration of BPDE. 2) There is no significant difference in the repair of BPDE-DNA adducts between the coding and noncoding regions in either amplified or nonamplified DHFR gene domains. 3) Repair in the nonamplified DHFR gene is more efficient (30-40%) than in the amplified DHFR genes. 4) There are no significant differences in repair in the transcribed or nontranscribed strands of the DHFR gene. 5) BPDE-DNA adduct formation and repair in the APRT gene in B-11 and AT3-2 cells are the same. These results contrast those for the repair of cyclobutane pyrimidine dimers, which occurs preferentially in the transcribed strand of the DHFR gene and in which gene amplification appears to play no role.

Benzo(a)pyrene (BP) is a strong mutagen and carcinogen. The mutagenic and carcinogenic effects of BP exposure are presumably a consequence of the covalently bonding of metabolically activated BP derivatives such as benzo(a)pyrene diol epoxide (BPDE) with cellular DNA. Although more than a dozen BPDE isomers have been identified in vivo, the (+)-BPDE-I isomers are by far the most abundant and carcinogenic. The bonding of BPDE-I with DNA is primarily through C10 of the BPDE with the exocyclic amine of guanine residues, or to a lesser extent, with adenine residues (for review, see: Weinstein (1981), Harvey (1981), Pelkonen and Nebert (1982), and Beland and Porier (1988)). The covalently bound BPDE-I adducts tend to be positioned externally to the DNA helix and induce unwinding of the helix (Gecanitov et al., 1978; MacLeod et al., 1982; Harvey and Gecanitov, 1988; Undemann et al., 1983).

Although the BPDE-I adduct conformation and its impact on DNA helix structure are very different from ultraviolet (UV) light-induced DNA damage-cyclobutane pyrimidine dimers (CPD), these two kinds of DNA damage appear to be repaired by the same mechanisms (Seeberg et al., 1983; Tang et al., 1992). It has been found that both mammalian and prokaryotic mutant cells which are sensitive to UV-induced cytotoxicity are also sensitive to BPDE-I treatment. Moreover, those mutant cells, which are deficient in removal of CPD, are also deficient in removal of BPDE-I-DNA adducts (Yang et al., 1982; MacLeod et al., 1988).

Using T4 endonuclease (endo) V as a probe, Bohr et al. (1985) have demonstrated that the cultured Chinese hamster ovary (CHO) cells repair CPD significantly more efficiently in the coding region of the DHFR gene domain than in noncoding regions. Mellon et al. (1987) further demonstrated that the repair of CPD in DHFR gene preferentially occurs in the transcribed strand. These findings suggest that cells have the capacity to remove DNA damage in transcriptionally active genes and that the transcription process may facilitate repair. It has been suggested that biological end points such as mutation and viability may be better related to the ability of cells to conduct gene- and strand-specific repair than the overall levels of DNA repair (Bohr et al., 1986; Bohr, 1991). Indeed, results from Chen et al. (1990), Carothers et al. (1991), Menichini et al. (1991), and Vrielig et al. (1989, 1992) suggest that mutations occur preferentially in the nontranscribed DNA strand in UV- or bulky chemical-treated mammalian cells.

We have recently demonstrated that the uvr system in Escherichia coli cells is the major system for repairing BPDE-I-DNA adducts and that the E. coli UvrA, UvrB, and UvrC proteins, working together (we term the collective function of these Uvr proteins the UvrABC nucleasae), incise BPDE-I-DNA adducts specifically and quantitatively (Tang et al., 1992). Using these enzymes and Southern DNA transfer hybridization techniques, we have developed a method to quantify BPDE-I-DNA adduct formation and repair in defined sequences in mammalian cells. We have investigated the effect of gene amplification on the BPDE-DNA adduct formation and repair by examining these processes in the coding and noncoding regions of the DHFR gene domain in cultured B-11 CHO cells, which are amplified 50-fold for the DHFR gene, and in AT3-2 CHO cells, which are diploid for the DHFR locus. As an internal standard, we have also examined adduct formation and repair in another nonamplified APRT gene in these two cell lines. We have found that both gene amplification and transcription have significant different effects on the repair of BPDE-DNA adducts and UV-induced photoproducts.

MATERIALS AND METHODS

Cell Culture and Carcinogen Treatment—The Chinese hamster ovary cell line, B-11 (Kaufman and Schinke, 1981) was grown in Ham's F-12...
medium without glycine, hypoxanthine, and thymidine and supplemented with 10% fetal calf serum and 500 ng/ml methotrexate to maintain the selection pressure for DHFR gene amplification. CHO AT5-2 cells were grown in a 9-cm Eagle's medium with 10% fetal calf serum. Fresh confluent cells were split 1:10 with fresh medium. When the cells were grown to about 50–70% confluence, cells were washed and resuspended in DPBS buffer (4.7 mM MgCl₂, 8.5 mM CaCl₂, 0.65 mM NaCl, 1.94 mM KCl, 1.07 mM KH₂PO₄, 6.16 mM Na₂HPO₄, pH 7.4). The cells were then either irradiated with UV or treated with DPBS. For BPDE treatment different amounts of BPDE (with or without H₂O₂ label) in dimethyl sulfoxide were added, and the cultures were incubated at 37 °C for 30 min. After the end of incubation, the buffer with BPDE was removed, and the cells were incubated in fresh growth medium with 5-bromo-2′-deoxyuridine (10 μM) and 5-fluorodeoxyuridine (1 μM). After further incubation at 37 °C for 0, 2, 6, and 24 h, the cells were harvested and DNA was isolated.

For UV irradiation, GE1518 germicidal lamp (major emission, 254 nm) was used as the UV source. The cells were irradiated with UV fluence rate of 0.6–0.8 J/m²s.

DNA Isolation—For DNA isolation cells were washed with DPBS buffer three times and lyed by incubation with lysing solution (0.5% SDS, 10 mM Tris, pH 7.8, 10 mM EDTA, and 10 mM NaCl) for 2 min. The cell lysates were treated with proteinase K (100 μg/ml) at room temperature overnight followed by washing with moderate salt solution (250 mM NaCl, 1 mM MgCl₂, 50 mM Tris, pH 7.5) and then resolution of nucleic acids was achieved by incubation at 37 °C for 90 min. At the end of incubation, the protein-DNA were ethanol-precipitated with 15 μg of tRNA as carrier and washed with ethanol, and resuspended in 10 μl of TE solution.

DNase Treatment—DNase I (1 unit/mg of DNA) at 37 °C overnight. The digestion was checked for completion by electrophoresis of samples on agarose gel. Replicated and nonreplicated DNAs were separated by CaCl₂ gradient centrifugation in Ti 50 rotor, 37,000 rpm for 64 h at 21 °C. For determining the relationship between BPDE concentration and the DNA adduct formation, total DNA isolated from treated cells was used. For determining the kinetics of adduct removal, only the nonreplicated DNAs were used. This precautional step is important, since a round of semiconservative DNA replication will dilute the adduct per unit length of DNA to one-half. Moreover, it has been found that treatment of cells with DNA damaging agent induces significant amount of semiconservative DNA replication (Cohn et al., 1984).

The UvrABC Nuclease Treatment—The UvrABC proteins were prepared as described previously (Tang et al., 1992). A typical 10 × (protein/14-kb DNA molar ratio) UvrABC reaction contains 330 nmol of UvrA, UvrB, and UvrC proteins and 3 μg of DNA with 3 ng of internal standard of linearity for a final volume treatment with mixture contains 50 mM Tris, pH 7.5, 100 mM KCl, 1 mM ATP, 10 mM MgCl₂, and 1 mM dithiothreitol. The reaction mixtures were incubated at 37 °C for 90 min. At the end of incubation, the protein-DNA were ethanol-precipitated with 15 μg of tRNA as carrier and washed with ethanol, and resuspended in 10 μl of TE solution.

DNA Denaturation and Gel Electrophoresis—DNAs were denatured by neutral formamide treatment (Ross and Tang, 1985). A 90-μl aliquot of fresh deionized formamide solution was added to 10 μl of DNA solution, and the mixtures were incubated at 37 °C for 60 min. This treatment dissociated proteins from the protein-DNA complex. Immediately after incubation the mixtures were electrophoresed at 5 V/cm for 3 h in a preformed 0.5% agarose horizontal gel in 0.5 × TBE buffer (25 mM Tris, pH 7.9, 25 mM NaOH, 2.5 mM EDTA) with 0.5 μg/ml ethidium bromide. After electrophoresis, the DNA in the gels was deproteinized according to the procedure of Mannisa et al. (1982) and denatured and transferred to a Zeta-probe or Zeta-probe membrane in 0.5 M NaOH and 0.6 M NaCl solution. The DNA in the membrane was subsequently hybridized with 32P-labeled DNA or strand-specific riboprobes as described by Bohr et al. (1985) and Mellon et al. (1987). The densitometric scans of DHFR and APRT gene domains are shown in Fig. 1.

RESULTS

Detection of BPDE-DNA Adducts in the DHFR Gene Domain in CHO Cells by UvrABC Nuclease Incision Method—Using BPDE-modified DNA fragments, we have previously demonstrated that purified UvrA, UvrB, and UvrC proteins working together incise 6–7 bases 5′ and 4 bases 3′ to a BPDE-modified guanine. Moreover, these enzymes incise BPDE-DNA adducts quantitatively regardless of whether the adducts are in linear or supercoiled DNA (Tang et al., 1992). These results suggest that our UvrABC nuclease incision method may be used to quantify BPDE-DNA adduct formation and repair at defined genomic sequences.

The approaches used to detect BPDE adduct formation are similar to those we have previously used to detect N-(deoxyguanosin-5′-yl)-8-aminofluorene adducts at defined sequences in mammalian cells (Tang et al., 1989). DNA isolated from BPDE-treated cells was digested with restriction enzyme and then reacted with optimal levels of UvrABC nuclease. The resultant DNAs were denatured in neutral formamide solution and then separated by agarose gel electrophoresis in buffer solution with moderate pH (7.9); this method eliminates the possibility of introducing nonenzymatic DNA degradation at BPDE adduct sites and abasic sites. The separated DNAs were then transferred to a nylon membrane and hybridized with 32P-labeled probes. Fig. 2, a and b, show the results of BPDE-DNA adduct formation in DHFR gene coding and 3′ downstream noncoding sequences of B-11 cells treated with concentrations of BPDE ranging from 0 to 8 μM. DNA isolated from these cells treated with higher BPDE concentrations were more susceptible to UvrABC nuclease incision, for both coding and noncoding regions. Densitometric analyses indicated that the number of UNSS per unit length was the same in both coding and noncoding regions of the DHFR gene (Fig. 2). Furthermore, the final level of UNSS formation displays a linear relation to the amount of BPDE up to 4 μM, used for treating the cells. This relation is the same as the result obtained from the determination of tritium-labeled BPDE adducts and 14C-labeled DNA. These results demonstrate that optimal levels of UvrABC nuclease can incise BPDE-DNA adducts quantitatively in DNAs isolated from mammalian cells. Treatment with 4 μM BPDE produced an average of 2.5 UNSS/14-kb length of B-11.
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**Fig. 2.** Formation of UNSS in the coding region and 3'-downstream noncoding region of the DHFR gene domain as a function of BPDE concentration. DNA were prepared from B-11 cells (a and b) or AT3-2 cells (c and d) treated with different concentrations of BPDE (0, 2, 4 and 8 μM), restricted with Asp78, reacted with 6 and 8 x UvrABC nuclease (i x of UvrABC nuclease renders a molar ratio of cellular DNA. We observed no significant difference of BPDE-DNA adduct formation between coding and noncoding regions (Fig. 2) or between transcribed and nontranscribed DNA strands (data not shown).

A slightly lower level of BPDE-DNA adduct formation in the DHFR gene domain was observed in AT3-2 cells; 4 μM BPDE treatment produces an average of 1.6 UNSS/14-kb length of DNA. However, as in B-11 cells, UNSS formation in the DHFR gene domain of AT3-2 cells is proportional to the BPDE concentration (Fig. 2). The Removal of BPDE-DNA Adducts in the Coding and Noncoding Regions of DHFR Gene Domain—Having established that the UvrABC nuclease incision method can quantify the BPDE adduct at defined sequences, we then used this method to examine the repair of BPDE-DNA adducts in the coding and noncoding regions of the DHFR gene. Nonreplicated DNAs were isolated from cells at different incubation times after being exposed to 4 μM BPDE. Fig. 3A shows the typical autoradiographs for measurements of UNSS for coding and noncoding regions of DHFR gene domain in both B-11 and AT3-2 cells. Quantification by densitometry of these autoradiographs is shown in Fig. 3B. These results demonstrate that the repair of BPDE-DNA adducts in both coding and noncoding regions of protein/14-kb DNA equals one), electrophoresed, transferred to nylon membrane, and hybridized with 32P-labeled probe, pMB5 (a and c).

After removal of the hybridized probes, the blots were hybridized with 32P-labeled probe, cs-14 (b and d). Signs (.) represent DNA without UvrABC nuclease treatment. A, typical autoradiographs; B, quantitations. The number of UNSS in the coding (.) and noncoding (C, ) sequences were calculated by Poisson distribution based on densitometric scanning of the autoradiographs. Symbols (Δ) represent the number of BPDE/14-kb fragment calculated from H specific activity. The solid line represents results from B-11 cells and the broken line results from AT3-2 cells. The results are the average of two to three experiments. For the purpose of clarity, some points are not precisely aligned.
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Fig. 4. Removal of UNSS from transcribed (T) and nontranscribed (NT) strands of DHFR gene. DNA isolated from 4 μM BPDE-treated B-11 or AT3-2 cells with different post-treatment incubation periods (2, 6, and 24 h) were probed with 32P-labeled riboprobes specific for either the transcribed or nontranscribed DNA strand, as described under “Materials and Methods.” Signs are the same as in Fig. 2. A, typical autoradiographs; B, quantitations. The BPDE-DNA adducts in the DNA sequences are the UNSS calculated from the densitometric scanning of autoradiographs (average of two to three experiments, except the 40-h points, which are from a single experiment). The percent of UNSS remaining in DNA was calculated based on the assignment of the number of DNA adducts at time 0 as 100%. Solid lines represent results from B-11 cells and the broken line results from AT3-2 cells. For the purpose of clarity some points are not precisely aligned.

The DHFR gene domain in AT3-2 cells (which are diploid for the DHFR locus) is more efficient than in B-11 cells (which are amplified 50-fold for the DHFR gene). Furthermore, there appears to be no significant difference in repair of BPDE-DNA adducts in coding and noncoding regions of the DHFR gene domain in both types of cells. These results contrast sharply with the repair of cyclobutane pyrimidine dimers (CPD) in CHO cells in two aspects: one is that the repair of CPD is faster and more efficient in the coding region of the DHFR gene than in noncoding regions and the other is that the rate of repair of CPD in the DHFR gene appears to be the same in cells which have DHFR gene amplified and cells which are diploid for the DHFR locus (Bohr et al., 1985).

The Removal of BPDE-DNA Adducts in the Transcribed and Nontranscribed Strand of DHFR Gene—It has been shown that CHO cells preferentially repair CPD in the transcribed strand of DHFR gene rather than those in the nontranscribed strand (Mellon et al., 1987). These workers have concluded that the process of transcription itself may facilitate the removal of CPD. In order to determine whether there is a similar strand bias of repair of BPDE-DNA adducts, we have hybridized the same membranes used for assaying repair of coding and noncoding regions of DHFR gene domain with the strand-specific riboprobes as described by Mellon et al. (1987). The autoradiographic results are shown in Fig. 4A, and densitometric scanning results for these autoradiographs are shown in Fig. 4B. Although each individual experiment consistently shows that in B-11 cells, repair of BPDE-DNA adducts is slightly more efficient (10–15%) for the transcribed strand than the nontranscribed strand, this difference is much smaller than that observed for CPD repair (Mellon et al., 1987; Table I). No significant strand bias of repair is observed in AT3-2 cells, and repair in these cells appears to be significantly more efficient than repair in B-11 cells.

Detection of the Formation and Removal of BPDE-DNA Adducts in the APRT Gene—The more efficient BPDE-DNA adduct removal observed for the unamplified DHFR gene of AT3-2 cells in comparison with the amplified DHFR genes in B-11 cells could either be due to a difference in repair efficiency between two CHO cell lines or the effect of gene amplification. If the former is the case, then we would expect that the differences observed in the case of the DHFR gene would also be observed for other genes and DNA regions between these two cell lines. In order to distinguish these two possibilities, we examined the repair of BPDE-DNA adducts at another nonamplified gene locus in these two cell lines, the APRT gene. The same membranes used for detection of BPDE-DNA adducts in the DHFR gene were deprobed and later reprobed with 32P-labeled APRT gene DNA. Typical autoradiograms are shown in Fig. 5A, and densitometric scanning results are shown in Fig. 5B. These figures demonstrate that the repair of BPDE-DNA adducts in the APRT gene is the same in both B-11 and AT3-2 cells, even though the kinetics of repair for this gene are different from those for the DHFR gene. Therefore, the lower extent of removal of BPDE-DNA adducts in DHFR genes of B-11 cells in comparison with AT3-2 cells appears to be a consequence of DHFR gene amplification in these cells.

Detection of UV-induced DNA Damage in DHFR Gene Domain by UvrABC Nuclease and T4 endo V Incision Methods—Since Bohr et al. (1985) and Mellon et al. (1987) have unambiguously demonstrated that CHO cells preferentially repair T4 endo V-sensitive CPD in the transcribed strand of active genes, how does one explain the fact that we detected a much smaller extent of strand-specific preferential repair for BPDE-DNA adducts in B-11 cell and that we detected no preferential repair at all in AT3-2 cells? One possible explanation could be that the transcription process has less effect on the removal of BPDE-DNA adducts than for CPD. A second possibility is that the UvrABC nuclease incision method is relatively insensitive in detecting gene- or strand-specific preferential repair. A third possibility is that, for unknown reasons, cells grown under our conditions do not have the ability to repair DNA damage in transcriptionally active genes. To date, the most distinct strand- and gene-specific repair is observed when T4 endo V is used for CPD detection. It is possible that transcription may have a profound effect on the removal of CPD but much less of an effect on the removal of other kinds of DNA modifications. On the other hand, since UvrABC nuclease is capable of recog-
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**DISCUSSION**

We have previously demonstrated that UvrABC nuclease can incise BPDE-DNA adduct specifically and quantitatively (Tang et al., 1992). In this report, we have used UvrABC nuclease as a reagent to quantify the BPDE-DNA adduct formation in defined genomic sequences in mammalian cells. This approach has been successfully used for measuring DNA damage induced by N-acetoxy-2-acetylaminofluorene (NAAAF) (Tang et al., 1989) and (6-4) photoproducts (Bohr et al., 1991) in defined sequences in mammalian cells. However, Thomas et al. (1988) reported that under their conditions, UvrABC nuclease detected only one-third of CPD and psoralen-DNA adducts. It is possible that some of these discrepant results may reflect the differential efficiency of UvrABC nuclease in incising different kinds of DNA damage. In order to obtain quantitative results, it is imperative to optimize reaction conditions so that reactions can go to completion. It has been shown that in the absence of DNA repair synthesis and ligation, UvrABC nuclease remains bound to damaged DNA after the incision reaction; consequently, under *in vitro* reaction conditions, incision by UvrABC nuclease is stoichiometric rather than catalytic (for review, see Sancar and Tang (1993)). Therefore, to insure complete incision of all DNA adducts, the nuclease must be present in significant excess over the number of DNA adducts. Empirically, we have found that reactions containing molar ratio 6 to 8 of UvrABC nuclease over 14-kb DNA fragment yield quantitative results.

Our finding that BPDE-DNA adducts are repaired with the same efficiency in coding and noncoding regions of the DHFR gene domain, in both B-11 and AT3-2 cells, contrasts sharply with the results for repair of CPD, but is consistent with results for the repair of NAAAF (Tang et al., 1989), dimethyl sulfate (Scicchitano and Hanawalt, 1989, 1990; Wassermann et al., 1990), and 4-nitroquinoline-1-oxide (Snydewine and Bohr, 1992)-induced damage in the same genomic regions. These findings suggest that "gene-specific preferential repair" may be highly dependent upon the structures of the DNA damage. Our results showed that BPDE-DNA adducts are repaired much more efficiently in the amplified DHFR gene domain than in bulk DNA (MacLeod et al., 1988), and the degree of repair is even higher in the DHFR gene of AT3-2 cells, which are diploid for the DHFR gene locus. If gene expression induces openness of a chromosomal domain, including the regions flanking the expressed gene region, and if this openness of chromosome increases the accessibility of repair complexes, then perhaps this is the reason that higher repair is observed in active genes such as DHFR, as well as their nearby noncoding regions. Since the amplified DHFR genes are not necessarily all located in the same chromosomal domain, and may not be uniformly transcribed, the overall repair level observed for the...
amplified DHFR genes may depend on the proportion of the genes which are actively transcribed. Our observation that the efficiency of repair of BPDE-DNA adducts in amplified DHFR genes is higher than that of bulk DNA, but lower than that of a nonamplified DHFR locus, suggests that many of the DHFR genes in an amplified array may not be transcriptionally active. Our finding that B-11 and AT3-2 CHO cell lines repair BPDE-DNA adducts in APRT gene with the same kinetics and efficiency rule out the trivial explanation that the observed differences in BPDE-DNA adduct removal in amplified and nonamplified DHFR genes is simply due to variations in repair efficiency between cell lines.

Previously, we also observed neither gene- nor strand-specific repair for NAAAP-induced DNA damage (Tang et al., 1989). However, using the same UvrABC method, we were able to detect strand-specific repair for UV-induced DNA damage.

It is also possible that BPDE treatment has less effect on the replication leading strand which is the transcription template. This could reflect intrinsic DNA damage, its impact on the helix structure, and the location of the damage relative to the direction of DNA replication. These factors together may determine the lethality of the DNA damage.

Using the UvrABC nuclease and the same neutral formamide denaturating method as we described previously (Tang et al., 1989), Chen et al. (1992) have reported a greater extent of transcribed strand biased repair (11–29%) for BPDE-DNA adducts in hypoxanthine-guanine phosphoribosyltransferase (HPRT) gene in human fibroblasts. This could reflect intrinsic difference in repair between human fibroblasts and CHO cells and/or from differences in repair between HPRT and DHFR genes. It is also possible that BPDE treatment has less effect on the transcription of HPRT gene than on that of DHFR gene in these two cell systems. However, consistent with their findings of preferential repair of BPDE-DNA adducts in the transcribed strand of HPRT gene, these workers observed a higher frequency of mutations in the nontranscribed strand than in the transcribed strand (Chen et al., 1990). They concluded that strand-biased repair is the cause of this strand-biased mutation. Intriguingly, Vrieling et al. (1989, 1992) and Menichini et al. (1991) found that the UV-induced mutations in the HPRT gene of UV-sensitive CHO mutant cells preferentially occurs in the replication leading strand which is the transcription template strand. Recently, Mellochier et al. (1992) reported that in the prokaryotic system the aromatic amine-DNA adducts induce the same mutation frequency in the transcribed and nontranscribed strands, and they also found that the major mutation (G to T transversions) induced by 1-aminopyrene and acetylbenzidine occur preferentially at the transcribed strand. These results indicate that factors such as the structure of the DNA damage, its impact on the helix structure, and the location of the damage relative to the direction of DNA replication may all contribute greatly to mutagenesis. Furthermore, strand-biased effects on mutagenesis may reflect either a strand bias in fidelity of DNA replication or a strand bias in repair. These factors together may determine the lethality of the DNA damage. Our results that UvrABC nuclease detects significantly less UV damage than T4 endo V at 3' downstream region and nontranscribed strand of DHFR gene in UV-irradiated cells with 24-h post-irradiation incubation, whereas both enzyme systems detect the same amounts of damage within the DHFR gene domain in cells without post-irradiation incubation, are intriguing. It is possible that T4 endo V and UvrABC nuclease incise different photoproducts, and cells repair the T4 endo V sensitive photoproducts and UvrABC nuclease-sensitive photoproducts at different rates. The number of T4 endo V-sensitive photoproducts and the number of UvrABC nuclease-sensitive photoproducts in the DNA isolated from cells immediately after UV irradiation may be fortuitously the same. Alternatively, both of these two enzymes may primarily detect CPD, but modification of CPD during the post-irradiation period may result in modified CPDs which are still sensitive to T4 endo V incision but resistant to UvrABC nuclease. The ability of the rodent cells to ressume DNA replication before a significant fraction of the T4 endo V-sensitive sites have been removed has long been a puzzle. Perhaps such a CPD modification mechanism could serve as a means of increasing the probability of cell survival by allowing the DNA replication to proceed at the expense of fidelity.

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REFERENCES

Beland, F. A., and Pouliert, M. C. (1989) in The Pathobiology of Neoplasia (Sirica, A. E., ed.) pp. 57–76, Plenum Press, New York.

Bohr, V. A. (1991) Carcinogenesis 12, 1983–1992.

Bohr, V. A., Smith, C. A., Okamoto, D. S., and Hansawalt, P. C. (1985) Cell 40, 359–369.

Bohr, V. A., Okamoto, D. S., and Hansawalt, P. C. (1986) Proc. Natl. Acad. Sci. U.S.A. 83, 3830–3833.

Carothers, A. M., Mucha, J., and Grunberger, D. (1991) Proc. Natl. Acad. Sci. U.S.A. 88, 5749–5753.

Chen, R.-H., Maher, V. M., and McCormick, J. J. (1990) Proc. Natl. Acad. Sci. U.S.A. 87, 8690–8694.

Chen, R.-H., Maher, V. M., Brewer, J., van de Putte, P., and McCormick, J. J. (1992) Proc. Natl. Acad. Sci. U.S.A. 89, 5413–5417.

Cohn, R. M., Krawisz, B. R., Dresler, S. L., and Lieberman, M. W. (1984) Proc. Natl. Acad. Sci. U.S.A. 81, 4828–4832.

Friedberg, E. C., Ganaan, A. K., and Seawell, P. C. (1980) Methods Enzymol. 65, 191–201.

Geacintov, N. E., Gagliano, A., Ivonievo, V., and Weinstein, I. B. (1978) Biochemistry 17, 5259–5392.

Harvey, R. (1981) Acc. Chem. Res. 14, 218–226.

Harvey, R. S., and Geacintov, N. E. (1988) Acc. Chem. Res. 21, 66–73.

Kaufman, R. J., and Schinske, R. T. (1981) Mol. Cell. Biol. 1, 1069–1076.

MacLeod, M. C., Mansfield, B. K., and Sdiski, J. R. (1982) Carcinogenesis (Land.) 3, 1031–1037.

MacLeod, M. C., Adair, G., and Humphrey, R. M. (1986) Mutat. Res. 199, 243–254.

Manstia, T., Pritsok, E. F., and Garsai, J. (1992) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

Melchior, W. J., Jr., Lindsey, L. A., and Beld, F. A. (1992) Proc. Am. Assoc. Cancer Res. 33, 175.

Mellon, I., Spivak, G., and Hansawalt, P. C. (1997) Cell 41, 241–249.

Menichini, P., Vrieling, H., van Zeeelend, A. A. (1991) Mutat. Res. 265, 145–155.

Pelkmans, O., and Neber, D. W. (1992) Pharmacol. Res. 36, 189–221.

Roes, J., and Tang, M. (1989) Anal. Biochem. 144, 212–217.

Sancar, A., and Tang, M. (1993) Photochem. Photobiol. 57, 905–921.

Scicchitano, D. A., and Hansawalt, P. C. (1989) Proc. Natl. Acad. Sci. U.S.A. 86, 3050–3054.

Scicchitano, D. A., and Hansawalt, P. C. (1990) Mutat. Res. 233, 31–37.

Seeberg, E., Steinwall, A. L., Nordenskjold, M., Soderhall, S., and Jernstrom, B. (1983) Mutat. Res. 112, 139–145.

Snyderwine, E. B., and Bohr, V. A. (1992) Cancer Res. 52, 4183–4189.

Tang, M., Bohr, V. A., Zhang, X., Pierce, J., and Hansawalt, P. C. (1988) J. Biol. Chem. 263, 14455–14462.

Tang, M., Pierce, J. R., Doisy, R. P., Nazimiec, M. E., and MacLeod, M. C. (1992) Biochemistry 31, 8429–8436.

Thomas, D. C., Morton, A. G., Bohr, V. A., and Sancar, A. (1988) Proc. Natl. Acad. Sci. U.S.A. 86, 3723–3727.

Udendam, O., Lyckel, P., Graslund, A., Attlund, T., Ehrenberg, A., Jernstrom, B., Tarrfeld, P., and Norden, B. (1993) Cancer Res. 43, 1851–1860.

Vrieling, H., van Rooijen, M. L., Groen, N. A., Zdzienicka, M. Z., Simons, J. W. I. M., and Zdzienicka, M. Z. (1992) Mutat. Res. 274, 17–155.

Wassermann, K., Kohn, K. W., and Bohr, V. A. (1990) J. Biol. Chem. 265, 13906–13913.

Weinstein, I. B. (1981) J. Supramol. Struct. Cell. Biochem. 17, 99–120.

Yang, L. L., Maher, V. M., and McCormick, J. J. (1982) Mutat. Res. 84, 435–447.

2 M.-S. Tang and I. Mellon, unpublished results.