MOLECULAR AND CELLULAR BIOLOGY, Aug. 1990, p. 3945–3951
0270-7306/90/083945-07$02.00/0

Vol. 10, No. 8

Effect of Nucleotide Excision Repair in Human Cells on Intrachromosomal Homologous Recombination Induced by UV and 1-Nitrosopyrene

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Received 16 January 1990/Accepted 30 April 1990

To study the role of nucleotide excision repair in the induction of intrachromosomal homologous recombination in mammalian cells, we introduced a plasmid containing a substrate for recombination into three human cell lines that differ in their repair capacity and compared the frequency of recombination induced by UV radiation and by 1-nitrosopyrene. One strain had a normal capacity for nucleotide excision repair, the second exhibited an intermediate rate of repair, and the third, derived from a patient with xeroderma pigmentosum, had no ability to repair UV- or 1-nitrosopyrene-induced DNA damage. The endogenous thymidine kinase genes in these cell strains had been inactivated, and the cells contained an integrated copy of a plasmid carrying duplicated copies of the herpes simplex virus type 1 thymidine kinase (H*tk*) gene, each inactivated by an 8-base-pair *Xho*I site inserted at a unique site. A functional *tk* gene can only be generated by a productive recombination event between the two H*tk* genes. In all three stains, UV and 1-nitrosopyrene induced dose-dependent increases in the frequency of recombinants. However, the doses required to cause a specific increase in recombination in the repair-deficient strains were 10 to 30 times lower than the dose required for the cell strain with a normal capacity for repair. These results strongly suggest that unexcised DNA lesions, rather than excision repair per se, stimulate intrachromosomal homologous recombination. Southern blot analysis of DNA from representative recombinants indicated that in all cases one of the two H*tk* genes had become wild type (*Xho*I resistant). The majority (90%) retained the H*tk* duplication, consistent with nonreciprocal transfer of genetic information (gene conversion).

Evidence points to genetic recombination as a mechanism for the loss of wild-type alleles of critical genes involved in the pathogenesis of several types of human cancers. For example, the attainment of homozygosity of loci on the short arm of chromosome 17 by mitotic homologous recombination has been shown to play a role in development of astrocytomas (8), and the homozygous condition of the *Rb* locus on chromosome 13, which is involved in the development of retinoblastomas, has in some cases resulted from mitotic homologous recombination (3). Indirect evidence of the causal involvement of recombination in tumor development is the finding that cells from a patient with Bloom's syndrome, characterized by an inherited predisposition to various kinds of cancer, exhibited an abnormally increased frequency of extrachromosomal recombination between homologous viral genes (10). In addition, nonhomologous genetic recombination resulting in the translocation of cellular *myc* proto-onogenes to positions within immunoglobulin genes has been implicated in the development of Burkitt's lymphoma (11).

Because homologous recombination may be involved as one step in the carcinogenesis process, we and our colleagues investigated the ability of a series of six chemical carcinogens and UV radiation to induce recombination between two herpes simplex virus type 1 thymidine kinase (H*tk*) genes carried on a plasmid stably integrated in the genome of a thymidine kinase (tk)-deficient mouse L-cell line (1, 18). Each H*tk* gene had been inactivated by an 8-base-pair (bp) *Xho*I linker inserted at a unique site, so that only by undergoing a productive recombinational event between the two nonfunctional genes could a functional Htk protein be produced and the cell be able to survive selection. Using this system, we showed that each of the carcinogens tested induced a dose-dependent increase in the frequency of homologous recombination (1, 18) and that the increase caused by four radiolabeled, polycyclic aromatic carcinogens was directly related to the number of residues covalently bound to the DNA of the cells (1).

The four carcinogens, i.e., 1-nitrosopyrene (1-NOP), the 7,8-diol-9,10-epoxide of benzo[*a*]pyrene, 4-nitroquinoline-1-oxide, and *N*-acetoxy-2-acetylaminofluorene, differed significantly in their ability to induce recombination, even when compared on the basis of equal amounts of DNA adducts formed. The specific nature of the adducts may account for the observed differences in the ability of these agents to induce recombination. Alternatively, recombination may be stimulated by nucleotide excision repair processes by which the cell removes such adducts from DNA. A third possibility is that the various kinds of DNA adducts remaining in the DNA are equally potent in inducing recombination, but that there are differences in the rate of repair of the various types of lesions. Bhattacharyya et al. (1) found no significant difference in the overall rates of removal of the various adducts (25 to 30% removed in 24 h), but certain rodent cell lines are known to selectively remove some forms of DNA damage from actively transcribed genes faster than from the total genome.

It was not possible to distinguish between these possibilities with the mouse L-cell line. Therefore, to determine whether the intermediates generated during nucleotide excision repair of the DNA damage caused by such agents stimulate intrachromosomal homologous recombination, we

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introduced the plasmid pJS-3, containing the recombination substrate (i.e., the duplicated H*tk* genes inactivated by *Xho*I linkers), into a series of tk-deficient human cell lines that differ significantly in their capacity for nucleotide excision repair. One cell line, 143 tk^-, has a normal rate of excision repair of UV-induced DNA damage; the second, RD tk^-, repairs at a rate <30% of that of normal cells; and the third, derived from a patient with xeroderma pigmentosum (XP), XP12ROSV40 tk^-, complementation group A, is incapable of this type of repair. Cell strains with closely matched low rates of spontaneous recombination were identified, and three cell strains, one derived from each parental cell line, were compared for the frequency and kinds of recombination induced by UV (254 nm) radiation and by 1-NOP. We here report that both agents induced a dose-dependent increase in recombination in all three human cell strains, and that the doses required to cause a specific increase in the repair-deficient strains were 10 to 30 times lower than the dose required for the cells with a normal capacity for repair. Since the XP cell strain does not even begin to carry out excision repair of UV-induced photoproducts, the increase in recombination observed with that strain cannot be the result of intermediates generated during repair. The data suggest that unexcised DNA lesions, rather than repair per se, stimulate intrachromosomal homologous recombination.

MATERIALS AND METHODS

Cell strains. Data on the origins, characteristics, rates of spontaneous recombination, and capacities for UV-induced nucleotide excision repair of the three tk-deficient human cell strains containing the recombination substrate that were used in this comparative study have been published (2). The relative excision repair capacity of the different cell strains was measured by comparing their ability to incorporate tritiated thymidine during 3 h of UV-induced unscheduled DNA synthesis. The extent of incorporation was determined from the amount of label incorporated into acid-precipitable DNA and from the number of lightly labeled nuclei seen with autoradiography (2). In summary, cell strain 143-7 has 1.5 integrated copies of the plasmid, recombines spontaneously at a rate of 1×10^{-6} cells per cell generation, and repairs UV-induced DNA damage at the same rapid rate as normal diploid human fibroblasts (2). Cell strain RD-12 has one copy of the plasmid, recombines spontaneously at a rate of 1×10^{-6} cells per cell generation, and exhibits an excision repair rate that is <30% of that of the 143-7 cells (2). Cell strain XP-7, derived from XP12ROSV40 cells complementation group A, contains one copy of the plasmid, recombines spontaneously at a rate of 3.5×10^{-6} cells per cell generation, and exhibits no capacity whatsoever for nucleotide excision repair. There was no UV-induced incorporation of labeled thymidine above background with this strain, and no lightly labeled nuclei were observed (2).

Culture conditions. Cells were routinely cultured in Eagle minimal essential medium supplemented with 10% fetal bovine serum and modified as described previously (18) (culture medium). The H*tk*$^+$ recombinants were selected by using culture medium supplemented with deoxycytidine (2×10^{-5} M), hypoxanthine (1×10^{-4} M), amethopterin (4×10^{-7} M), and thymidine (3×10^{-5} M) (CHAT medium). All work with cell cultures was done under gold fluorescent lights.

Assay of cell survival (cytotoxicity). The cytotoxic effect of exposure of the cells to UV or 1-NOP was determined from the decrease in colony-forming ability of the treated populations as compared with that of the unirradiated or solvent-treated control cells (1, 18). Cells to be UV irradiated were plated at cloning densities (i.e., densities adjusted to give 35 to 60 macroscopic colonies per 100-mm diameter dish; four to six dishes per determination). Cells to be treated with 1-NOP were plated at 10^4 cells per cm^2. After exposure to 1-NOP, cells were trypsinized and plated at cloning densities as above. After 12 to 14 days with one feeding with culture medium, the colonies were stained and counted, and the survival was calculated from the relative cloning efficiency (18). The cloning efficiency of untreated 143-7 cells ranged from 40 to 70%, that of the RD-12 cells ranged from 5 to 25%, and that of the XP-7 cells ranged from 20 to 50%.

Assay of carcinogen-induced recombination. Cells growing exponentially in culture medium were plated into 100-mm-diameter dishes at a density of 10^4 cells per cm^2 (5×10^5 cells per dish). Sufficient dishes were used per determination to have a minimum of 2×10^6 viable cells after the exposure to carcinogen. A set of cells was also plated at cloning densities to determine the extent of cell killing by UV. After 18 h the culture medium was removed, and the cells to be irradiated with UV light were rinsed with phosphate-buffered isotonic saline. The excess buffer was removed, and the cells were irradiated as described previously (16) and fed with fresh culture medium. For 1-NOP treatment, the culture medium was exchanged for serum-free medium, and 1-NOP dissolved in dimethyl sulfoxide or solvent alone was introduced into the dishes by micropipette. After 1 h, the medium containing 1-NOP was exchanged for fresh culture medium. To determine the cytotoxity of the 1-NOP treatment, the cells in one dish from each concentration were trypsinized and plated at cloning densities as above. These cells were allowed 2 weeks to form colonies, and cell survival was calculated as above.

Selection for recombinants (i.e., cells resistant to CHAT medium) was begun at the designated time after exposure to carcinogen. The CHAT medium was renewed two or three times during the 2- to 3-week growth period until macroscopic H*tk*$^+$ colonies formed. The colonies were located by using a focused beam of light, and representative clones were isolated for further analysis as described (2). The frequency of recombination was determined from the number of CHAT-resistant colonies observed divided by the number of viable cells plated (as determined from the accompanying cytotoxicity assay). The frequency of induced recombination was determined by subtracting the background frequency observed in the control cells in each experiment from the total frequency.

Assay of recombinants for the loss of the *neo* gene. CHAT-resistant colonies were isolated and transferred to 60-mm-diameter dishes containing CHAT medium. The medium was later exchanged for CHAT medium lacking amethopterin. As soon as the cells were nearing confluence, they were assayed for resistance to Geneticin (loss of the *neo* gene) as described (2) to get information about the products of the recombination events.

Southern blot analysis of recombination products. The methods used to analyze CHAT-resistant clones for the kinds of recombination products in their DNA by Southern blotting have been described (2). Briefly, restriction enzyme digestion with *Bam*HI, *Hin*dIII, and *Xho*I was carried out as recommended by the supplier (New England BioLabs, Beverly, Mass.), and the digested DNA (12 μg) was electrophoresed on 0.8% agarose gels. The DNA was probed with the ^{32}P-labeled 2.5-kbp *Bam*HI fragment of the original plasmid containing an H*tk* gene. The pJS-3 plasmid contain-

ing the recombination substrate was originally constructed by Liskay et al. (12) in such a way that one H*tk* gene is released on a 2.5-kbp *Bam*HI restriction fragment and the other is released on a 2.0-kbp *Hin*dIII fragment. If the *Xho*I linker is still present in the H*tk* genes, digestion with *Xho*I releases a 1.5-kbp fragment and a 1.0-kbp fragment from the larger gene and a 1.5-kbp fragment and a 0.5-kbp fragment from the smaller H*tk* gene.

RESULTS

Sensitivity of the cell strains to killing by UV radiation. Before comparing the frequency of homologous recombination induced by UV in the three human cell strains, we compared their sensitivity to killing by this agent. The nucleotide excision repair-proficient cell strain (143-7) was the most resistant, exhibiting a shouldered survival curve (Dq = 3 J/m^2) and a D_{37} of 6 J/m^2 (Fig. 1A), values very similar to what is found with normal diploid human fibroblasts (17). (Dq is the dose at which the extrapolation of the exponential portion of the curve intersects the 100% survival line; D_{37} is the dose required to reduce the survival to 37% of the untreated control.) As expected, the XP-7 cell strain, which is virtually devoid of excision repair capacity (2), was extremely sensitive to UV-induced cell killing (D_{37} = 0.3 J/m^2). This UV survival curve was comparable to that found previously in this laboratory for the parental cell line XP12ROSV40 (data not shown) and for XP12BE (16), a diploid fibroblast cell line from complementation group A that also is virtually devoid of nucleotide excision repair. The RD-12 cell strain, which exhibits an intermediate rate of excision repair (2), exhibited an intermediate sensitivity to UV (no shoulder; D_{37} = 2 J/m^2).

Optimization of experimental conditions for measuring recombination. Before measuring recombination frequencies, we determined the optimal expression time for UV-induced CHAT resistance. Htk$^-$ cells were plated at 10^4 cells per cm^2 and irradiated with doses known to reduce survival to 33% of the unirradiated control, and the populations were selected for CHAT resistance at various times postirradiation. The results showed there was no difference in the frequency of CHAT-resistant colonies when selection was begun 12, 18, or 24 h postirradiation (data not shown). Therefore, an 18-h expression period was used for all subsequent experiments.

Since the frequency of recombination is calculated from the observed number of CHAT-resistant colonies divided by the number of viable target cells, we carried out reconstruction experiments with H*tk*$^+$ recombinants derived spontaneously from each of the three cell strains to see whether H*tk*$^+$ and H*tk*$^-$ cells are equally sensitive to killing by UV and to check on recovery of H*tk*$^+$ cells under the experimental conditions. CHAT-resistant H*tk*$^+$ recombinants (50 to 150) from each cell strain were seeded into dishes containing a lawn of the corresponding H*tk*$^-$ target cell strain plated at 10^4 cells per cm^2 as well as into dishes lacking the lawn of H*tk*$^-$ cells. The cells were irradiated with doses giving 33% survival, and the medium was exchanged for CHAT medium after 18 h. There was no difference in sensitivity to UV between H*tk*$^+$ and H*tk*$^-$ cells in the presence or absence of the lawn of cells (data not shown), and the recovery of H*tk*$^+$ cells under these experimental conditions was 100% for 143-7 and XP-7 cells and 60% for RD-7.

Sensitivity of the cell strains to induction of recombination by UV. The results of the comparative studies of UV-induced recombination in the three cell strains are shown in Fig. 1B. Data from a representative experiment for each strain are given in Table 1. The excision repair-deficient XP-7 and RD-12 cell strains were significantly more sensitive to induction of homologous recombination by UV than was the repair-proficient 143-7 cell strain. All three cell strains gave a dose-dependent increase in the frequency of recombinants. The doses required to cause a specific increase in recombination in the repair-deficient strains were 10- to 30-fold lower than the dose required for the 143-7 cell strain.

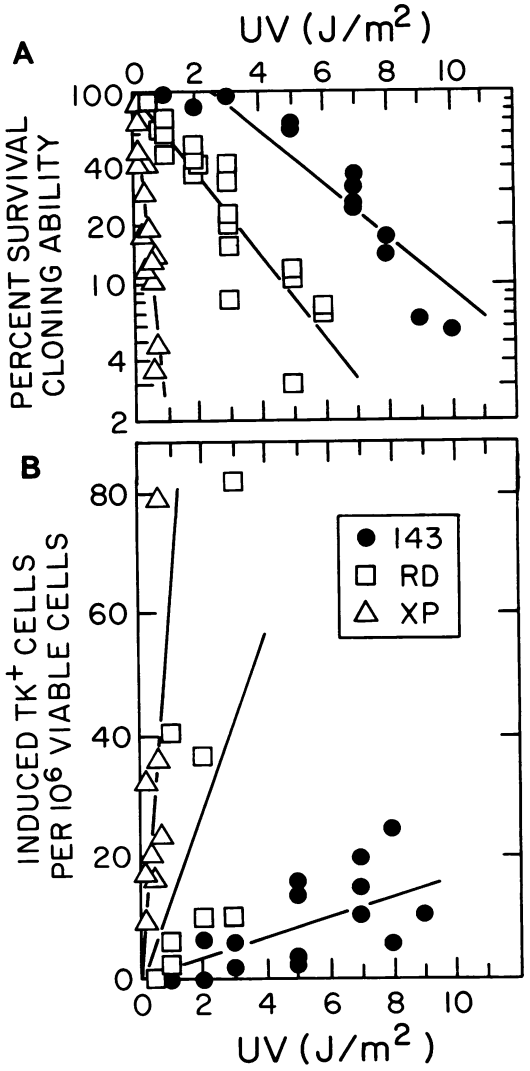

FIG. 1. Cytotoxicity (A) and frequency of homologous recombination (B) induced by UV in three cell strains that differ in nucleotide excision repair capacity. The cytotoxicity data include the results of experiments determining survival only as well as the survival results from each of the recombination experiments measuring the induction of CHAT-resistant cells by UV. The background frequency of CHAT-resistant cells in the untreated populations was determined for each experiment. These values per 10^6 cells ranged from 4 to 18 for the 143-7 cells, from 2 to 29 for the RD-12 cells, and from 3 to 37 for the XP-7 cells. These values have been subtracted to yield the induced frequencies. Lines were drawn by the method of least squares.

Sensitivity of the cell strains to killing and recombination induced by 1-NOP. Patton et al. (15) showed that XP12BE fibroblasts from complementation group A are significantly more sensitive than normal fibroblasts to the killing and mutagenic effects of 1-NOP. XP12BE cells cannot remove

TABLE 1. Representative example of data used to determine frequency of UV- and 1-NOP-induced recombination in the three cell strains

Agent and dose	Cell strain	Survival (% of control)	No. of target cells (10^6)	No. of viable cells (10^6)	CHAT-resistant colonies	CHAT-resistant cells per 10^6 viable cells[a]	Induced frequency per 10^6 viable cells
UV (J/m^2)							
0	XP-7	100	2	2	14	7.0	
0.2		42	4	1.68	35	20.8	13.8
0.4		31	6	1.86	51	27.4	20.4
0.6		13.7	10	1.37	54	42.3	35.3
0	RD-12	100	2	2	35	17 (28.2)	
1.0		60	4	2.4	100	41.7 (69.2)	41
2.0		55	6	3.3	127	38.5 (63.9)	35.7
3.0		16	10	1.6	106	66.3 (110)	82
0	143-7	100	2	2	13	6.5	
2		84.2	2	1.68	22	13.1	6.6
5		66.2	4	2.65	54	20.4	13.8
7		32.0	6	1.92	41	21.6	15.1
1-NOP (μM)							
0	XP-7	100	2	2	78	39 (54.8)	
0.05		77	3	2.3	143	61.9 (89.5)	34.7
0.10		42	6.5	2.73	350	128.2 (118.4)	63.6
0.15		20	8.5	1.7	319	187.6 (165.2)	110.4
0	RD-12	100	2	2	66	33	
0.05		60	3	1.8	97	53.9	20.9
0.10		34	4	1.36	97	71.3	38.3
0.15		22	9	1.98	197	99.5	66.5
0	143-7	100	2	2	13	6.5	
0.5		49.6	6	2.98	67	22.5	16
0.8		12.7	8	1.01	47	46.3	39.8
1.2		4.8	10	0.48	29	60.4	53.9

[a] The frequencies of CHAT resistant cells for the RD-12 cell strain have been corrected for 60% recovery. The corrected values are in the parentheses.

covalently bound 1-NOP residues from their DNA (unpublished data). In contrast, normally repairing human fibroblasts readily excise such adducts, i.e., >75% are removed in 12 h (13, 19). Therefore, 1-NOP represents a multiringed chemical carcinogen that forms bulky DNA adducts that are repaired by an excision process, i.e., nucleotide excision repair, that is defective in XP cells. For this reason, we also compared the frequency of recombination induced in the three cell strains by this carcinogen.

The repair-deficient cell strains were significantly more sensitive than 143-7 cells to killing by 1-NOP (Fig. 2A). The concentration needed to reduce the survival of the 143-7 cells to 37% of that of the untreated control was 0.6 μM, a value equal to that found previously with normal diploid human fibroblasts (15). For RD-12 and XP-7 cells, the D_{37}s were 0.06 and 0.035 μM, respectively. Data on the frequency of 1-NOP-induced recombination are shown in Fig. 2B and Table 1. All three strains exhibited a concentration-dependent increase in frequency of recombinants. The slope of the line showing the relationship between 1-NOP-induced recombinants and applied concentration for the RD-12 cells was 10 times steeper than for the repair-proficient 143-7 cells; for the XP-7 cells, it was 20 times steeper.

Characteristics of the UV- and 1-NOP-induced recombinants. If a cell contains only a single integrated copy of the recombination substrate as these cell strains do, inference as to the type of recombinational event that has taken place can be made simply by analyzing the CHAT-resistant recombinants for resistance to Geneticin. This is because the *neo* gene, coding for resistance to Geneticin, is located between the two H*tk* genes on the plasmid used to construct the strains (12). If the recombinational event involves a single reciprocal exchange within a chromatid or a single unequal exchange between chromatids, only a single *Xho*I-resistant copy of the H*tk* gene will be present and the *neo* gene will be lost. On the other hand, if the event consists of a nonreciprocal transfer of wild-type information, i.e., gene conversion, the H*tk* gene duplication and the *neo* gene will be retained (18). The presence of a single wild-type (*Xho*I-resistant) H*tk* gene or of two H*tk* genes, only one of which is wild type, can be confirmed by Southern blot hybridization of DNA from CHAT-resistant recombinants with the H*tk* gene as a probe.

Both of these approaches were used to infer the nature of the UV- or 1-NOP-induced recombinational events from an analysis of the recombination products. The data for Geneticin resistance in UV-induced recombinants are shown in Table 2. Approximately 93% of the CHAT-resistant recombinants still remained resistant to Geneticin, indicating that only 7% of the recombinational events represented single reciprocal exchanges. To date, we have tested 10 CHAT-resistant clones taken from XP-7 populations exposed to high doses of 1-NOP. In all cases, these CHAT-resistant clones were also Geneticin resistant.

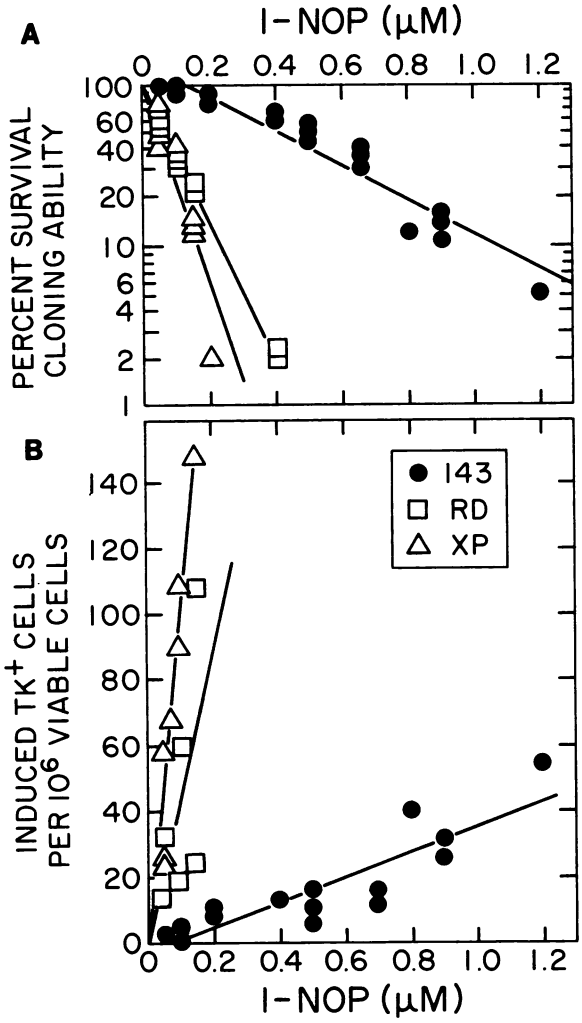

FIG. 2. Cytotoxicity (A) and frequency of homologous recombination (B) induced by 1-NOP in the three cell strains. The cytotoxicity data include results from studies of survival only as well as those from each recombination experiment. The background frequencies per 10^6 cells ranged from 6 to 9 for the 143-7 cells, 10 to 55 for the RD-12 cells, and 29 to 39 for the XP-7 cells. These values have been subtracted to give the induced frequencies. Lines were drawn by the method of least squares.

Genomic DNA from several CHAT-resistant recombinants was analyzed by Southern blot hybridization. Representative data obtained from such analyses of UV- or 1-NOP-induced recombinants in the XP-7 cell strain are shown in Fig. 3. This cell strain before recombination exhibits two H*tk* genes: the 2.5-kbp fragment released by *Bam*HI digestion and the 2.0-kbp fragment released by

TABLE 2. Fraction of UV-induced recombinants resistant to Geneticin

Cell strain	No. of recombinants tested	No. resistant to Geneticin	Fraction resistant to Geneticin
143-7	19	18	0.95
RD-12	10	9	0.90
XP-7	27	24	0.89

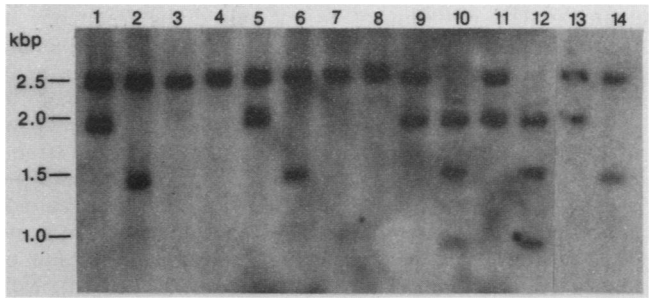

FIG. 3. Southern blotting analysis of genomic DNA from representative XP-7 recombinants to determine the type of recombinational product. To determine the number of copies of the H*tk* gene, the DNA was digested with *Bam*HI and *Hin*dIII (odd-numbered lanes). These two restriction enzymes liberate the H*tk* gene carried on a 2.5-kbp fragment and a 2.0-kbp fragment, respectively (2). To determine which of the two H*tk* genes had become wild type (*Xho*I resistant), the DNA was digested with *Bam*HI, *Hin*dIII, and *Xho*I (even-numbered lanes). The DNA was hybridized with a ^{32}P-labeled probe consisting of the 2.5-kbp *Bam*HI fragment of the pJS-3 plasmid containing the H*tk* gene (12) as described previously (2). Lanes: 1 through 8, UV-induced recombinants; 9 through 14, 1-NOP-induced recombinants.

*Hin*dIII digestion (2). Both of these genes are *Xho*I sensitive, yielding 1.5- and 1.0-kbp bands and 1.5- and 0.5-kbp bands, respectively (data not shown). As expected, all of the Geneticin-resistant recombinants tested contained two H*tk* genes (a 2.5-kbp band and a 2.0-kbp band). In every case, one of these was wild type (*Xho*I resistant) and the other was still *Xho*I sensitive. For example, in the DNA analyzed in lanes 1 and 2 of Fig. 3, the 2.5-kbp H*tk* gene is *Xho*I resistant; in the DNA in lanes 9 and 10, the 2.0-kbp gene is *Xho*I resistant. There is an 0.5-kbp faint band in lanes 2, 6, and 14 that cannot be distinguished in the photograph. The DNA analyzed in lanes 3 and 4 and lanes 7 and 8 was isolated from Geneticin-sensitive recombinants. The presence of a single wild-type H*tk* gene is evident from the *Xho*I-resistant 2.5-kbp band observed in lanes 4 and 8. These results confirm the conclusion that Geneticin sensitivity occurs from a single reciprocal exchange, whereas resistance results from gene conversion.

DISCUSSION

We have shown (Fig. 1A and 2A) that, as expected, sensitivity of the three cell strains to killing by UV or 1-NOP is correlated with their nucleotide excision repair capacity. The XP-7 cells, which are completely lacking in excision repair, were the most sensitive. Their UV and 1-NOP survival curves are virtually identical to what was found previously with the diploid XP fibroblast cell line XP12BE, which also lacks the capacity to repair UV- or 1-NOP-induced DNA damage (15, 16). The 143-7 cells, which are proficient in excision repair, were the most resistant. Their survival curves were equal to those found previously with excision repair-proficient diploid human fibroblasts derived from normal neonates (13, 15, 16). The RD-12 cells showed intermediate survival curves, consistent with the low but detectable level of excision repair capability that was observed in these cells (2).

Our data (Fig. 1B and 2B) show that the sensitivity of these three cell strains to homologous recombination induced by UV or 1-NOP is also inversely correlated with their capacity to excise DNA damage caused by these agents. For

example, UV radiation at 1 J/m^2 raised the frequency of recombinants per 10^6 cells in the XP-7 cell strain to 60, in the RD-12 cell strain to 15, and in the 143-7 cell strain to 2. Similarly, 0.05 μM 1-NOP increased the frequency per 10^6 cells in the XP-7 strain to 60 and in the RD-12 strain to 24 but did not cause any detectable increase in the 143-7 cell strain. Since the 143-7 cells can rapidly remove lesions in DNA caused by UV or 1-NOP, whereas the XP-7 cells cannot carry out such repair and the RD-12 cells do so only at a low rate, the observed recombination cannot be the result of intermediates generated by nucleotide excision repair. Neither can the differences in induced frequency be caused by differences in the rate of spontaneous recombination in these cell strains, since their rates are so similar (2).

The recombinational events cannot simply be the result of damage induced, since for a given dose of UV, all three strains received equal amounts of UV-induced damage. Rather, the results indicate that recombination between the duplicated H*tk* genes is stimulated by the presence of lesions remaining unexcised in the DNA at some later time after treatment, after the 143-7 cells have had time to remove most of the lesions and the RD-12 cells have been able to remove some of them.

The unexcised lesions may stimulate recombination directly, for example, by blocking DNA synthesis, leading to the generation of discontinuities, single-stranded regions, that initiate recombination. We favor this hypothesis. It is, of course, possible that the presence of the unexcised lesions interferes with DNA synthesis, RNA synthesis, or other cellular processes, and that such interference indirectly results in homologous recombination by inducing an as yet unidentified process.

Since the cell strains containing the recombination substrate are not derived from a single parental cell line, there is also the possibility that the observed differences in frequency of UV- or 1-NOP-induced recombination are not causally related to their nucleotide excision repair capacity but reflect some other unknown differences between the strains. If so, whatever these differences are, they do not produce the relationships seen in Fig. 1B and 2B when homologous recombination is induced in these strains by an agent that causes DNA damage that is not repaired by nucleotide excision repair, e.g., a simple methylating agent (unpublished observations).

There is evidence in *Escherichia coli* and *Saccharomyces cerevisiae* for the existence of recombinational repair as an integral step in the process of bypassing unexcised DNA damage (7), but little is known about recombinational repair mechanisms in mammalian cells. In an attempt to understand how DNA replication can proceed on a damaged template, Fornace (6) assayed UV-irradiated human cells for the presence of photoproducts (as revealed endonuclease-sensitive sites) in the daughter strands of DNA. He found that for a given dose of UV, the number of endonuclease-sensitive sites in the daughter strands was higher for XP cells than for normal human cells. Evidence for the presence of pyrimidine dimers in the daughter strand of simian virus 40 DNA has also been obtained in UV-irradiated monkey cells (4). Our data showing that UV-induced recombination frequencies, including single reciprocal exchanges, are higher in cells that cannot remove such lesions or excise them only slowly are consistent with the hypothesis that a pathway for transferring lesions from parental strands to daughter strands (by recombination) functions in these human cells.

DasGupta and Summers (5) infected normal and XP cells with UV-irradiated herpes virus particles and found a higher frequency of extrachromosomal recombination in the XP cells. These results and our own are consistent with what has been found in assays of UV-induced recombination in repair-deficient bacterial cells and in yeast cells (9), but in an assay measuring extrachromosomal recombination between two plasmids carrying defective H*tk* genes in nucleotide excision repair-proficient and deficient Chinese hamster ovary (CHO) cells, no difference in frequency was observed (14). The difference between the results in rodent cells and those of DasGupta and Summers and our own in human cells probably reflects differences in the repair pathway gene(s) that is defective in the cells used for the studies. This could also explain why DNA damage enhances DNA-mediated transformation of human cells by nonhomologous plasmid incorporation in human cells but does not do so in CHO cells (17).

The ratio of gene conversions to single reciprocal exchanges observed in the recombinants induced by UV or 1-NOP was similar to what we found for spontaneous recombinational events between the H*tk* genes in these same cells strains (2). Similar ratios of spontaneous and induced gene conversions and reciprocal exchanges were found with a mouse L-cell line (1, 18). This suggests that the mechanisms involved in carcinogen-induced recombination are similar to those operating spontaneously in these cells.

In summary, we have shown that photoproducts and bulky adducts that distort DNA and block DNA synthesis stimulate intrachromosomal homologous recombination in human cells, and that the frequency of such recombination is significantly lower in nucleotide excision repair-proficient cells than in repair-deficient cells. Most probably, excision repair lowers the frequency of such events by removing prerecombinagenic lesions. The recent recognition that mitotic recombination can play a role in carcinogenesis and our present funding that carcinogens induce genetic recombination more frequently in repair-deficient cells than in normal cells suggest that the increased risk of cancer in XP patients may also reflect increased recombination.

ACKNOWLEDGMENTS

The excellent technical assistance of Neil Davis is gratefully acknowledged.

The research was supported in part by Public Health Service grant CA21253 from the National Institutes of Health and by contract 87-2 from the Health Effects Institute, an organization jointly funded by the U.S. Environmental Protection Agency (assistance agreement x812059) and automotive manufacturers.

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