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Cell Cycle-Dependent Strand Bias for UV-Induced Mutations in the Transcribed Strand of Excision Repair-Proficient Human Fibroblasts but Not in Repair-Deficient Cells

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To study the effect of nucleotide excision repair on the spectrum of mutations induced in diploid human fibroblasts by UV light (wavelength, 254 nm), we synchronized repair-proficient cells and irradiated them when the *HPRT* gene was about to be replicated (early S phase) so that there would be no time for repair in that gene before replication, or in G_1 phase 6 h prior to S, and determined the kinds and location of mutations in that gene. As a control, we also compared the spectra of mutations induced in synchronized populations of xeroderma pigmentosum cells (XP12BE cells, which are unable to excise UV-induced DNA damage). Among the 84 mutants sequenced, base substitutions predominated. Of the XP mutants from S or G_1 and the repair-proficient mutants from S, ~62% were G · C→A · T. In the repair-proficient mutants from G_1, 47% were. In mutants from the repair-proficient cells irradiated in S, 71% (10 of 14) of the premutagenic lesions were located in the transcribed strand; with mutants from such cells irradiated in G_1, only 20% (3 of 15) were. In contrast, there was no statistically significant difference in the fraction of premutagenic lesions located in the transcribed strand of the XP12BE cells; ~75% (24 of 32) of the premutagenic lesions were located in that strand, i.e., 15 of 19 (79%) in the S-phase cells and 9 of 13 (69%) in the G_1-phase cells. The switch in strand bias supports preferential nucleotide excision repair of UV-induced damage in the transcribed strand of the *HPRT* gene.

Populations of diploid human fibroblasts, synchronized by release from the density-inhibited G_0 state and plated at 10^4 cells per cm^2, begin DNA synthesis after approximately 16 h (26). If populations of nucleotide excision repair-proficient cells are irradiated with UV light (wavelength, 254 nm) 17 h after release from confluence, the frequency of 6-thioguanine (TG)-resistant (TG^r) mutants (resulting from loss of function of the gene coding for hypoxanthine [guanine] phosphoribosyltransferase [HPRT]) is significantly higher than in cells irradiated in early G_1 phase (9). This difference in frequency cannot be attributed to differences in the physical state of the DNA, since no such difference in mutation frequency is found when diploid xeroderma pigmentosum cells (XP12BE, complementation group A), which are virtually devoid of nucleotide excision repair (18), are irradiated in early S phase or in early G_1 phase (9). These data suggest that S-phase replication is centrally involved in the conversion of potentially mutagenic DNA damage into mutations (fixation) and that excision repair prior to the onset of S phase decreases the frequency of mutants by eliminating such lesions.

Of the two major classes of potentially mutagenic UV photoproducts, i.e., cyclobutane pyrimidine dimers (Py-Py dimers) and 6-4 pyrimidine-pyrimidone (6-4 Py-Py) lesions, the latter have been shown to be removed from human genomic DNA more rapidly than the former (15). It has also been shown that Py-Py dimers are excised more rapidly from an actively transcribed gene of human cells, the dihydrofolate reductase gene (*DHFR*), than from bulk DNA (1, 12). Moreover, Mellon et al. (14) demonstrated that there is a strand bias in the rate of removal of Py-Py dimers from the *DHFR* gene in human cells. The majority of such lesions are removed within 24 h, but dimers in the transcribed strand are excised much faster than those in the nontranscribed strand. Information on strand-specific excision repair of UV-induced damage from the *HPRT* gene of human cells is not yet available, but if such repair occurs, *HPRT* mutations induced by UV in excision repair-proficient human cells irradiated at the onset of S phase should arise from 6-4 Py-Py lesions and Py-Py dimers located in either strand of DNA. In contrast, the lesions responsible for the mutations induced in such cells allowed 6 h for repair before the onset of S phase should arise mainly from Py-Py dimers, and these should be located primarily in the nontranscribed strand. This switch in strands should not occur in XP12BE cells.

There are no published data on the kinds and locations (i.e., the spectrum) of UV-induced mutations in an endogenous gene of human cells, nor are there data showing whether excision repair in such cells alters the spectrum. This study was designed to examine the spectrum of mutations induced by UV in the *HPRT* gene of diploid human cells and to determine, at the DNA sequence level, the effect of nucleotide excision repair of UV-induced photoproducts on the induction of such mutations. We were particularly interested in determining whether there was biological evidence of strand-specific repair of UV photoproducts in diploid human fibroblasts and comparing the results with what was found in V79 Chinese hamster cell lines by Vrieling et al. (25).

Although the time of *HPRT* replication has not been measured directly, studies of the mutation frequency of TG^r mutants induced by UV irradiation at different times across the S phase of synchronized populations of human fibroblasts showed that the highest frequency occurred when cells were irradiated in the first quarter of S phase (7). A

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similar finding was reported for Syrian hamster cells by Tsutsui et al. (23). To investigate the effect of nucleotide excision repair on the spectrum of UV-induced lesions, we irradiated repair-proficient and repair-deficient diploid human cells in early S phase, just before the *HPRT* gene is replicated, or in G_1 phase, 6 h before replication, and determined the kinds and locations (spectrum) of the mutations in the coding region of the gene. We then analyzed the data to determine in which strand the premutagenic dipyrimidine photoproduct was located. The distribution of such sites in the coding region of the human *HPRT* gene is 59% transcribed strand/41% nontranscribed strand. If their mutagenic potentials are comparable and their distribution in *HPRT* sequences coding for critical parts of the protein is random, then the mutations induced in repair-proficient cells irradiated in early S phase and in XP12BE cells ought to be 59% arising from lesions in the transcribed strand and 41% arising from lesions in the nontranscribed strand. Mutations in repair-proficient cells irradiated in G_1 phase might not show this pattern.

The XP12BE cells showed a transcribed/nontranscribed strand ratio of premutagenic lesions of 79:21 in S-phase mutants and 69:31 in G_1-phase mutants. The repair-proficient cells also showed a 71:29 ratio in the S-phase mutants, but the G_1-phase mutants showed a 20:80 ratio. These data indicate bias toward UV-induced mutations resulting from lesions in the transcribed strand of the *HPRT* gene in the absence of repair and preferential repair of such lesions in that strand.

MATERIALS AND METHODS

Cells and media. Finite-life-span diploid human fibroblasts, designated NFSL89, were explanted from the foreskin of a normal newborn (11). These cells have normal nucleotide excision repair capacity. XP12BE cells were obtained from the American Type Culture Collection (Rockville, Md.). Early-passage cells were used and were routinely cultured in a modified MCDB-110 medium (20) containing 10% supplemented bovine calf serum (HyClone, Logan, Utah) (culture medium). For selection of TG^r cells, the same medium but lacking adenine and containing 5% fetal bovine serum, 5% supplemented calf serum, and 40 μM TG was used. For thymidine incorporation experiments, the medium was changed to Eagle's minimal essential medium to eliminate thymidine.

Mutagenesis and isolation of $HPRT^-$ mutants. (i) Cell synchronization. Cells were driven into the G_0 state as described previously (26). To stimulate the cells to enter the cell cycle, they were plated in culture medium at a density of 10^4 cells per cm^2. The time of onset of S phase following release from G_0 was determined by the incorporation of [^{3}H]thymidine into acid-insoluble material as described previously (26).

(ii) Exposure to UV light and isolation of $HPRT^-$ mutants. A series of independent populations (1.5×10^6 cells each, plated in 150-mm-diameter plastic dishes) was irradiated 17 h after release from G_0 (at the beginning of S phase) or 11 h after release from G_0 (in G_1 phase). The culture medium was aspirated, and the cells were washed with sterile phosphate-buffered saline (PBS; pH 7.4). The cells were irradiated as described previously (17) at UV fluences of 6.5 J/m^2 for repair-proficient cells and 0.5 J/m^2 for repair-deficient cells and were refed with culture medium. Cells plated at cloning density were similarly exposed and used to determine survival of colony-forming ability. The target cells were allowed an 8-day expression period before selection of 2.5×10^5 cells from each population with TG was begun as described previously (11). When macroscopic drug-resistant clones had developed 14 days later, they were located, isolated, and expanded into cultures composed of $\sim 2.0 \times 10^4$ cells, or the cDNA was amplified directly from the original clone.

Synthesis of first-strand cDNA directly from mRNA in cells. Cells were trypsinized and suspended in culture medium; if the cells in a colony had been expanded, the cell number was determined by electronic counting, and the cells were diluted in cold PBS (pH 7.4). From 100 to 500 cells in PBS were transferred to a 0.5-ml Eppendorf tube and centrifuged for 10 min at 4°C. The supernatant was removed, and the cell pellet was resuspended in 5 μl of the cDNA cocktail described in detail by Yang et al. (28). The reverse transcriptase reaction was performed at 37°C for 1 h to allow the cell membranes to be lysed by detergent and first-strand cDNA to be synthesized from total cytoplasmic poly(A) mRNA (28).

Amplification of *HPRT* cDNA and DNA sequencing. The experimental conditions, optimized for preparing second-strand *HPRT* cDNA, amplifying the cDNA 10^{11}-fold by using two polymerase chain reaction stages of 30 cycles each, and sequencing the product directly by using three sequencing primers and a modified Sanger dideoxynucleotide procedure, have been described elsewhere (28).

RESULTS

Determining onset of S phase. Repair-proficient NFSL89 fibroblasts and repair-deficient XP12BE fibroblasts were synchronized by release from confluence (density-induced G_0 state) and plated into a series of culture dishes in Eagle's minimal essential medium at 10^4 cells per cm^2. At various times postrelease, the cells were pulse-labeled with tritiated thymidine (5 μCi/ml of medium) for 20 min and the acid-precipitable counts were determined. DNA synthesis in both cell lines began 15 to 16 h following release (Fig. 1). By 17 h, both cell populations were actively incorporating tritiated thymidine.

Survival and mutation frequency. Synchronized populations of XP12BE cells were irradiated with UV light (0.5 to 0.6 J/m^2) 17 h after release from confluence at the beginning of S phase, or in G_1 phase 6 h prior to S, and assayed for survival and the frequency of TG^r cells. Eight replicate cultures in either S or G_1 phase of the cell cycle were irradiated in each experiment together with nonirradiated controls, and three experiments were conducted, for a total of 24 independent populations of cells irradiated at the beginning of S phase and 24 populations irradiated 6 h before the onset of S phase. Cell survival ranged from 10 to 34%. The frequency of TG^r mutants per 10^6 clonable cells in populations irradiated in early S phase ranged from 223 to 324; that in populations irradiated 6 h prior to the onset of S ranged from 253 to 323. The background frequency (in the absence of UV treatment) was less than 10 TG^r mutants per 10^6 clonable cells. The cloning efficiency of the cells at the time of selection was 15 to 22%.

Repair-proficient diploid human fibroblasts were synchronized and irradiated as described above but at a fluence of 6.5 J/m^2. Their survival, which was the same whether irradiation was in early S phase or in G_1 phase, ranged from 18 to 22%. The frequency of TG^r mutants per 10^6 clonable cells in populations irradiated in early S phase ranged from 145 to 162; that in populations irradiated 6 h prior to the onset of S phase ranged from 67 to 76. The frequency observed in unirradiated cells was 5 to 10 mutants per 10^6

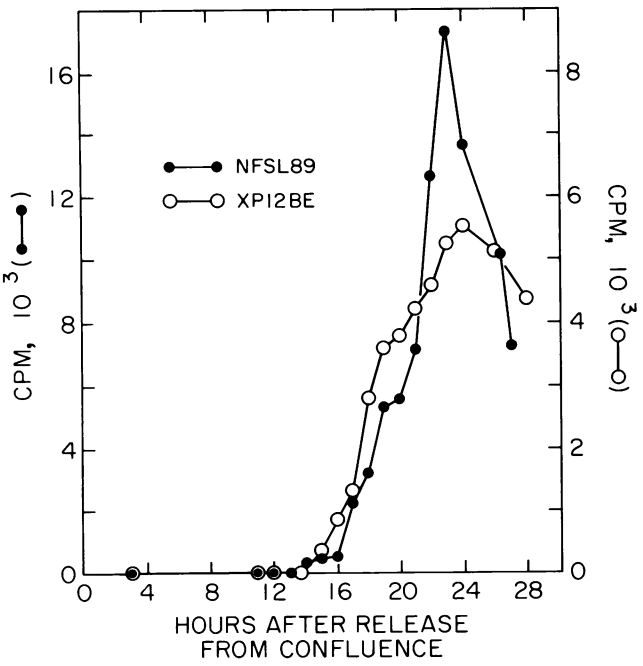

FIG. 1. Incorporation of [^{3}H]thymidine into acid-insoluble material following a 20-min pulse-label at the indicated times. Counts have been normalized to 10^5 cells, and the background (300 cpm/10^5 cells) has been subtracted. Populations were released from density-induced G_0 at T = 0 h.

clonable cells. The cloning efficiency of the repair-proficient cells ranged from 50 to 60%.

Nucleotide sequence alterations in xeroderma pigmentosum mutants. The coding region of the *HPRT* gene of 42 independent TG^r mutants was amplified by polymerase chain reaction, and the product was directly sequenced. Of this number, 22 were derived from populations of XP12BE cells irradiated in S phase and 20 were derived from cells irradiated in G_1 phase. The mutants could be distinguished as independent either because the sequence alterations were unique or because they had been generated in separate populations. The kinds of mutations seen in mutants derived from cells irradiated in S phase, their locations in the coding region, and the consequence for the primary structure of the protein are presented in Table 1. The majority were base substitutions. Among these, transitions predominated, accounting for 68% (15 of 22) of the total. One mutant had an insertion of 62 bp from intron 5, which very probably resulted from inactivation of the 5′ splice site of intron 5 by a G · C→A · T transition and use of a downstream cryptic splice donor site (16). Three mutants had an exon completely deleted. These deletions probably resulted from base substitutions in splice sites, but because we sequenced only the coding region of the gene, the nature of the presumed intron mutations that led to such exon deletions has not been evaluated.

The base substitutions were distributed throughout the exons, but with more located in exon 8 than would be expected on a random basis (Table 1; Fig. 2). The premutagenic dipyrimidine photoproducts presumed to be responsi-

TABLE 1. Kinds and locations of mutations induced in the coding region of the *HPRT* gene of XP12BE cells irradiated in S phase

Type of change and mutant	Position	Exon	Deleted exon	Type of mutation	Surrounding sequence	Amino acid change	Strand with affected dipyrimidine[a]
Base substitution							
XUS39	74	2		C · G→T · A	ATA CCT AAT	Pro → Leu	NT
XUS27	118, 119 tandem	2		G · C→A · T G · C→A · T	CAT GGA CTA	Gly → Lys	T
XUS9	125	2		T · A→A · T	CTA ATT ATG	Ile → Asn	NT
XUS53	126	2		T · A→G · C	CTA ATT ATG	Ile → Met	NT
XUS15	135	3		G · C→T · A	GAC AGG ACT	Arg → Ser	T
XUS42	209	3		G · C→A · T	AAG GGG GGC	Gly → Glu	T
XUS75	403	4		G · C→A · T	GAA GAT ATA	Asp → Asn	T
XUS40	471	6		G · C→A · T	AAG ATG GTC	Met → Ile	T
XUS61	478	6		G · C→T · A	AAG GTC GCA	Val → Phe	T
XUS19	532	7		T · A→C · G	GAC TTT GTT	Phe → Leu	NT
XUS51	539	8		G · C→A · T	GTT GGA TTT	Gly → Glu	T
XUS70	544	8		G · C→C · G	TTT GAA ATT	Glu → Gln	T
XUS77	568, 569 tandem	8		G · C→A · T G · C→A · T	GTA GGA TAT	Gly → Lys	T
XUS20	580	8		G · C→A · T	CTT GAC TAT	Asp → Asn	T
XUS35	599	8		G · C→A · T	TTC AGG GAT	Arg → Lys	T
XUS8	600, 601 tandem	8		G · C→A · T G · C→A · T	TTC AGG GAT TTC AGG GAT	No change Asp → Asn	T T
XUS52	601	8		G · C→A · T	AGG GAT TTG	Asp → Asn	
XUS69	649	9		A · T→C · G	TAC AAA GCC	Lys → Gln	T
Splice site mutation							
XUS3	Splice donor site, intron 5			G · C→A · T	GAAgtaagt	62-bp insertion	T
Putative splice site mutation							
XUS31			8				
XUS44			5				
XUS47			5				

[a] NT, Nontranscribed; T, transcribed.

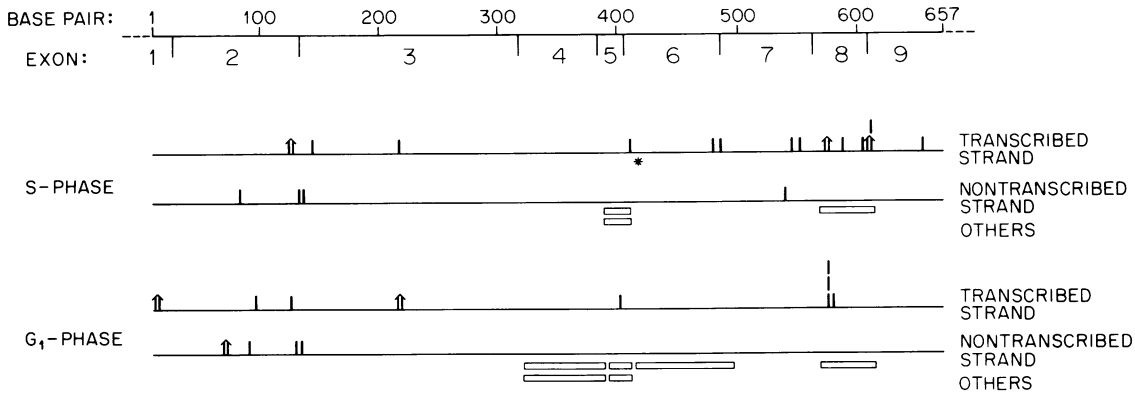

FIG. 2. Locations of independent *HPRT* mutations in XP12BE cells irradiated with UV light (254 nm) at a fluence of 0.5 J/m^2 in early S phase (22 mutants) or 6 h before S phase, in G_1 phase (19 mutants). Mutation XUG20, which did not occur opposite a dipyrimidine sequence, has been excluded. Symbols: □, exon deletions; |, base substitutions; (⇑), tandem mutations; *, base substitution in intron 5 of the transcribed strand. Strand assignments were made on the basis of which strand contained the dipyrimidine sequence that presumably targeted the mutation. There was no statistically significant difference between cells irradiated in S phase or in G_1 phase in the kinds of mutations induced or in the strand distribution of the potentially mutagenic photoproducts.

ble for the observed base substitutions were located primarily in the transcribed strand (79%; 15 of 19).

Table 2 lists the sequence alterations found in 20 mutants isolated from XP12BE cells irradiated 6 h before the onset of S phase. The kinds of mutations did not differ significantly from those induced at S phase, nor did their locations in the gene (Fig. 2). The majority contained base substitutions, predominantly G · C→A · T (56%; 9 of 16), and the corresponding dipyrimidine photoproducts were primarily located in the transcribed strand (69%; 9 of 13). There were 6 exon deletions resulting from putative splice site mutations. One mutant (XUG20) contained a mutation that did not occur at a dipyrimidine site but involved a base substitution and a deletion at an ACA site. This sequence has been reported to form a complex photoproduct following UV radiation (2), but we cannot be certain that this unusual photoproduct was the premutagenic lesion; therefore, this mutation has been excluded from analysis of the strand location of the premutagenic lesions.

Nucleotide sequence alterations in mutants from repair-

TABLE 2. Kinds and locations of mutations induced in the coding region of the *HPRT* gene of XP12BE cells irradiated in G_1 phase

Type of change and mutant	Position	Exon	Deleted exon	Type of mutation	Surrounding sequence	Amino acid change	Strand with affected dipyrimidine[a]
Base substitution							
XUG44	3⌇ 4⌡ tandem	1		G · C → A · T G · C → A · T	ATG GCG ACC ATG GCG ACC	Met → Ile Ala → Thr	T
XUG22	61⌇ 62⌡ tandem	2		T · A → A · T T · A → A · T	GAT TTA TTT	Leu → Lys	NT
XUG23	82	2		T · A → G · C	CAT TAT GCT	Tyr → Asp	NT
XUG21	88	2		G · C → T · A	GCT GAG GAT	Glu → Stop	T
XUG33	119	2		G · C → A · T	CAT GGA CTA	Gly → Glu	T
XUG42	122	2		T · A → C · G	GGA CTA ATT	Leu → Pro	NT
XUG31	125	2		T · A → A · T	CTA ATT ATG	Ile → Asn	NT
XUG11	208⌇ 209⌡ tandem	3		G · C → A · T G · C → A · T	AAG GGG GGC	Gly → Lys	T
XUG20	243 244	3		C · G A · T → T · A	GAT TAC ATC	Frameshift	—[b]
XUG16	393	5		G · C → T · A	GTC TTG ATT	Leu → Phe	T
XUG4	568	8		G · C → A · T	GTA GGA TAT	Gly → Arg	T
XUG13	568	8		G · C → A · T	GTA GGA TAT	Gly → Arg	T
XUG14	568	8		G · C → A · T	GTA GGA TAT	Gly → Arg	T
XUG10	569	8		G · C → A · T	GTA GGA TAT	Gly → Glu	T
Putative splice site mutation							
XUG34			4				
XUG5			4				
XUG27			5				
XUG12			5				
XUG28			6				
XUG40			8				

[a] T, Transcribed; NT, nontranscribed.

[b] We cannot be certain that the premutagenic lesion involved ACA; therefore, the strand involved in the mutation cannot be determined. The nature of the base substitution also cannot be determined since either base pair could have been involved.

TABLE 3. Kinds and locations of mutations induced in the coding region of the *HPRT* gene of repair-proficient diploid cells irradiated in S phase

Type of change and mutant	Position	Exon	Deleted exon	Type of mutation	Surrounding sequence	Amino acid change	Strand with affected dipyrimidine[a]
Base substitution							
NUS13	84	2		A · T → C · G	CAT TAT GCT	Tyr → Stop	—[b]
NUS8	122	2		T · A → C · G	GGA CTA ATT	Leu → Pro	NT
NUS1	123	2		A · T → G · C	GGA CTA ATT	No change	T
	568	8		G · C → A · T	GTA GGA TAT	Gly → Arg	T
NUS21	208, 209 tandem	3		G · C → A · T G · C → A · T	AAG GGG GGC	Gly→ Lys	T
NUS9	447	6		G · C → C · G	TCC TTG GTC	Leu → Phe	T
NUS16	463, 464 tandem	6		C · G → T · A C · G → T · A	AAT CCA AAG	Pro→ Leu	NT
NUS14	471	6		G · C → A · T	AAG ATG GTC	Met → Ile	T
NUS19	498, 499 tandem	7		A · T → G · C A · T → G · C	GTG AAA AGG	No change Arg →Gly	T
NUS7	500, 501 tandem	7		G · C → A · T G · C → A · T	AAA AGG ACC	Arg → Lys	T
NUS4	505	7		C · G → T · A	ACC CCA CGA	Pro → Ser	NT
NUS15	596	8		T · A → C · G	TAC TTC AGG	Phe → Ser	NT
NUS5	599 600	8		G · C G · C → A · T	TTC AGG GAT	Frameshift	T
NUS6	600	8		G · C → C · G	TTC AGG GAT	Arg → Ser	T
NUS3	601	8		G · C → A · T	AGG GAT TTG	Asp → Asn	T
Putative splice site mutation							
NUS2			4				
NUS18			5				
NUS17			5				
NUS22			7				
NUS11			7				
NUS10			8				
NUS12			8				
Other							
NUS20				10-bp deletion, 536–545[b]			

[a] NT, Nontranscribed; T, transcribed.
[b] The strand containing the premutagenic lesion cannot be determined since this mutation did not occur at a dipyrimidine site.

proficient cells. Our hypothesis predicts that the mutations induced in the repair-proficient cells irradiated at the onset of S will resemble those found in the XP12BE cells treated in S or G_1 phase. As shown in Table 3, which presents the *HPRT* sequence alterations found in 22 mutants derived from such populations, this is the case. The majority (15 of 22) of the mutants contained single or tandem base substitution, for a total of 18 base substitutions in the 15 mutants; of this number, 11 (61%) were G · C→A · T transitions. All base pair substitutions except the A · T→C · G base substitution in mutant NUS13 occurred at dipyrimidine sites where photoproducts could form, and 71% (10 of 14) of these photoproducts were located in the transcribed strand. The substitution in mutant NUS13 may represent a preexisting background mutation, since eight of the nine base substitutions that we found in unirradiated background mutants involved A · T base pairs (27).

One S-phase mutant had a 10-bp deletion in exon 8; the premutagenic lesion resulting in this deletion could not be determined. Seven putative splice site mutations that resulted in complete exon deletions were found. The strand distribution of the S-phase mutations (Fig. 3) resembled that found in mutants from either population of XP12BE cells (Fig. 2).

Table 4 lists the spectrum of mutations analyzed from 20 mutants isolated from populations of repair-proficient fibroblasts irradiated in G_1 phase, 6 h before the onset of scheduled DNA synthesis. The majority were base substitutions, mainly G · C→A · T (47%), but the fraction of the premutagenic photoproducts located in the transcribed strand was greatly reduced, i.e., 20% rather than 71%. There were few mutations resulting from lesions in the transcribed strand (Fig. 3). For convenience, Table 5 compares the frequencies of the various kinds of base substitutions observed in the four different populations.

DISCUSSION

The mutation and survival data for the repair-proficient fibroblasts and the XP12BE cells are consistent with previously published reports from this laboratory (9). As discussed by those investigators, the higher survival of the colony-forming ability in the repair-proficient cells than in XP12BE cells indicates that following irradiation, there is time for DNA repair before reproductive death occurs. However, the lack of variation in survival with the cell cycle indicates that the time available for such repair is not time prior to S phase. Instead, it probably reflects time before the cells experience a need for certain critical proteins that cannot be produced because of blocks in transcription.

The data presented here show that in human cells that have no time (or no ability) to excise UV photoproducts, the strand distribution of potentially mutagenic lesions averages 75% transcribed/25% nontranscribed, but in repair-proficient

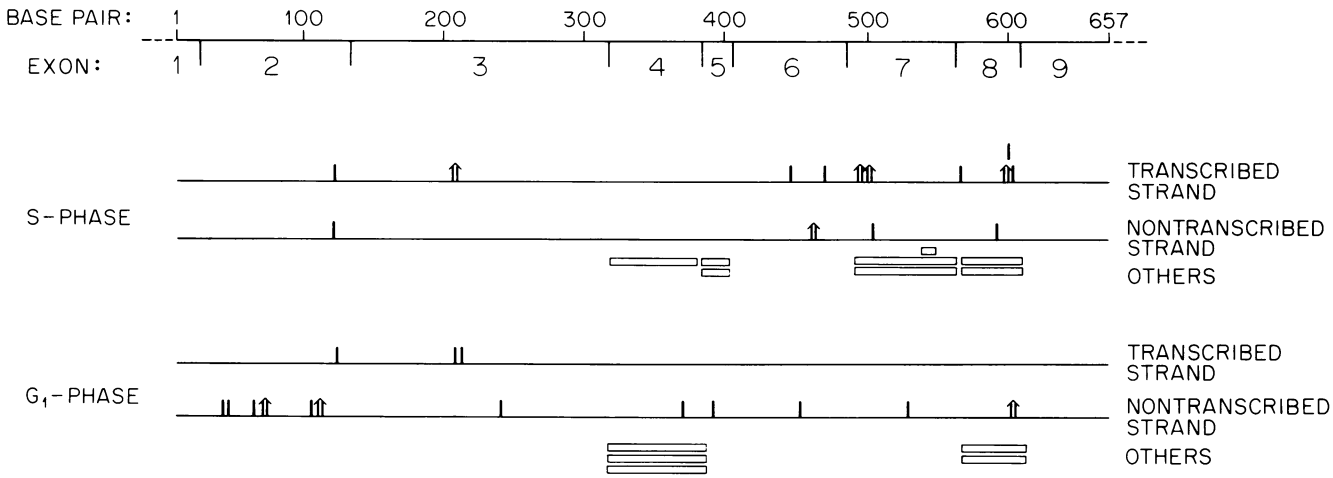

FIG. 3. Locations of independent *HPRT* mutations in repair-proficient human fibroblasts irradiated with UV light (6.5 J/m^2) in early S phase (21 mutants) or in G_1 phase, 6 to 7 h before S phase (20 mutants). Mutation NUS13, which did not occur opposite a dipyrimidine sequence, has been excluded. Mutations are indicated as in Fig. 2. In 71% of the mutants from cells irradiated in S phase, the premutagenic lesion was located in the transcribed strand; in only 20% of the mutants from cells irradiated in G_1 phase was the premutagenic lesion located there. This switch in strand bias indicates preferential repair of the transcribed strand of the *HPRT* gene.

cells given 6 h for repair before S, the distribution is reversed to 20:80. We argue that such a switch in strand distribution is best explained by preferential repair of lesions from the transcribed strand of the *HPRT* gene. There was no statistically significant difference in the frequency or kinds of mutations induced or in the strand distribution of the premutagenic lesions in the mutants derived from populations of XP12BE cells irradiated in either phase of the cell cycle. The data in Fig. 2 suggest that in S-phase cells, mutations induced by UV are more likely to occur in the 3′ half of the *HPRT* gene (72%) and that in G_1-phase cells they are more evenly distributed (47% in the 3′ end). This apparent change cannot be due to DNA excision repair since XP12BE cells are virtually devoid of such repair. This difference was not seen in XP12BE cells following exposure to benzo(a)pyrene diol epoxide (3), and so it might reflect differential accessibility of the gene to UV damage during S and G_1 phases. Nevertheless, it is important to note that there was no cell

TABLE 4. Kinds and locations of mutations induced in the coding region of the *HPRT* gene of repair-proficient diploid cells irradiated in G_1 phase

Type of change and mutant	Position	Exon	Deleted exon	Type of mutation	Surrounding sequence	Amino acid change	Strand with affected dipyrimidine[a]
Base substitution							
NUG2	43	2		C · G → G · C	GAA CCA GGT	Pro → Ala	NT
NUG15	44	2		C · G → T · A	GAA CCA GGT	Pro → Leu	NT
NUG10	67	2		T · A → A · T	TTT TGC ATA	Cys → Ser	NT
NUG3	73, 74 tandem	2		C · G → T · A C · G → T · A	ATA CCT AAT	Pro → Phe	NT
NUG7	108	2		T · A → A · T	GTG TTT ATT	Phe → Leu	NT
NUG14	112, 113 tandem	2		C · G → T · A C · G → T · A	ATT CCT CAT	Pro → Phe	NT
NUG4	124	2		A · T → G · C	CTA ATT ATG	Ile → Val	T
NUG12	209	3		G · C → A · T	AAG GGG GGC	Gly → Glu	T
NUG5	212	3		G · C → T · A	GGG GGC TAT	Gly → Val	T
NUG11	241	3		T · A → C · G	GAT TAC ATC	Tyr → His	NT
NUG6	371	4		C · G → T · A	TCA ACT TTA	Thr → Ile	NT
NUG18	392	5		T · A → C · G	GTC TTG ATT	Leu → Ser	NT
NUG19	464	6		C · G → T · A	AAT CCA AAG	Pro → Leu	NT
NUG20	532	7		T · A → C · G	GAC TTT GTT	Phe → Leu	NT
NUG1	603, 604 tandem	8		T · A T · A → C · G	GAT TTG AAT	Frameshift	NT
Putative splice site mutation							
NUG16			4				
NUG8			4				
NUG9			4				
NUG13			8				
NUG17			8				

[a] NT, Nontranscribed; T, transcribed.

TABLE 5. Types of base substitutions induced in the coding region of the *HPRT* gene in repair-deficient XP12BE fibroblasts and repair-proficient cells irradiated in S phase and in G_1 phase

Type of base substitution	No. of substitutions observed			
	XP12BE fibroblasts		Repair-proficient cells	
	S phase	G_1 phase	S phase	G_1 phase
Transition				
G · C → A · T	15	9	11	8
A · T → G · C	1	1	5	5
Transversion				
G · C → C · G	1	0	2	1
G · C → T · A	2	2	0	1
A · T → C · G	2	1	0	0
A · T → T · A	1	3	0	2
Total	22	16	18	17

cycle-related change in the strand distribution of UV-induced premutagenic lesions in these XP12BE cells. Furthermore, there was no statistically significant difference in the strand distribution of premutagenic lesions between the XP12BE cells in S or G_1 and the NFSL89 cells in S. Therefore, the switch in strand bias observed in mutants derived from the repair-proficient cells irradiated in S or G_1 phase can be attributed to preferential repair of the transcribed strand.

This conclusion, which is based on a comparison of repair-proficient diploid human fibroblasts irradiated in two different phases of the cell cycle, agrees with that of Vrieling et al. (25). These investigators based their conclusion on a comparison of mutants from asynchronous cultures of a repair-proficient and a repair-deficient Chinese hamster cell line. Both studies support the work of Mellon et al. (14), indicating that UV-induced dipyrimidine dimers are preferentially removed from the transcribed strand of an actively transcribed gene in a human cell line. Although it is not yet clear how such targeting of the excision repair complex to a particular strand occurs, preferential repair of the transcribed strand is abolished in mammalian cells by inhibition of RNA polymerase II with α-amanitin (4). Conversely, in *Escherichia coli*, induction of a gene has been shown to induce preferential repair of the transcribed strand (13). These studies suggest that there is a coupling of transcription with nucleotide excision repair of UV-induced lesions. As suggested by Mellon et al. (14), it is possible, for instance, that the stalled RNA polymerase acts as a signal to target the repair complex to the site of the transcription-blocking lesion on the transcribed strand.

Glickman and colleagues (5, 6) have suggested from data in *E. coli* and in CHO rodent cells that UV-induced G · C→A · T transitions result predominantly from 6-4 Py-Py lesions. However, Vrieling et al. (25) suggest that such transitions result primarily from Py-Py dimers because they found that 100% (15 of 15) of the UV-induced base substitutions were G · C→A · T in a Chinese hamster cell line (VH-1) that is incapable of repairing Py-Py dimers but repairs 6-4 Py-Py lesions at a rate 50% that of their repair-proficient V-79 hamster cell line. Since in human cells virtually all 6-4 Py-Py lesions are repaired within 6 h (15), if 6-4 Py-Py lesions uniquely cause a particular kind of base substitution, and if these substitutions constitute a substantial fraction of the UV-induced mutations seen in these cells, we should have observed a significant difference in the kinds of base substitutions in mutants from repair-proficient cells irradiated in S and those irradiated in G_1. This was not observed in our study. Therefore, we conclude that in human cells G · C→A · T transitions result from both unexcised Py-Py dimers and 6-4 photoproducts. This could explain why such transitions are the most common mutations induced by UV radiation in a variety of organisms (5, 8, 10, 21, 24).

As noted above, the distribution of dipyrimidine sites between the transcribed and the nontranscribed strands in the coding region of the human *HPRT* gene is 59:41. If all lesions were equally mutagenic, and if each mutation resulted in a nonfunctional protein, 59% of the mutations in XP12BE cells or repair-proficient cells irradiated in S should have arisen from photoproducts in the transcribed strand and 41% should have arisen from lesions in the complementary strand. Instead, we found a distribution of 75:25. Vrieling et al. (25) found a still larger shift, 90:10, and suggested that the bias reflected a more error-prone polymerase (i.e., polymerase delta) replicating the transcribed strand of the gene. As discussed by these investigators, the origin of replication of the *HPRT* gene is putatively located in the first intron (22), implying that the transcribed strand of most of this gene is also the leading strand during DNA replication and therefore is synthesized by DNA polymerase delta (19). If polymerase delta were more error prone than polymerase alpha, lesions in the transcribed strand would be more likely to result in mutations than would lesions in the complementary strand. However, there is at least one other possible explanation. If photoproducts involving cytosine were more mutagenic than those involving thymine, and if in the coding region of the *HPRT* gene mutations involving a C · G base pair with the C in the transcribed strand were particularly likely to result in a nonfunctional enzyme, this would explain the observed bias. We found that 71% (52 of 73) of the UV-induced base substitutions involved C · G base pairs, and among mutations targeted to lesions in the transcribed strand, 89% (40 of 45) involved C · G base pairs. In contrast, only 43% (12 of 28) of the mutations targeted to lesions in the nontranscribed strand involved C · G base pairs. Furthermore, Chen et al. (3), using the same system, recently showed that when guanine substituted with a bulky residue of benzo(a)pyrene diol epoxide was the premutagenic lesion in the transcribed strand of *HPRT*, the distribution of premutagenic lesions was lower than predicted, i.e., 25% instead of 40%. This finding argues against an error-prone delta polymerase causing the increase above the expected strand distribution observed in the UV studies. Rather, the increase seen in the XP12BE cells and the repair-proficient cells in S more likely reflects selection for nonfunctional HPRT protein.

In summary, we have shown that if diploid human fibroblasts are able to excise UV-induced photoproducts before DNA replication occurs, they exhibit a significant switch in the strand distribution of premutagenic lesions. The ratio of lesions in the transcribed to nontranscribed strand is reversed from 75:25 to 20:80, consistent with preferential excision repair of UV photoproducts from the transcribed strand of the *HPRT* gene.

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ADDENDUM IN PROOF

After this research was completed, A. R. Lehmann (University of Sussex, Brighton, United Kingdom) shared a preprint of a paper describing results of a study of the spectrum of mutations induced in *HPRT* by UV irradiation of asynchronously growing simian virus 40-transformed xeroderma pigmentosum cell line group A (Dorado et al., J. Mol. Biol., in press). In their study, nine base substitutions in the coding region could be attributed to a dipyrimidine lesion in the transcribed strand, compared with only one in the nontranscribed strand.

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