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Mutagenic specificity of solar UV light in nucleotide excision repair-deficient rodent cells

(sunlight mutagenesis/adenine phosphoribosyltransferase gene/xeroderma pigmentosum/p53 tumor-suppressor gene)

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ABSTRACT **To investigate the role of nucleotide excision repair (NER) in the cellular processing of carcinogenic DNA photoproducts induced by defined, environmentally relevant portions of the solar wavelength spectrum, we have determined the mutagenic specificity of simulated sunlight (310–1100 nm), UVA (350–400 nm), and UVB (290–320 nm), as well as of the "nonsolar" model mutagen 254-nm UVC, at the adenine phosphoribosyltransferase (*aprt*) locus in NER-deficient (ERCC1) Chinese hamster ovary (CHO) cells. The frequency distributions of mutational classes induced by UVB and by simulated sunlight in repair-deficient CHO cells were virtually identical, each showing a marked increase in tandem CC → TT transitions relative to NER-proficient cells. A striking increase in CC → TT events was also previously documented for mutated p53 tumor-suppressor genes from nonmelanoma tumors of NER-deficient, skin cancer-prone xeroderma pigmentosum patients, compared to normal individuals. The data therefore indicate that the *aprt* gene in NER-deficient cultured rodent cells irradiated with artificial solar light generates the same distinctive "fingerprint" for sunlight mutagenesis as the p53 locus in NER-deficient humans exposed to natural sunlight *in vivo*. Moreover, in strong contrast to the situation for repair-competent CHO cells, where a significant role for UVA was previously noted, the mutagenic specificity of simulated sunlight in NER-deficient CHO cells and of natural sunlight in humans afflicted with xeroderma pigmentosum can be entirely accounted for by the UVB portion of the solar wavelength spectrum.**

The incidence of both melanoma and nonmelanoma skin cancer has been rising steadily, especially in industrialized nations with primarily fair-skinned populations (1). This trend is often attributed to stratospheric ozone depletion, resulting in increased levels of highly genotoxic solar UVB (290–320 nm), although it is becoming increasingly evident that life-style changes, including more frequent recreational suntanning, may be the major determinant (especially for melanoma) (2). In either case, it is clear that mutations generated in critical growth-control genes by the UV component of sunlight are primary factors in the initiation of multistage photocarcinogenesis (3). Most notably, mutated p53 tumor-suppressor genes recovered from a majority of human nonmelanoma skin cancers are characterized by the "signature" of UV mutagenesis—i.e., a predominance of C → T base substitutions at pyrimidine runs, as well as some tandem CC → TT mutations (4). This DNA sequence specificity is consistent with a role, in sunlight-induced mutagenesis and carcinogenesis, for dipyrimidine photoproducts—i.e., cyclobutane pyrimidine dimers (CPD), pyrimidine(6-4)pyrimidone photoproducts (P6-4P), and/or Dewar photoisomers of P6-4P, which all form at adjacent pyrimidine bases subsequent to direct absorption of UVB photons by DNA (5).

The removal of genetic damage by nucleotide excision repair (NER) represents a vital molecular strategy used by mammalian cells to counteract the genotoxic and carcinogenic effects of diverse environmental agents, including sunlight (6). In fact, the physiological importance of NER is exemplified by the autosomal recessive disease xeroderma pigmentosum (XP), for which affected individuals manifest defective removal of UV-induced dipyrimidine photoproducts and a concomitant 2000-fold increase in the frequency of sunlight-induced skin cancer (7). Moreover, reduced NER capacity among otherwise phenotypically normal individuals has been correlated with predisposition to basal cell carcinoma of the skin (8, 9). The situation is complicated, however, because NER deficiency alone may not be sufficient to explain a cancer-prone phenotype. Indeed, many patients afflicted with trichothiodystrophy present the same genetic repair defect as XP complementation group D but without any accompanying susceptibility to cutaneous tumors (10).

It is therefore of considerable interest to further elucidate the role of NER in the mutagenic processing of sunlight-induced carcinogenic photoproducts in mammalian cells. Investigations on diverse prokaryotic and eukaryotic systems, which compared the types of DNA sequence alterations induced by the model mutagen 254-nm UVC in strains of differing repair capacity, have been extremely useful for probing the overall basic mechanism of mutagenesis (11–14). However, wavelengths in the UVC region are vastly attenuated at the surface of the earth by stratospheric ozone (15), and, to date, no investigations of mutational specificity have been done with NER-deficient mammalian cells exposed to environmentally relevant portions of the sunlight spectrum (i.e., $\lambda > 290$ nm). This consideration may be important, in view of previous data from our laboratory showing the inadequacy of monochromatic UVC, or even of "environmentally relevant" UVB sources, as models for broad-spectrum sunlight-induced mutagenesis. Indeed, using the adenine phosphoribosyltransferase (*aprt*) gene in NER-proficient Chinese hamster ovary cells (CHO) cells, we characterized the mutagenic specificity of "nonsolar" 254-nm UVC (16, 17), as well as of defined regions of the terrestrial sunlight spectrum, including simulated solar light (SSL; 310–1100 nm) (18) and purified polychromatic UVA (350–400 nm) and UVB (290–320 nm) (19). The data revealed that T → G transversions, a class of mutation only rarely recovered in cultured cells either spontaneously or after treatment with a wide variety of mutagens, constituted a high proportion of DNA sequence alterations

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Abbreviations: aprt, adenine phosphoribosyltransferase; CHO, Chinese hamster ovary; CPD, cyclobutane pyrimidine dimer; P6-4P, pyrimidine (6-4)pyrimidone photoproduct; NER, nucleotide excision repair; SSL, simulated sunlight; XP, xeroderma pigmentosum.

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generated by purified UVA. These mutations were also induced significantly more frequently by SSL relative to UVB or UVC, indicating an important role for UVA in solar mutagenesis at the *aprt* locus in CHO cells. This finding on NER-proficient rodent cells highlighted the need for further studies on the genotoxic effects of broad-spectrum sunlight, as opposed to sources emitting only UVC or UVB. We now present the molecular-level characterization of *aprt*$^-$ mutations induced by each of UVA, UVB, UVC, and SSL in an NER-deficient (ERCC1) CHO strain. Our overall results at the *aprt* locus in repair-proficient and -deficient rodent cells, in conjunction with previous data cataloguing DNA sequence alterations in mutant p53 genes from nonmelanoma tumors of XP patients (20), shed further light on the genotoxic basis of skin cancer in humans.

MATERIALS AND METHODS

Cell Strains, Irradiation Conditions, and Sequence Characterization of Mutant *aprt* Alleles. Protocols for routine maintenance of the NER-deficient (ERCC1) CHO *aprt* hemizygote UVL-9 (provided by G. Adair, University of Texas), for irradiation of these cells with defined regions of the solar wavelength spectrum, and for subsequent selection and characterization of *aprt*$^-$ mutants are identical to those described in detail for the isogenic, NER-proficient counterpart (strain AT-3) (16–19). Briefly we used standard 25-W fluorescent lamps emitting primarily 254-nm UVC, polychromatic UVB, or polychromatic UVA, as well as a 2500-W xenon arc lamp to produce broad-spectrum SSL. For UVB, UVA, and SSL, the light was passed through glass cut-off filters to attenuate wavelengths <290, 350, and 310 nm, respectively. [As such, the terms UVB, UVA, and SSL will hereafter refer to the filtered wavelengths incident upon cells—i.e., UVB (290–320 nm), UVA (350–400 nm), and SSL (310–1100 nm)]. For each mutagenic treatment, ≈50 clonally independent *aprt*$^-$ mutants were isolated from NER-deficient cells in medium containing 8-azadenine. Mutant alleles were amplified by using PCR, and the coding regions of the nontranscribed strand were directly sequenced using a *Taq* polymerase-based cycle sequencing protocol (Bethesda Research Laboratories).

RESULTS

Mutation Induction by Solar UV. The repair-deficient CHO strain used in this study retains <5% of NER capacity relative to wild type and is 5- to 10-fold more sensitive to the cytotoxic and mutagenic effects of UV (21). The doses of UVC, UVB, and SSL used to irradiate NER-deficient cells reflect this difference (Table 1). As in the case of doses chosen for the NER-proficient strain, all yielded 80–100% relative survival and a significant induction of *aprt*$^-$ mutants over spontaneous background. For UVA treatment only, both strains were exposed to the same dose (500 kJ·m^{-2}). The relative survival for NER-deficient cells exposed to 500 kJ·m^{-2} UVA was reduced (59%), whereas the mutational yield (absolute number of mutants) was 7-fold higher than for wild type (Table 1).

Mutagenic Specificity of Solar UV-Induced Mutations at the *aprt* Locus in NER-Deficient Vs. -Proficient CHO Cells. A total of 199 independent *aprt*$^-$ mutants induced by UVC, UVB, UVA, or SSL were isolated from NER-deficient CHO cells and analyzed at the DNA sequence level. A number of significant trends are evident when this collection is compared to that previously characterized using the wild-type strain (n = 222). The combined data base is summarized in Table 2 and Fig. 1. The vast majority of mutations generated by all wavelength regions in both repair backgrounds occurred within pyrimidine runs (i.e., at potential sites of dipyrimidine photoproduct formation), and there was considerable overlap in the site specificity, and distribution by class, of mutations within strains (Fig. 1). In particular, the UVB- and SSL-induced mutational distributions in NER-deficient cells were very similar, each marked by approximately equal proportions of C → T and tandem CC → TT transitions and almost no transversions (Table 2). Furthermore, the incidence of tandem double events in UVB-exposed NER-deficient cells is much higher than in wild type (35% vs. 5%; $P < 0.0001$, Fisher's exact test). A similar striking increase in CC → TT events was previously observed for mutated p53 genes from nonmelanoma skin tumors of XP patients, relative to normal individuals (Table 2), although the ratio of single/tandem-double C → T transitions was lower in XP p53 vs. CHO *aprt*. The frequency of SSL-induced CC → TT was also relatively high for NER-deficient (42%) vs. -proficient CHO cells (25%); however, this difference was not statistically significant (P = 0.16). [In this latter respect, we note that the incidence of CC → TT events was already significantly elevated in SSL- vs. UVB-exposed NER-proficient cells (Table 2)]. The overall frequency distributions of UVA- and UVC-induced mutational classes in NER-deficient CHO cells were also surprisingly comparable, although the incidence of CC → TT events in both cases was significantly lower than for UVB and SSL ($P < 0.02$). Interestingly, three UVA-induced C → T mutational hot spots in the NER-deficient strain were also recovered at low frequency in NER-proficient cells after exposure to UVA or UVC (Fig. 1). Finally, T → G mutations were induced significantly more frequently in UVA-irradiated NER$^+$ vs. NER$^-$ cells ($P < 0.002$), even when the strong UVA-induced T → G hot spot (located at positions 243–244; Fig. 1) in the NER-proficient strain is counted only once. In the case of SSL exposure, the disparity in T → G events between repair-proficient and -deficient cells was also significant ($P < 0.002$).

In accord with previously published reports on UVC mutagenesis in NER-proficient vs. -deficient prokaryotic and eukaryotic cells (see *Discussion*), we have observed a striking reversal in the strand bias for mutation induction between NER$^+$ and NER$^-$ CHO strains treated with solar UV. Indeed, in NER-proficient CHO cells, the vast majority of mutations induced by UVB, UVA, and SSL could be attributed to dipyrimidine photoproducts located on the nontranscribed strand of the *aprt* gene (Table 2; Fig. 1). Nevertheless, *aprt*$^-$ mutations induced by all wavelength regions in the NER-deficient strain were mostly attributable to dipyrimidine photoproducts on the transcribed strand. In contrast to this general

Table 1. Frequency of mutations induced by UVA, UVB, UVC, and SSL at the *aprt* locus in NER-proficient and -deficient CHO cells

	Mutation frequency/viable cell × 10^6 ± SEM*	
	NER-proficient	NER-deficient
UVA (WG360)		
Control	2.4 ± 0.2	9.9 ± 0.4
500 kJ·m^{-2}	7.7 ± 0.9	47 ± 0.2
UVB (WG305)		
Control	1.8 ± 0.5	6.8 ± 0.2
20 J·m^{-2}	ND	130 ± 7
100 J·m^{-2}	44 ± 3	ND
UVC (unfiltered)		
Control	1.7 ± 0.3	0.9 ± 0.1
0.5 J·m^{-2}	ND	22 ± 2
5 J·m^{-2}	75 ± 0.6	ND
SSL (WG320)		
Control	2.2 ± 0.4	1.4 ± 0.3
100 kJ·m^{-2}	ND	101 ± 10
500 kJ·m^{-2}	69 ± 0.5	ND

*SE for each treatment was calculated by using mutation-frequency data from at least 10 independent experiments. ND, not determined.

Table 2. Classes of mutation induced by UVA, UVB, UVC, and SSL at the *aprt* locus in NER$^+$ and NER$^-$ CHO cell strains

			Mutation, no. (%)							
			UVC		UVB		UVA		SSL	
	−UV and NER$^+$	Natural sunlight* and p53 from XP tumors	NER$^+$	NER$^-$	NER$^+$	NER$^-$	NER$^+$	NER$^-$	NER$^+$	NER$^-$
Transition										
G·C→A·T	22 (26%)	5 (22%)	33 (57%)	29 (57%)	49 (66%)	26 (51%)	12 (22%)	32 (65%)	13 (36%)	23 (48%)
A·T→G·C	9 (10%)	1 (4%)	4 (7%)	1 (2%)	0	0	5 (9%)	3 (6%)	1 (3%)	0
Transversion										
G·C→T·A	11 (13%)	0	0	2 (4%)	1 (1%)	0	2 (4%)	2 (4%)	0	0
G·C→C·G	9 (10%)	0	5 (9%)	3 (6%)	1 (1%)	0	5 (9%)	0	0	0
A·T→T·A	2 (2%)	1 (4%)	3 (5%)	1 (2%)	2 (3%)	0	1 (2%)	1 (2%)	2 (6%)	0
A·T→C·G	2 (2%)	2 (8%)	3 (5%)	2 (4%)	7 (9%)	2 (4%)	20 (37%)	0	9 (25%)	1 (2%)
Double										
Tandem	0	14 (61%)	6 (10%)	10 (20%)	5 (10%)	19 (37%)	6 (11%)	7 (14%)	11 (31%)	23 (48%)
Nontandem	1 (1%)	0	4 (7%)	3 (6%)	6 (7%)	1 (2%)	2 (4%)	1 (2%)	0	1 (2%)
CC→TT†	0	14 (61%)	3 (5%)	7 (14%)	4 (5%)	18 (35%)	3 (5%)	2 (4%)	9 (25%)	20 (42%)
Insertion	1 (1%)	0	0	0	0	0	0	0	0	0
Deletion	18 (21%)	0	0	0	0	3 (6%)	0	3 (6%)	0	0
Frameshift	6 (7%)	0	1 (2%)	0	2 (2%)	0	1 (2%)	0	0	0
Duplication	5 (6%)	0	0	0	1 (1%)	0	0	0	0	0
Strand‡		(95%)	(64%)	(29%)	(86%)	(33%)	(85%)	(23%)	(94%)	(17%)
Total	86	23	59	51	74	51	53	49	36	48

*Data taken from ref. 20.
†Data also included as part of the Tandem category.
‡Percentage of point mutations attributable to dipyrimidine photoproducts (i.e., either CPD or P6-4P) located on the nontranscribed strand.

situation for UV-irradiated NER-deficient cultured cells, we note that a large majority of natural sunlight-induced mutations at the p53 locus in NER-deficient XP patients were recovered opposite photoproducts on the nontranscribed strand (ref. 20; Table 2). However, this result reflects the exceptional situation that most of these mutations were isolated from XP-C individuals, who are completely proficient in preferential repair of active genes but are deficient in the repair of bulk DNA.

DISCUSSION

We previously used NER-proficient CHO cells to analyze the DNA sequence specificity of *aprt*$^-$ mutations generated by defined regions of the terrestrial solar wavelength spectrum, including SSL (310–1100 nm), UVA (350–400 nm), and UVB (290–320 nm), as well as by the "nonsolar" model mutagen 254-nm UVC. The data revealed unique patterns of mutation induction for each wavelength region, which together were consistent with a significant role for UVA in SSL-exposed rodent cells. To elucidate the role of NER in sunlight mutagenesis, as well as the respective contributions of UVA and UVB in the absence of repair, we have now characterized the molecular nature of mutations induced by UVA, UVB, UVC, and SSL at the *aprt* locus in an isogenic, NER-deficient CHO strain. The frequency distribution of mutational classes induced by UVB and by SSL in NER$^-$ CHO cells was virtually identical, each being characterized by a considerably higher proportion, compared to NER$^+$ cells, of CC → TT tandem double "UV signature" mutations (Table 2). A similar increase in CC → TT events has also been observed for mutated p53 tumor-suppressor genes in sunlight-induced nonmelanoma tumors isolated from NER-deficient, skin cancer-prone XP patients, relative to normal individuals (Table 2; ref. 20). These data indicate important mechanistic similarities between rodent cells irradiated *in vitro* with SSL and human skin exposed *in situ* to natural sunlight. Moreover, the UVA-dependent surplus of T → G transversions previously observed in NER$^+$ cells is absent in the repair-deficient strain (Table 2), indicating that this surplus depends upon a functional NER pathway, as well as the presence of UVA wavelengths. Thus, in contrast to the situation for NER-proficient CHO cells, our results strongly indicate that the mutagenic specificity of SSL in the NER$^-$ CHO strain, and, by analogy, of natural sunlight in repair-deficient humans, can be entirely accounted for by the UVB portion of the solar wavelength spectrum.

Two distinct mutagenesis pathways are theoretically available to sunlight-exposed repair-proficient cells—i.e., mutagenic bypass of photoproducts during semiconservative DNA synthesis (replicative bypass) and/or error-prone filling of nucleotide excision repair gaps involving closely opposed adducts (repair bypass) (6). Therefore, the observed disparities in the types and distribution of solar UV-induced mutations between NER-deficient vs. -proficient rodent cells at the *aprt* locus *in vitro*, as well as between normal individuals vs. XP patients at the p53 locus *in vivo*, might reflect different specificities of nucleotide misincorporation opposite UV photoproducts for DNA polymerases involved in each of these processes (22, 23). Consequently, the replicative polymerase complex(es), the only one(s) operative in NER-deficient cells, may tend to misincorporate adenine across either one or both bases of highly mutagenic CC dipyrimidine photoproducts but would perform translesion synthesis opposite dimerized T residues in a relatively error-free manner. On the other hand, the high frequency of T → G transversions in repair-proficient cells (which depends on both NER and the presence of UVA) could result from the propensity of an error-prone repair polymerase, either induced or modified by UVA, to insert cytosine opposite thymine-containing dipyrimidine photoproducts. In this respect, we note emerging data demonstrating UVA-mediated induction of particular proteins that are not also up-regulated by UVB (24–26).

The divergent patterns of DNA sequence alterations induced by solar UV in NER-proficient vs. -deficient cells may also reflect different premutagenic lesions predominating in each case. For example, wild-type rodent cells excise P6-4P much more rapidly relative to CPD (27), providing some indication that CPD are the preeminent genotoxic lesions in NER-proficient cells. Nonetheless, P6-4P appear intrinsically more mutagenic than CPD in bacteria and yeast (28) and may account for a significant fraction of UVC-induced mutations in hamster cells even under repair-proficient conditions (29). The persistence of P6-4P (or of its Dewar valence photoisomer) in sunlight-exposed, NER-deficient cells may therefore

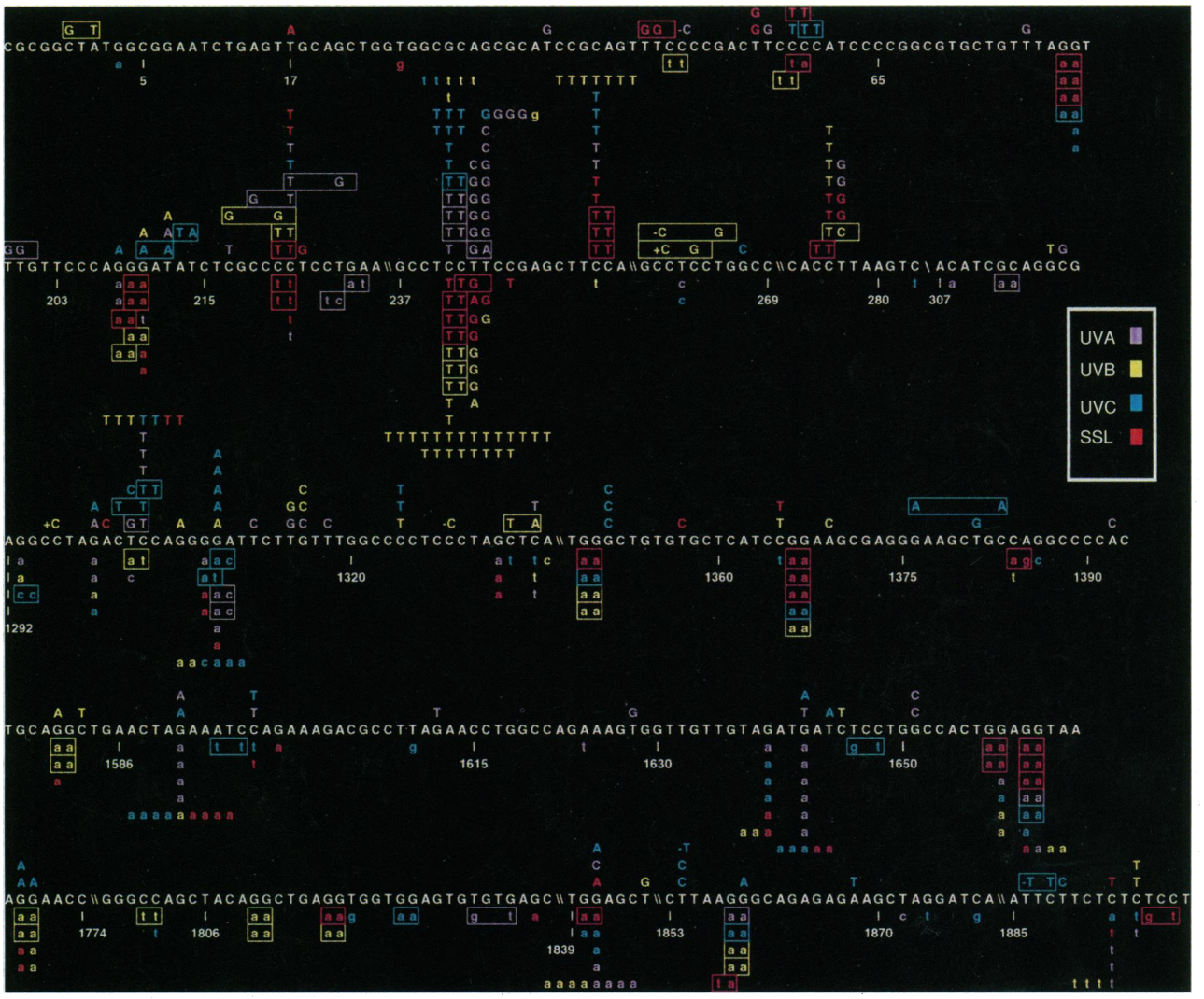

FIG. 1. DNA sequence specificity of mutations induced by UVC, UVB, UVA, and SSL at the CHO *aprt* locus in NER-proficient and -deficient strains. The five sequences from top to bottom represent portions of the nontranscribed strand for exons 1–5 respectively, and compose 80% of the *aprt* coding region. Mutations above the line (uppercase) were isolated from NER$^+$ cells (strain AT-3), whereas those below the line (lowercase) were isolated from NER$^-$ cells (strain UVL-9). The sole exception to this is pyrimidine run 238–246, where mutations derived from AT-3 cells are depicted both above and below the line and those derived from UVL-9 are above the line. A double backslash indicates a break in the sequence. Tandem and nontandem double events are boxed.

result in a much more important mutagenic contribution by this photoproduct relative to CPD. This mechanism could (*i*) suppress the yield of SSL-induced T → G transversions, considering that these events are probably attributable to CPD (as opposed to P6-4P) as discussed previously (19), and (*ii*) lead to an increase in CC → TT transitions, given that this is consistent with the coding properties of CC P6-4P. Finally, it is also possible that the surplus of T → G mutations in SSL-irradiated NER$^+$ CHO cells may be induced by a rare, but highly mutagenic, UVA-specific nondimer lesion. Relative to dipyrimidine photoproducts, this lesion might also be refractory to NER. In repair-competent cells, such an adduct could make a significant contribution to the mutagenic burden imposed by broad-spectrum sunlight but might be completely overwhelmed by the plethora of UVB-induced dipyrimidine photoproducts that would persist in the absence of NER.

It is also noteworthy that while CC → TT events are recovered more frequently at the CHO *aprt* and *hprt* loci in UVC-irradiated NER$^-$ vs. NER$^+$ strains (this study; ref. 13), these increases are not statistically significant. Furthermore, the present data show a much higher frequency of CC → TT mutations in UVB vs. UVC-irradiated repair-deficient CHO cells (Table 2; $P < 0.02$), perhaps reflecting the increased yield of CC CPD recovered in DNA irradiated with UVB, compared with UVC (18, 30). Finally, in strong contrast to the situation for the human p53 gene from normal individuals vs. XP patients exposed to natural sunlight *in vivo* (20), there is no difference in the frequency of UVC-induced CC → TT events at the endogenous *hprt* locus in human diploid fibroblasts from normal individuals vs. XP patients (14) or in the *supF* target gene carried on an extrachromosomal shuttle vector passaged through normal human vs. XP cells (12). These observations reinforce the notion that cultured rodent or human cells irradiated with UVC do not provide an adequate mechanistic paradigm for natural sunlight-induced mutagenesis *in vivo*.

It is now well established in UVC-irradiated bacteria, yeast, and mammalian cells that CPD and P6-4P located on the transcribed strand of active genes are preferentially repaired relative to the nontranscribed strand (31) and, indeed, that the processes of transcription and NER are mechanistically coupled (32). In accord with this situation, a clear majority of *aprt*$^-$ mutants induced by environmentally relevant UVA, UVB, and SSL in NER-proficient CHO cells were presumably fixed across dipyrimidine photoproducts on the nontranscribed

strand (Table 2). In striking contrast, a preponderance of events induced by all wavelength regions in the NER-deficient strain can be attributed to photoproducts located on the transcribed strand (Table 2; Fig. 1). A series of investigations of UVC mutagenesis at various target loci in repair-deficient bacterial (11, 33), rodent (this study; 34, 35), and human cells (14, 36) have also revealed a bias for mutation induction toward photoproducts located specifically on the transcribed strand. The seemingly universal occurrence of this phenomenon is clearly consistent with a mechanistic link to transcription. Accordingly, a plausible model was invoked to explain the repair-status-dependent reversal in strand bias for UVC-induced mutations in the *lacI* gene of *Escherichia coli* (33). This model was based on previous evidence indicating that stalled transcription-repair complexes can shield lesions on the transcribed strand from other critical repair enzymes—e.g., photolyase (37). Therefore, in the absence of NER, repair of the transcribed strand via alternative pathways would be preferentially inhibited relative to the nontranscribed strand, ultimately favoring mutation induction due to lesions on the transcribed strand.

The striking reversal in strand bias for mutation induction at the *aprt* locus in NER-proficient vs. -deficient cells is highlighted by the extensive redistribution of mutational hot spots between the two strains (Fig. 1). Most notably, the frequency of base substitutions recovered within the pyrimidine run CCTCCTTCC 238–246 in exon 2, where 30% of all solar UV-induced mutations was recovered in NER-proficient cells, was significantly diminished ($P < 10^{-4}$) for the repair-deficient strain, wherein new hot spots appeared (Fig. 2). Interestingly, three of these (located at positions 1591, 1641, and 1841) are characterized by a particularly high incidence of UVA-induced G·C → A·T events and were also recovered as sites of mutation at low frequency in UVC- and UVA-irradiated NER-proficient cells. This result is noteworthy, as numerous reports have claimed that dipyrimidine photoproducts do not form in biologically significant yield after irradiation of cells with purified UVA. (DNA only barely absorbs wavelengths >320 nm.) On the other hand, a role for reactive oxygen species has been demonstrated in UVA-induced cytotoxicity (24) and mutagenesis (38). Nonetheless, the present study clearly indicates the preeminent premutagenic potential of dipyrimidine photoproducts in the formation of UVA-induced mutational hot spots in the absence of repair.

Perhaps the most critical issue in carcinogenicity testing and risk assessment concerns the validity of extrapolations from model systems to the human situation. It is evident that previous investigations on the mutagenic specificity of 254-nm UVC in repair-proficient cultured cells have provided a useful model for probing the genotoxic basis of photocarcinogenesis in humans (39). We now show that the *aprt* gene in NER-deficient CHO cells irradiated with environmentally relevant UVB or SSL, but not with UVC, may constitute a relevant mechanistic paradigm for at least one critical process involved in the initiation of cutaneous tumors in XP patients—i.e., error-prone replicative bypass of solar UV-induced photoproducts at the p53 locus, leading to a strikingly high proportion of CC → TT mutations relative to repair-proficient cells. In short, natural or artificial sunlight generates the same distinctive mutational "signature" in NER-deficient rodent and human cells of diverse anatomical origin, whether *in vitro* or *in vivo*. Such findings are of great practical importance in terms of choosing convenient experimental systems that precisely reflect various aspects of natural sunlight mutagenesis in human oncogenes and tumor-suppressor genes.

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1. Glass, A. G. & Hoover, R. N. (1989) *J. Am. Med. Assoc.* **262,** 2097–2100.
2. Setlow, R. B. & Woodhead, A. D. (1994) *Mutat. Res.* **307,** 365–374.
3. Ananthaswamy, H. N. & Pierceall, W. E. (1990) *Photochem. Photobiol.* **52,** 1119–1136.
4. Brash, D. E., Rudolph, J. A., Simon, J. A., Lin, A., McKenna, G. J., Baden, H. P., Halperin, A. J. & Pontén, J. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 10124–10128.
5. Sage, E. (1993) *Photochem. Photobiol.* **57,** 163–174.
6. Friedberg, E. C., Walker, G. C. & Siede, W. (1995) *DNA Repair and Mutagenesis* (Am. Soc. Microbiol., Washington, DC), pp. 536–544.
7. Kraemer, K. H., Lee, M. & Scotto, J. (1984) *Carcinogenesis* **5,** 511–514.
8. Alcalay, J., Freeman, S. E., Goldberg, L. H. & Wolf, J. E. (1990) *J. Invest. Dermatol.* **95,** 506–509.
9. Wei, Q., Matanoski, G. M., Farmer, E. R., Hedayati, M. A. & Grossman L. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 1614–1618.
10. Stefanini, M., Lagomarsini, P., Arlett, C. F., Borrone, C., Crovato, F., Trevisan, G., Cordone, G. & Nuzzo, F. (1986) *Hum. Genet.* **74,** 107–112.
11. Schaaper, R. M., Dunn, R. L. & Glickman, B. W. (1987) *J. Mol. Biol.* **198,** 187–202.
12. Bredberg, A., Kraemer, K. H. & Seidman, M. M. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 8273–8277.
13. Menichini, P., Vreiling, H. & van Zeeland, A. A. (1991) *Mutat. Res.* **251,** 143–155.
14. McGregor, W. G., Chen, R. H., Lukash, L., Maher, V. M. & McCormick J. J. (1991) *Mol. Cell. Biol.* **11,** 1927–1934.
15. Latarjet R. (1935) *Rev. Opt. Theor. Instrum.* **16,** 398–414.
16. Drobetsky E. A., Grosovsky A. J. & Glickman, B. W. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 9103–9107.
17. Drobetsky, E. A., Grosovsky, A. J., Skandalis, A. & Glickman, B. W. (1989) *Somatic Cell Mol. Genet.* **15,** 401–409.
18. Drobetsky, E. A., Moustacchi, E., Glickman, B. W. & Sage, E. (1994) *Carcinogenesis* **15,** 1577–1583.
19. Drobetsky, E. A., Turcotte, J. & Chateauneuf, A. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 2350–2354.
20. Dumaz, N., Drougard, C., Sarasin, A. & Daya-Grosjean, L. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 10529–10533.
21. Hoeijmakers, J. H. J. (1993) *Trends Genet.* **9,** 211–217.
22. Kunkel, T. A. (1992) *Bioessays* **14,** 303–308.
23. Feig, D. I. & Loeb, L. A. (1994) *J. Mol. Biol.* **235,** 33–41.
24. Tyrrell, R. M. & Keyse, S. M. (1990) *J. Photochem. Photobiol.* **4,** 349–361.
25. Matsui, M. S. & DeLeo, V. A. (1990) *Carcinogenesis* **11,** 229–234.
26. Peterson, M. J., Hanson, C. & Craig, S. (1992) *J. Invest. Dermatol.* **99,** 440–444.
27. Mitchell, D. L. & Nairn, R. S. (1989) *Photochem. Photobiol.* **49,** 805–819.
28. Lawrence, C. W., Gibbs, P. E. M., Borden, A., Horsfall, M. J. & Kilbey, B. J. (1993) *Mutat. Res.* **299,** 157–163.
29. Mullenders, L. H. F., Hazekamp-van Dokkum, A. M., Kalle, W. H. J., Vrieling, H., Zdzienicka, M. Z. & van Zeeland, A. A. (1993) *Mutat. Res.* **299,** 271–276.
30. Mitchell, D. L., Jen, J. & Cleaver, J. E. (1992) *Nucleic Acids Res.* **20,** 225–229.
31. Bohr, V. A. (1991) *Carcinogenesis* **12,** 1983–1991.
32. Selby, C. P. & Sancar A. (1993) *Science* **260,** 53–58.
33. Oller, A. R., Fijalkowska, I. J., Dunn, R. L. & Schaaper, R. M. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 11036–11040.
34. Vreiling, H., Zhang, L.-H., van Zeeland A. A. & Zdzienicka, M. Z. (1992) *Mutat. Res.* **274,** 147–155.
35. Tasseron-de Jong, J., den Dulk, H., Lichtenauer-Kaligis, E. G. R., Kroone, R. C., Giphart-Gassler, M. & van de Putte, P. (1993) *Mutagenesis* **8,** 399–406.
36. Dorado, G., Steingrimsdottir, H., Arlett, C. F. & Lehmann A. R. (1991) *Mol. Cell. Biol.* **217,** 217–222.
37. Selby, C. P., Witkin, E. M. & Sancar, A. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 11574–11578.
38. Applegate, L. A., Lautier, D., Frenck, E. & Tyrrell, R. M. (1992) *Carcinogenesis* **13,** 1557–1560.
39. Greenblatt, M. S., Bennet, W. P., Hollstein, M. & Harris, C. C. (1994) *Cancer Res.* **54,** 4855–4878.