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Unique DNA Repair Properties of a Xeroderma Pigmentosum Revertant

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A group A xeroderma pigmentosum revertant with normal sensitivity was created by chemical mutagenesis. It repaired (6-4) photoproducts normally but not pyrimidine dimers and had near normal levels of repair replication, sister chromatid exchange, and mutagenesis from UV light. The rate of UV-induced mutation in a shuttle vector, however, was as high as the rate in the parental xeroderma pigmentosum cell line.

Xeroderma pigmentosum (XP) is a human recessive disorder that in the homozygote exhibits a large increase in skin cancer induced by sunlight (5). The sensitivity of XP cells in culture to UV light (254 nm) is 5- to 10-fold greater than normal (1, 15), and the cells fail to repair a wide range of radiation- and chemically induced DNA damage (5, 10, 27), including (5-5,6-6)cyclobutane pyrimidine dimers and (6-4) pyrimidine-pyrimidone photoproducts, measured by radioimmunoassay (4, 19–21) and enzymatic methods (7, 13, 14, 44). The relative proportions of cyclobutane dimers to other photoproducts determined from their photoreactivation suggest that (6-4) photoproducts may account for as much as 30% of the total lesions (24), and they are highly mutagenic (2). One of the problems encountered in attempts to clone the XP gene results from the reversion of the XP phenotype to UV resistance (16, 28, 29); therefore, we decided to fully characterize revertants themselves.

XP12RO (a simian virus 40-transformed group A cell line), GM637 (a simian virus 40-transformed normal cell line), and normal fibroblasts (HS27 and AG) were grown in Eagle minimal essential medium with fetal calf serum, penicillin, and streptomycin. The XP12RO cell line was cloned by choosing a single colony from among those that grew from a culture of 100 to 200 cells. Large cultures (10^7 to 10^8 cells) of XP12RO were exposed to ethyl methanesulfonate at a concentration of 1, 3, or 6 mM in culture medium for 16 h, after which the medium was replaced and the cultures were grown for 1 week. The cultures were subsequently transferred on regular occasions (approximately twice per week), based on the cell density. They were irradiated in phosphate-buffered saline with 1 to 3 J of UV light per m^2 (254 nm; 1.3 J/m^2 per s) at each transfer. After an accumulated dose of approximately 60 J/m^2, the cultures were given a dose of 6.5 J/m^2 and allowed to grow for 3 weeks to produce colonies. One colony was chosen for each ethyl methanesulfonate dose and grown into a large culture for subsequent analysis. Cell survival after UV irradiation was determined from the number of macroscopic colonies (>50 cells) formed in 21 days (J. E. Cleaver and G. H. Thomas, J. Invest. Dermatol., in press). Each of the cell lines derived from XP12RO proved to be resistant to UV light (Fig. 1A), and, accordingly, they were defined as revertants (designated XP129, XP322, and XP644). When similar-size cultures were not exposed to ethyl methanesulfonate, no surviving colonies were recovered. Revertants showed intermediate sensitivities to 4-nitroquinoline-1-oxide (4NQO)(Fig. 1B) but were still sensitive to *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) (Fig. 1C), a response characteristic of the mex$^-$ phenotype of XP12RO (8, 31). Normal, XP12RO, and XP129 cells all had similar sensitivity to mitomycin C, with a D_{37} concentration of 45 nM (Cleaver and Thomas, in press), consistent with the results of Fujiwara et al. (11) with normal and XP cells.

Cyclobutane pyrimidine dimers in DNA were quantified in purified DNA by digestion with *Micrococcus luteus* UV endonuclease, as previously described (7, 13, 14). On the basis of this assay, the XP129 revertant and the parental XP12RO cell line both appear to be defective in the removal of cyclobutane dimers (Table 1), whereas the two normal cell lines removed about 60% of the initial number of dimers in 24 h. When repair of cyclobutane and (6-4)pyrimidine-pyrimidone dimers was monitored by radioimmunoassays that specifically detect these lesions in DNA (18, 20, 21, 36), slightly different results were obtained. Normal cells removed 50% of the cyclobutane dimers within 6 h of irradiation compared with none for XP12RO cells (Fig. 2). In normal cells, repair of cyclobutane dimers usually appears faster when assayed by antigenicity than by enzymatic or chromatographic methods (19, 39, 41), suggesting that some modification of antigenicity precedes actual excision from the DNA (D. L. Mitchell et al., Mutat. Res., in press). In this antibody assay, there was no change in cyclobutane dimers in the DNA of the revertants at 6 h and a small reduction at 24 h. This suggests that some modification of dimer antigenicity still occurred in the revertant even though the enzymatic assay (Table 1) demonstrated that cyclobutane dimers were not excised. In contrast, the (6-4) photoproduct was excised to the same extent in both normal and revertant cells, with no removal evident in XP12RO (Fig. 2).

Repair replication after exposure to 10 J of UV light per m^2 was determined by labeling cultures for 4 h either with [^{3}H]thymidine (10 μCi/ml; 80 Ci/mmol) plus bromodeoxyuridine (BrdUrd) (10 μM), fluorodeoxyuridine (1 μM), and hydroxyurea (2 mM) or with [^{3}H]BrdUrd (10 μCi/ml; 1 Ci/mmol). Repair replication was quantified from the specific activities of normal-density DNA isolated from alkaline

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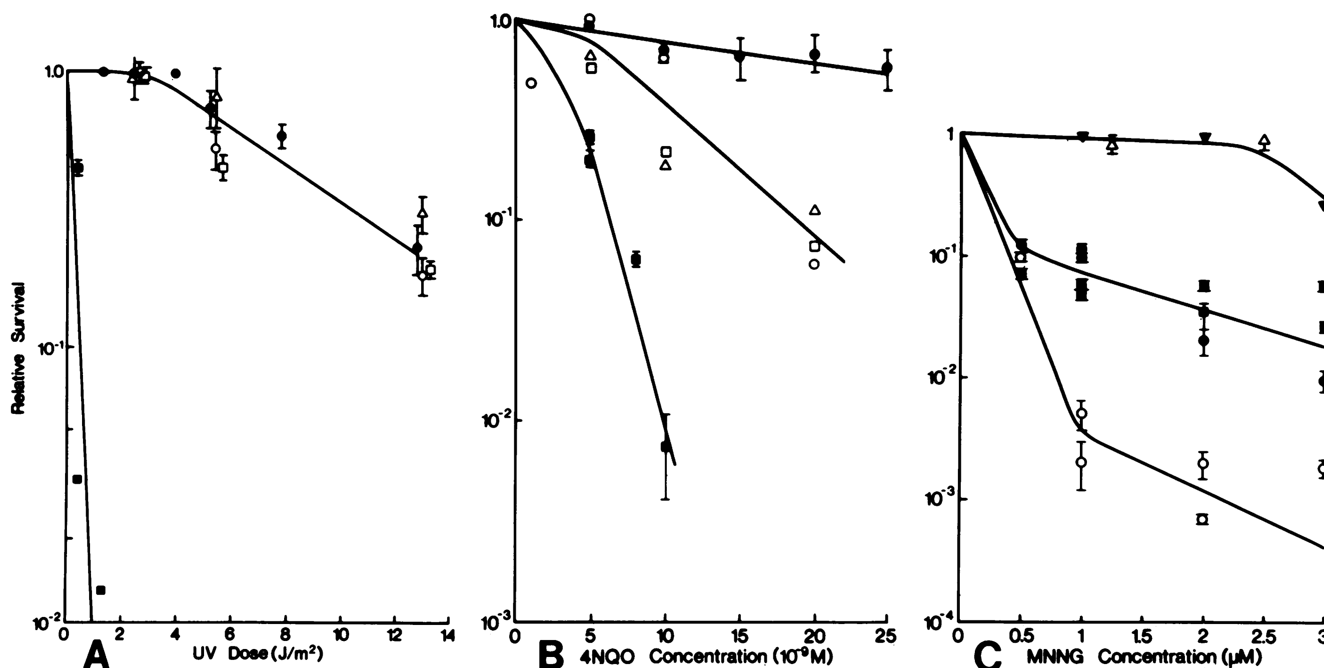

FIG. 1. Relative survival of normal, XP12RO, and XP revertant cell lines after exposure to UV light, 4NQO, or MNNG, as assayed by colony formation. (A) Survival after exposure to UV light. Symbols: ●, GM637; ○, □, and △, XP revertants XP129, XP322, and XP644, respectively; ■, XP12RO. (B) Survival after exposure to 4NQO. Symbols: ●, GM637; ○, □, and △, XP revertants XP129, XP322, and XP644; ■, XP12RO. (C) Survival after exposure to MNNG. Primary human fibroblasts: ▼, FS; △, HS27. Simian virus 40-transformed human fibroblasts: ■, XP12RO; ●, A13-6F. XP revertant: ○, XP129. Means and standard errors are shown.

cesium chloride-cesium sulfate isopycnic gradients (6) and normalized to values obtained from one of the normal cell lines (GM637) for comparison. The revertant XP129 showed levels 50 to 100% of normal (Table 2).

Sister chromatid exchanges (SCEs) were assayed as described previously (22, 42). After trypsinization, asynchronous cells (5×10^5) were plated in 50-mm petri dishes for 90 min. Cultures of each cell type were then exposed to UV light and incubated in fresh medium containing BrdUrd (20 μM) either immediately or after 14 h in fresh medium. The cultures were grown for two complete cell cycles (48 to 52 h), and Colcemid (10 μM) was added for 6 h before harvest. Mitotic cells were collected and processed for SCE analysis as previously described (42). The average numbers of chromosomes were similar for the normal, XP12RO, and revertant XP129 cell lines. Irradiation with low UV doses produced a large increase in SCE frequencies in XP12RO cells and only small increases in normal and XP129 cells (Fig. 3). Above 1 J/m², the SCE frequency in XP12RO cells was too high to score.

Mutations were scored in a shuttle vector system by the method of Seidman et al. (30), except that plasmid pZ189 (a gift of K. Kraemer) was transfected into the mammalian cells by the calcium phosphate coprecipitation procedure (40). Extracellular irradiation of the pZ189 plasmid followed by propagation for 48 h in human cells and subsequent analysis in *Escherichia coli* (12) produced the anticipated results (3)

TABLE 1. Excision of UV endonuclease-sensitive sites (cyclobutane pyrimidine dimers) 24 h after exposure to 13 J of UV light per m²

Cell line	% Sites removed[a] (mean ± SE)
Normal	
GM637	60.0 ± 10.0
HS27[b]	78.0 ± 8.0
XP12RO	5.0 ± 3.5
XP129 (XP revertant)	6.0 ± 2.6

[a] The percentage of enzyme-sensitive sites removed was calculated from the molecular weights of DNA in alkaline sucrose gradients after treatment with UV endonuclease. The number of enzyme-sensitive sites removed in 24 h is expressed as a percentage of the number present immediately after irradiation.

[b] Data described in detail in a previous publication (7).

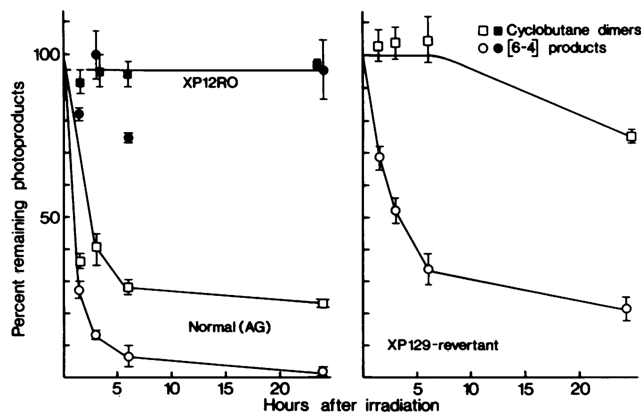

FIG. 2. Percentage of photoproducts remaining in normal human fibroblasts (AG) (●), XP129 (○), and XP12RO (■) after exposure to UV light. Means and standard errors are shown.

TABLE 2. Relative repair replication in normal, XP, and XP revertant cells after exposure to 10 J of UV light per m^2 and incubation with [^{3}H]BrdUrd or [^{3}H]thymidine and BrdUrd

Cell line	% Repair replication (mean ± SE)	
	[^{3}H]BrdUrd	[^{3}H]thymidine + BrdUrd
GM637 (normal)	100	100
XP12RO	0	8.2 ± 1.5
XP129 (XP revertant)	52.9 ± 16.2	91.4 ± 4.3

for normal and XP12RO cells (Table 3). XP12RO and its revertant XP129 both showed an elevated mutation frequency and reduced survival. Preliminary experiments determining the rates of mutation to 6-thioguanine resistance in genomic DNA (17) produced a UV-induced mutation frequency after a dose of 1.3 J/m^2 of 0.15 mutant per 10^5 survivors for GM637 and 0.42 mutant per 10^5 survivors for XP129, whereas a dose of 0.4 J/m^2 to XP12RO produced 8.3 mutants per 10^5 survivors. These results are consistent with the more extensive SCE data and suggest that the XP revertant removes mutagenic lesions from chromosomal DNA but not from shuttle vector DNA. An extensive comparison of mutagenesis in the host genome and the shuttle vector is being made for these cells to determine the basis for the different responses of the two mutational systems.

The procedure we used to derive XP revertants involved mutagenic treatment followed by multiple UV doses and may have produced cell lines with multiple genetic changes. Direct reversal of the original mutation in XP12RO would be an unlikely or very rare event. Reversion by a second-site

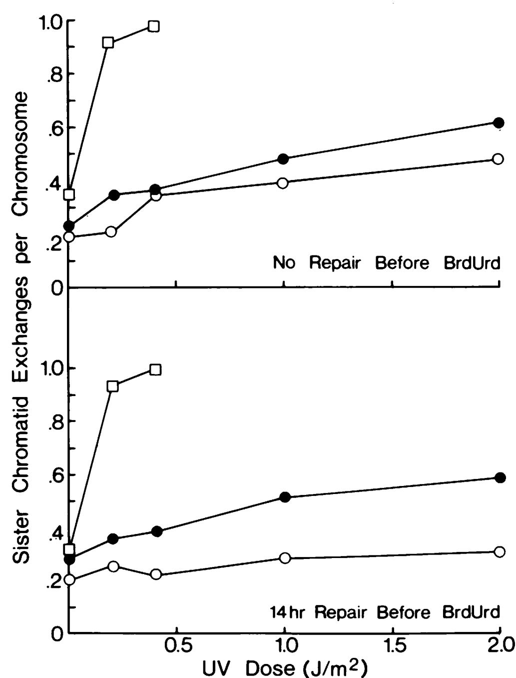

FIG. 3. Frequency of SCEs in normal GM637 (○), XP12RO (□), and XP129 (●) cells irradiated with UV light and grown in BrdUrd for 48 h with or without an intervening period of 14 h of growth in fresh medium. Fifty second-division metaphases were scored for each datum point.

TABLE 3. Survival and mutation frequency in plasmid pZ189 irradiated with UV light (320 J/m^2)[a]

Cell line	UV dose (J/m^2)	Relative survival[b] (%)	Mutation frequency (per 1,000 colonies)
GM637	0		5.5
	320	110.1	5.0
XP12RO	0		3.0
	320	58.2	21.0
XP129	0		16.0
	320	40.0	83.0

[a] Plasmid was propagated for 48 h in human cells, isolated, and assayed in *E. coli* MBM7070 on LB plates containing ampicillin (50 μg/ml), 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside (100 μg/ml), and isopropyl-β-D-thiogalactopyranoside (50 μg/ml). A total of 7,032 transformants were recovered and enumerated.

[b] Survival of irradiated plasmid relative to that of unirradiated plasmid in the same cell line.

mutation is more probable because a variety of mutations could conceivably suppress the effects of the original mutation. Alternative mechanisms for reversion could involve amplification of the region of the DNA containing XP12RO-related genes, but amplified regions are generally unstable in the absence of selection. These cells have remained stable in culture for 1 to 2 years with no further selection pressure. Increased expression of the repair gene *ERCC-1* identified on human chromosome 19 is also unlikely to produce the properties seen in our revertant because this gene increases excision of cyclobutane dimers (43).

These results indicate that the (6-4) photoproduct may play a more important role in the lethal and mutagenic effects of UV light on human chromosomal DNA than do cyclobutane dimers. Experiments showing the importance of cyclobutane dimers in human cells usually involve exogenously irradiated plasmids or exogenously supplied microbial genes or gene products (9, 24, 25, 34, 35, 37) (Table 3). These experiments may not accurately reflect how cells repair their own chromosomes with their endogenous enzyme systems.

The properties of XP129 indicate that it may more readily repair lesions that exhibit relatively greater distortions in the DNA (23, 26, 38). This property may reflect the occurrence of multiple photoproduct-specific excision enzymes, of which only one has reverted, or broad specificity in an excision enzyme complex (38) that has partially reverted allowing detection of only a few important lesions. The high immunogenicity of the (6-4) photoproduct supports the notion that this may be a more readily detectable lesion (20).

If the XP revertant we analyzed is typical of revertants obtained during DNA transfection and from other sensitive mutants (32, 33), some of its properties may be exploited in strategies for cloning XP genes. A selection scheme that kills revertants but not cells containing a normal gene, for example, would be a distinct advantage. The revertants described here had intermediate sensitivity to 4NQO and are as sensitive as XP12RO to monofunctional psoralen derivatives (L. Vuksanovic and J. E. Cleaver, Mutat. Res., in press). Selection by a suitable chemical carcinogen might make it possible to distinguish true transfectants from revertants in attempts to clone XP genes.

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