Genetic and Biochemical Characterization of the CHO-UV-1 Mutant Defective in Postreplication Recovery of DNA

Patricia Hentosh, Andrew R. S. Collins, Laura Correll, Albert J. Fornace, Jr., Amato Giaccia, and Charles A. Waldren

Eleanor Roosevelt Institute for Cancer Research [P. H., L. C., C. A. W.] Denver, Colorado 80206; Department of Radiology, University of Colorado Health Sciences Center [C. A. W.], Denver, Colorado 80262; University of Aberdeen, Department of Biochemistry, Marischal College, Aberdeen AB9 1AS, United Kingdom [A. R. S. C.]; Laboratory of Pathology, National Institutes of Health, Bethesda, Maryland 20892 [A. J. F.]; and Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania 19104 [A. G.]

ABSTRACT

The CHO-UV-1 mutant, a Chinese hamster ovary cell with defective postreplication recovery of DNA, is 2- to 4-fold more sensitive than its wild-type counterpart (CHO-77256) to the lethal effects of ethylating agents and UV radiation; it is also hypersensitive (10- to 20-fold) to some DNA-methylating and cross-linking agents. We studied the CHO-UV-1 mutant further to define its phenotype in terms of DNA damage induction and repair, methyltransferase activity, and effects of caffeine on mutational and lethal responses. Both wild-type and CHO-UV-1 cells incurred similar levels and types of damage when exposed to UV radiation, N-methyl-N'-nitro-N-nitrosoguanidine, or N-methyl-N-nitrosourea. The rate and extent of repair of Micrococcus luteus endonuclease-sensitive sites after UV irradiation or treatment with N-methyl-N'-nitro-N-nitrosoguanidine were also equivalent in these two cell types. Twenty % of the initial endonuclease-sensitive sites induced in either cell line remained at 18 h after UV irradiation; approximately 8% of the sites after N-methyl-N'-nitro-N-nitrosoguanidine exposure were present in both parental and CHO-UV-1 cells after a 17-h repair period. Moreover, the ability of CHO-UV-1 to resynthesize and ligate DNA during excision repair was similar to that of its parent. Neither CHO-UV-1 nor CHO-77256 had appreciable levels of O6-methylguanine-DNA methyltransferase activity which ameliorates the cytotoxicity of alkylating agents. Caffeine, a known inhibitor of postreplication repair, decreased the frequency of mutation induction at the hypoxanthine-guanine phosphoribosyltransferase locus by 40-55% in CHO-77256 but not in CHO-UV-1. These results rule out defective excision repair as a factor in the hypersensitivity of the CHO-UV-1 mutant to DNA-damaging agents. Hence, this cell line appears to derive from a mutation affecting nonexcision repair processes and should be useful in clarifying the mechanisms of postreplication recovery of DNA in mammalian cells.

INTRODUCTION

DNA repair processes can influence the induction of human cancers and their response to chemotherapy (1-4). Current knowledge of repair pathways and enzymes has been gained largely through the production and characterization of repair-deficient mutants of bacteria and yeast, many of which were isolated on the basis of their hypersensitivity to cytoxic agents (5-8). Some mutants are defective in "error-free" repair, so named because of the accuracy with which damaged bases are excised and replaced, with the undamaged complementary strand serving as a template. Other repair mechanisms are much less exact, allowing particular kinds of lesions (noninstructional) to be bypassed during DNA synthesis or to be circumvented by recombination; cells containing these unrepaired lesions may survive and proliferate (9). Such mechanisms have been variously termed PRR, bypass recovery, and daughter strand gap synthesis (10). Bacteria that lack "error-prone" repair may be lessmutable than wild-type cells (11).

In mammalian cells, there is evidence for both error-free and error-prone repair, although the former is much better documented and better understood (12, 13). Excision repair-defective mutants derived from either human or rodent cells tend to be hypermutable, suggesting that excision repair in mammalian cells is also error-free and acts to reduce the rate of mutation (14, 15). The importance of these error-free repair mechanisms in human cells is well illustrated by patients with xeroderma pigmentosum, who have defective excision repair and tend to develop skin cancers and other neoplasms more readily than do normal persons (1).

Relatively little is known about nonexcision repair responses such as PRR, because natural mutants defective in these processes are rare. Cultured cells from patients with the variant form of xeroderma pigmentosum display normal excision repair activity but are thought to be defective in PRR (16, 17). Such persons have extreme photosensitivity and an increased risk of developing cancer, as do typical xeroderma pigmentosum patients. Thus, the absence of a defect in PRR pathways may have a role in the induction of human cancer.

Mutant mammalian cell lines may aid in understanding the biological importance of PRR and the pathogenic implications of defects in this process. We have described a mutant Chinese hamster ovary cell line, CHO-UV-1 (18), that exhibits wild-type levels of UV-induced excision repair, as judged by both unscheduled DNA synthesis and incision capacity, but is deficient in PRR after exposure to UV radiation (19). This was observed as a 4-fold slower rate of conversion of nascent CHO-UV-1 DNA into bulk high molecular weight DNA after UV irradiation, compared to CHO-77256. Thus, the ability to complete DNA replication on a UV-damaged template is abnormal in the mutant line (19). CHO-UV-1 displays moderate hypersensitivity to the lethal activity of many mutagenic agents, including UV radiation, EMS, N-nitroso-N-ethylurea, and N-acetoxy-2-acetylaminofluorene. It also is strikingly hypersensitive to methylating agents such as MNNG and MNU and to drugs such as MMC that create DNA-DNA cross-links (20, 21). CHO-UV-1 was shown to be less mutable than its parental cell line at the HGPRT locus after exposure to either EMS or UV radiation (19). Hoy et al. (21), by fusing CHO-UV-1 with excision repair-deficient hamster cells, also demonstrated that the CHO-UV-1 mutant is also more sensitive to cross-link and methylation damage, produced hybrids that were resistant to MMC but not to methyl methanesulfonate. They also isolated revertants of CHO-UV-1 that remained hypersensitive to MMC, suggesting that the CHO-UV-1 mutation, apart from its PRR deficiency, may affect other cellular processes that influence mutability.

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2 To whom requests for reprints should be addressed, at Department of Biochemical and Clinical Pharmacology, St. Jude Children's Research Hospital, P. O. Box 318, Memphis, TN 38101.

3 Present address: Department of Radiology and Radiation Biology, Colorado State University, Ft. Collins, CO 80523.

4 The abbreviations used are: PRR, postreplication recovery; EMS, ethyl methanesulfonate; MNNG, N-methyl-N'-nitro-N-nitrosoguanidine; MNU, N-methyl-N-nitrosourea; HGPRT, hypoxanthine-guanine phosphoribosyltransferase; ESS, endonuclease-sensitive sites; MMC, mitomycin C; HU, hydroxyurea; Ara-C, 1-β-D-arabinofuranosyl cytosine; 6-TG, 6-thioguanine; O6-MeGua, O6-methylguanine; PBS, phosphate-buffered saline.
sitive to MMC but were resistant to MNNG, suggesting among other possibilities that the phenotype of CHO-UV-1 was due to more than one genetic alteration, perhaps reflecting greater susceptibility to damage or a defect in excision repair in addition to PRR.

In this paper we further characterize the CHO-UV-1 mutant, demonstrating that its hypersensitivity to DNA-damaging agents cannot be attributed to defective excision repair but most likely reflects an inability to bypass DNA lesions during replication.

MATERIALS AND METHODS

Cell Culture. The CHO-UV-1 mutant was derived from CHO-77256, a glycine auxotrophic mutant of CHO-K1 (18). Cells were routinely cultured in complete growth medium [F12 medium supplemented with 7% heat-inactivated fetal bovine serum (HyClone, Inc., Logan, UT), 200 µM glycine, and antibiotics] at 37°C in a 5% CO2 atmosphere. Detailed life cycle analyses of both parental and CHO-UV-1 mutant cells have been described (18, 22).

Cell Survival and Mutagenesis. The cytotoxicity of MNU (Sigma, St. Louis, MO), EMS (Eastman Kodak, Rochester, NY), and UV radiation was determined from single-cell survival curves (23). Graded concentrations of MNU or EMS were added, in the presence or absence of 6 mM caffeine (Sigma), to specified numbers of cells that had been preincubated for 3 h in 60-mm tissue culture dishes. After removal of the drugs at 16 h, the cells were rinsed with PBS (1 mM KH2PO4, 5.6 mM Na2HPO4, 155 mM NaCl) and incubated an additional 7 days in complete F12 growth medium. The preparations were then fixed and stained, and the number of surviving colonies was determined.

For UV irradiation, cells suspended in growth medium at a concentration of 1.4 x 105 cells/ml were exposed to constant shaking to a 30-watt germicidal lamp that delivered 254-nm light at a dose rate of 0.33 J/m2/sec (18, 19). Aliquots of cells were transferred to tissue culture dishes containing fresh growth medium, to allow cellular repair to occur (27). For alkaline elution, approximately 8 x 105 cells were deposited by gravity onto polycarbonate filters (0.8-µm pore size). The cells were lysed with 2 ml of a solution of 2% sodium dodecyl sulfate, 0.1 mM glycine, and 0.02 M Na2EDTA (pH 9.6). The filter, containing DNA, was rinsed with 1 ml of endonuclease buffer (30 mM Tris, 40 mM NaCl, 1 mM EDTA, pH 8.0) without M. luteus (27). The M. luteus endonuclease crude extract was prepared as described by Carrier and Setlow (29). A 1.5-mL solution of fraction II, containing 0.17 mg/ml protein, was slowly filtered and the filter assembly was incubated at 37°C for 1 h. The DNA was then eluted from the filter, as above, at a flow rate of 0.8 ml/min (27, 30, 31). Eluted fractions were collected and assayed for radioactivity, as described by Kohn et al. (25).

Measurement of Dimer Exchange. To investigate the exchange of UV-induced dimers from parental to daughter DNA strands (30), we grew CHO-77256 and CHO-UV-1 cells in isoleucine-deficient medium for 48 h to synchronize the cells in G2 phase. The cells were then irradiated with 4 or 10 J/m2 UV, as above, and incubated in fresh growth medium to allow DNA synthesis. At 8 h postirradiation, growth medium plus [3H]thymidine (0.05 µCi/ml) was added to label newly replicating daughter strands; the cells were then incubated for 8 h, rinsed with PBS, and incubated for an additional 3 to 4 h in nonradioactive medium, to permit labeled DNA replication intermediates to become large molecular weight DNA. The presence of dimers in nascent daughter strands was assayed with M. luteus extract and the alkaline elution procedure, as described above and in previous reports (30).

DNA Incision and Ligation after UV Irradiation. Cells (2 x 106) were inoculated into Lab-Tek tissue culture chamber slides (VWR Scientific, Denver, CO) and incubated overnight with [3H]thymidine (42 Ci/mmol, 0.1 µCi/ml) to label DNA. The medium was then aspirated, and the cells were incubated for 30 min with 10 µM HU and 100 µM Ara-C (Sigma), to inhibit DNA synthesis further. After being washed with warm PBS, the cells were irradiated with UV, inhibitor-containing medium was added, and breaks in DNA were determined, as described by Collins et al. (22), at 40 and 80 min postirradiation. DNA ligation was examined by washing cells free of HU and Ara-C at 40 min after irradiation and incubating them with the four deoxynucleobases (100 µM each) for an additional 20 or 40 min, after which time the DNA breaks were measured (22).

O*-MeGua-DNA Methyltransferase Assay. O*-MeGua-DNA methyltransferase activity was measured as described by Myrnnes et al. (32).

RESULTS

Levels of DNA Damage Induced by MNU. We first investigated the amount of DNA damage induced by a methylating agent, MNU, in the CHO-UV-1 mutant and its parent. Cells were exposed to 50 µg/ml MNU for 1.5 h, and the induction of alkaline-sensitive DNA lesions was examined by alkaline elution. The resulting profiles (Fig. 1) were virtually indistinguishable, indicating that both types of cells incur the same initial extent of damage. No differences were detected in the elution profiles of untreated CHO-77256 and CHO-UV-1 (data not shown). However, the single-cell survival curves shown in Fig. 2 demonstrate that CHO-UV-1 cells were much more sensitive to killing than were the parental cells at any given dose of MNU, despite equivalent levels of MNU-induced damage. Thus, the hypersensitivity of CHO-UV-1 cannot be attributed to enhanced susceptibility to the damaging effects of MNU.

nonradioactive medium (27, 28). For UV irradiation, confluent monolayers of cells were rinsed with PBS and irradiated with a 254-nm GE germicidal lamp. The cells were then chilled immediately in PBS containing 15 mM Na2EDTA or incubated at 37°C for various times in fresh nonradioactive medium containing 10% serum plus 0.01 M 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, to allow repair. For MNNG treatment, [3H]labeled cells were exposed to 2.5 µM MNNG in the dark for 45 min, rinsed with PBS, and then either suspended in cold PBS plus 15 mM Na2EDTA or incubated further at 37°C in fresh medium, to allow cellular repair to occur (27). For alkaline elution, approximately 8 x 105 cells were deposited by gravity onto polycarbonate filters (0.8-µm pore size). The cells were lysed with 2 ml of a solution of 2% sodium dodecyl sulfate, 0.1 M glycine, and 0.02 M Na2EDTA (pH 9.6). The filter, containing DNA, was rinsed with 1 ml of endonuclease buffer (30 mM Tris, 40 mM NaCl, 1 mM EDTA, pH 8.0) without M. luteus extract (27). The M. luteus endonuclease crude extract was prepared as described by Carrier and Setlow (29). A 1.5-mL solution of fraction II, containing 0.17 mg/ml protein, was slowly filtered and the filter assembly was incubated at 37°C for 1 h. The DNA was then eluted from the filter, as above, at a flow rate of 0.8 ml/min (27, 30, 31). Eluted fractions were collected and assayed for radioactivity, as described by Kohn et al. (25).

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analyzed by alkaline elution. Ovary cells are reported to have low levels of O6-MeGua-DNA methyltransferase activity (Mer') and are unable to repair O6-MeGua lesions (36,37). Although Chinese hamster ovary cells are reported to have low levels of O6-MeGua-DNA methyltransferase activity, compared to findings in human cells (38), we reasoned that this deficiency might be more pronounced in CHO-UV-1 mutants, which could account for their enhanced susceptibility to killing by alkylating agents (33–35) and are unable to repair O6-MeGua lesions (36,37). After incubation of small amounts of the purified Escherichia coli methyltransferase with DNA containing O6-[3H]-MeGua, high levels of 3H were present in the transferase protein (Table 1). O6-MeGua-DNA methyltransferase activity was also expressed in MR1, a Mer human cell line, but was extremely low in CHO-77256 and CHO-UV-1 cells. This result suggests that a difference in methyltransferase levels does not account for the increased sensitivity of CHO-UV-1 cells to MNU.

Repair and Exchange of ESS Induced by UV and MNNG. We measured the induction of DNA damage after UV irradiation or MNNG treatment, the subsequent removal of lesions, and dimer exchange between parental and daughter strand DNA in CHO-UV-1 parental cells, using alkaline elution and a crude endonuclease preparation from M. luteus (27). In both cell lines, the amount of initial (0-h repair) ESS induction by UV radiation was 2.2–2.5 ESS/10⁶ daltons per 0.1 J/m². Low levels of ESS repair were observed at early times after irradiation; approximately 80% of the sites remained at 2 h after radiation exposure (data not shown). At longer repair times (17–40 h), however, only 20% of the sites remained in the parental strands of CHO-77256 and CHO-UV-1 (Table 2). Thus, the rate and extent of ESS removal were similar for both cell types. In cells irradiated during early G₁ phase of the division cycle, a fraction of the ESS induced initially in parental strand DNA appears in newly synthesized daughter strands after DNA replication (30). This process, thought to involve recombinational exchange during DNA synthesis (30), may be another pathway whereby discontinuities in nascent DNA strands are eliminated (39) and could potentially be defective in CHO-UV-1. For such experiments, high doses of UV radiation are necessary to aid in the detection of dimers that are exchanged to daughter strand DNA. Within parental DNA, a cellular dose of 4 or 10 J/m² induces ≈90 and 225 ESS/10⁶ daltons, respectively. Results in Table 2 for ESS exchange indicate that, at 20 h postirradiation, levels of ESS found in daughter strand DNA in both CHO-UV-1 and CHO-77256 were nearly equivalent, compared to the initial induction levels after either dose. Hence, CHO-UV-1 does not differ from wild-type cells in the exchange of M. luteus ESS between parental and daughter strand DNA after UV irradiation.

DNA repair in cells exposed to 2.5 μM MNNG for 45 min was also examined by measuring the loss of M. luteus ESS. The frequency of MNNG-induced ESS was approximately the same for both cell lines (Table 3). After a 2-h repair period, 60–80% of the sites remained unrepaired but, by 17 h, the frequency of ESS had been reduced to approximately 8% in both the parental and CHO-UV-1 cells. This result indicates that the sensitivity of CHO-UV-1 to MNNG killing does not reflect deficient
Table 2 Repair and exchange of M. luteus ESS after UV irradiation in CHO-77256 and CHO-UV-1 cell lines.

To examine repair of ESS in parental strand DNA, we cultured exponentially growing cells for 3 days in complete growth medium containing [³H]thymidine, followed by incubation in nonradioactive medium for 1-3 days. Confluent monolayers were UV irradiated and incubated for various repair periods. Lysed cells were then treated with M. luteus endonuclease extract and assayed by alkaline elution (see "Materials and Methods"). Efficiency of cellular ESS induction was then measured by growing cells in nonradioactive isoleucine-deficient medium for 48 h. Eight h after UV irradiation of cultures, complete growth medium containing [³H]thymidine was added, and incubation was continued for 8 h to label newly synthesized daughter strands. Cells were then chased in fresh nonradioactive medium for 4 h, lysed, and assayed for the sensitivity of DNA to incision by M. luteus endonuclease.

<table>
<thead>
<tr>
<th>UV dose (J/m²)</th>
<th>Repair time (h)</th>
<th>Parental strand ESS/10⁶ daltons</th>
<th>ESS remaining in parental strand (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.21</td>
<td>17</td>
<td>1.1</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.1</td>
<td>19</td>
</tr>
<tr>
<td>1.0</td>
<td>18</td>
<td>4.3</td>
<td>18</td>
</tr>
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<td></td>
<td></td>
<td>5.5</td>
<td>23</td>
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<tr>
<td>1.0</td>
<td>40</td>
<td>4.3</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.3</td>
<td>18</td>
</tr>
</tbody>
</table>

Table 3 Repair of M. luteus ESS after cellular treatment with MNNG.

CHO-77256 and CHO-UV-1 cells were radioactively labeled, as stated for parental strand DNA in Table 2, exposed to 2.5 µM MNNG in the dark for 45 min, rinsed, and then either suspended in cold PBS plus 15 mM Na₂EDTA or incubated in fresh medium to allow repair to occur. Following cell lysis, the presence of MNNG-induced ESS was assayed by incubation of DNA with M. luteus extract, followed by alkaline elution, as described in Table 2 and "Materials and Methods."

<table>
<thead>
<tr>
<th>UV dose (J/m²)</th>
<th>Exchange time (h)</th>
<th>Daughter strand ESS/10⁶ daltons</th>
<th>ESS exchanged to daughter strand (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.0</td>
<td>20</td>
<td>1.10</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.75</td>
<td>0.8</td>
</tr>
<tr>
<td>10.0</td>
<td>20</td>
<td>3.10</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.51</td>
<td>1.1</td>
</tr>
</tbody>
</table>

Fig. 3. Accumulation of DNA breaks introduced by repair endonucleases after UV irradiation of CHO-77256 (A) or CHO-UV-1 (B). Cells were labeled overnight in [³H]thymidine, incubated for 30 min with HU and Ara-C, and irradiated with 6 J/m². Medium with DNA repair inhibitors was then added. The number of breaks accumulated in the presence of HU and Ara-C (A, ••), as well as the breaks detected after HU and Ara-C were removed at 40 min after UV irradiation (A, □) are shown.

Fig. 4. Time after UV (min)

DNA Breaks/10⁶ Daltons

A

B

Table 3 Repair of M. luteus ESS after cellular treatment with MNNG.

CHO-77256 and CHO-UV-1 cells were radioactively labeled, as stated for parental strand DNA in Table 2, exposed to 2.5 µM MNNG in the dark for 45 min, rinsed, and then either suspended in cold PBS plus 15 mM Na₂EDTA or incubated in fresh medium to allow repair to occur. Following cell lysis, the presence of MNNG-induced ESS was assayed by incubation of DNA with M. luteus extract, followed by alkaline elution, as described in Table 2 and "Materials and Methods."

<table>
<thead>
<tr>
<th>Repair incubation time (h)</th>
<th>ESS/10⁶ daltons</th>
<th>ESS remaining (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CHO-77256</td>
<td>CHO-UV-1</td>
</tr>
<tr>
<td>0</td>
<td>8.6</td>
<td>10.5</td>
</tr>
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<td></td>
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<tr>
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<td>0.8</td>
</tr>
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</table>

Discussion

In this study, we were unable to distinguish CHO-UV-1 from its parent on the basis of levels of DNA damage or a DNA repair process (other than PRR). Similar numbers of lesions (i.e., alkali-sensitive DNA lesions induced by MNU, DNA dimers resulting from UV irradiation, and M. luteus ESS produced by MNNG treatment) were observed in CHO-UV-1 and parental cells exposed to DNA-damaging agents. However, the effects of MNNG, MNU, and UV irradiation were more lethal...
in CHO-UV-1 than in parental cells, suggesting that cytotoxicity cannot be attributed solely to an increased frequency of lesions per unit dose of DNA-damaging agent. That the hypersensitivity of CHO-UV-1 is not due to differential uptake of lethal chemicals, resulting in different amounts of DNA damage, has been demonstrated by Hoy et al. (21), who found equivalent specific activity for DNA isolated from CHO-UV-1 and CHO-77256 after exposure to 14C-labeled MNU. In addition, we have shown that neither CHO-UV-1 nor CHO-77256 contains appreciable levels of 06-MeGua-DNA methyltransferase, an enzyme that reduces the toxicity of MNU and MNNG in bacterial and human cells (33-35).

No defect in any step of excision repair could be demonstrated in the CHO-UV-1 mutant. Although the initial rate of UV-induced M. luteus ESS removal in the mutant and parental cells was slower than that in human cells (27), by 17 to 18 h postirradiation only 20% of the ESS remained in both cell lines. Thus, the final extent of ESS repair is almost equivalent to that of human cells and is much greater than that observed in mouse 3T3 cells (27). The known substrate specificity of the crude M. luteus preparation toward UV-irradiated DNA is primarily for pyrimidine dimers. The efficiency of both the incision and ligation steps of excision repair after UV irradiation were also approximately the same in both cell types. However, we cannot rule out the possibility of differences in the preferential repair of active genes compared to noncoding sequences or of transcribed compared to nontranscribed strands of DNA, both of which have been shown to correlate better with cell survival in Chinese hamster ovary cells than does overall genomic repair (46, 47).

Both CHO-UV-1 and wild-type cells showed similar capacities for dimer exchange between parental and daughter strands, a process that is thought to involve recombinational exchange during DNA synthesis (30). Repair of MNNG-induced adducts recognized by M. luteus extracts was also equivalent in both cell lines. M. luteus endonuclease activity recognizes only a fraction of the total alkylated bases in DNA, those believed to consist primarily of 3-methyl adenine (27). Hence, the increased killing of CHO-UV-1 by UV radiation or chemical agents cannot be attributed to defects in the above mentioned processes.

We previously reported (19) that the number of HGPRT+ mutants (per surviving cell) induced by UV light or EMS in CHO-UV-1 was substantially less than that in CHO-77256. These results are consistent with the idea that CHO-UV-1 is defective in an error-prone PRR process that functions in wild-type cells to increase mutagenesis in those cells that survive the mutagenic treatment. Caffeine, which is reported to inhibit PRR (44, 45), would be expected to reduce mutagenicity in
CHO-77256 cells but to have no such effect on CHO-UV-1. This is exactly what was found in our study. The higher mutation rate in wild-type cells most likely arises because the cells utilize PRR pathways to bypass damage caused by UV light. EMS, or other agents that create bulky adducts; during that process, DNA polymerase inserts an incorrect base opposite a noninstructive lesion. Our result concurs with a decrease in mutation frequency at the HGPRT locus observed after treatment with UV light and caffeine in another Chinese hamster cell line (43).

By the same reasoning, if caffeine and the mutation in CHO-UV-1 both affect a PRR mechanism that influences cell killing, then survival of wild-type cells exposed to cytotoxic drugs in the presence of caffeine might be similar to that of the mutant cells treated in the absence of caffeine. However, CHO-77256 cells treated with EMS and caffeine had greater survival than CHO-UV-1 cells not exposed to caffeine. This may reflect an insufficient intracellular concentration of caffeine in wild-type cells, such that PRR processes are incompletely inhibited, thus allowing DNA lesions to be bypassed to some extent and enabling the cells to survive. On the other hand, the finding that caffeine decreased the $D_e$ equally in both EMS-treated cell lines suggests that the effects of caffeine on mutation induction may not be responsible for or connected with the ability of caffeine to potentiate cell killing by EMS. The decreased cell survival in the presence of caffeine observed in Fig. 5 may be the result of a reversal of carcinogen-induced inhibition of replication initiation, premature mitosis, inhibition of the repair of potentially lethal lesions, or the reversal of agent-induced delay in DNA synthesis. These effects of caffeine have all been observed previously in cells exposed to DNA-damaging agents (see Ref. 48 for review). Lastly, the lesions leading to cell lethality may not be those responsible for mutagenesis.

One of the few examples of PRR-defective mutants derived from mammalian cells is an SV40-transformed Indian muntjac cell line (49). Like CHO-UV-1, it displays a wide spectrum of hypersensitivity to radiation and chemical agents, as well as defective synthesis of daughter strands on a damaged DNA template. In contrast to CHO-UV-1, the muntjac cell line displays an extremely high frequency of sister chromatid exchanges upon exposure to UV and high levels of induced and spontaneous chromosome aberrations (50). Cultured cells from the variant form of xeroderma pigmentosum appear defective in another aspect of PRR. The absolute size of nascent DNA strands synthesized shortly after UV irradiation is much smaller than that of irradiated normal human cells. Nascent DNA strands in such cells increase in size at the same rate as those in normal human cells (16). This contrasts with CHO-UV-1, in which the initial size of DNA postirradiation is the same as in wild-type CHO cells but the DNA increases in size at a 4-fold slower rate, compared to the wild type (19). However, xeroderma pigmentosum variant cells, unlike the CHO-UV-1 mutant, have a higher level of mutability than do their normal counterparts (51). Xeroderma pigmentosum variant cells, like CHO-UV-1 and the SV40-transformed muntjac cell lines, have normal levels of unscheduled DNA synthesis and normal rates of dimer removal, compared to their wild-type counterparts.

PRR-defective mutants of *Drosophila* (52, 53) and yeast (54) have also been reported. Yeast *rad6* mutants are proficient in excision repair but are defective in PRR after UV irradiation. They also exhibit decreased radiation-induced mutagenesis and extreme sensitivity to UV radiation, X-rays, and chemical mutagens and are defective in sporulation (55, 56). The *rad6* gene has been cloned and characterized (57) and it was recently identified as encoding an E2-type-conjugating protein that ubiquitinates protein, especially histone H2A and H2B, *in vitro* (58). It has been suggested that histone ubiquitination by the *rad6* gene product may induce a change in chromatin structure, making DNA lesions more accessible to repair enzymes or, alternatively, making template DNA more accessible to replicative, recombinational, and transcriptional enzymes (59).

The pleiotropic phenotype of the CHO-UV-1 mutant mimics certain aspects of *rad6* mutants, namely, its decreased induced mutation frequency, its PRR deficiency, and its hypersensitivity to UV and chemical mutagens. Thus, the mutated gene in CHO-UV-1 and the *rad6* gene may both play important cellular regulatory roles. Further characterization of the CHO-UV-1 cell and isolation of the gene(s) involved in its PRR deficiency should help to clarify the relationship with *rad6* mutants.

It has generally been assumed that excision repair processes are the most important repair pathways within cells. Our results suggest that postreplication recovery mechanisms are also involved in the prevention of both cell lethality and human disease.

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PHENOTYPIC CHARACTERIZATION OF CHO-UV-1 MUTANT


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Patricia Hentosh, Andrew R. S. Collins, Laura Correll, et al.


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