Genetic diversity of UV-sensitive DNA repair mutants of Chinese hamster ovary cells

(complementation analysis/mutagen sensitivity/tester strains/7-bromomethylbenz(a)anthracene)

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ABSTRACT Mutant lines of Chinese hamster ovary cells that show hypersensitivity to killing and mutagenesis by UV light were analyzed by genetic complementation analysis to determine whether defects in different gene loci might underlie a common cellular phenotype. To facilitate rapid screening of mutant clones, a procedure was devised that allowed presumptive complementation to be assessed on the basis of the frequency of UV-resistant cells after fusion by polyethylene glycol. Four classes were identified among 44 clones tested. By using drug-resistance markers for selection of hybrid cells in crosses between UV mutant and wild type, a mutant from each of the four classes was shown to behave as phenotypically recessive. Hybrids were also isolated from crosses between each of the pair combinations of the four mutants. All such hybrids were relatively resistant to UV killing, providing confirmation of the complementation classes. When mutants representing the four UV-complementation classes were tested with the polyaromatic hydrocarbon 7-bromomethylbenz(a)anthracene, complementation was again seen for all pair combinations. These results suggest that each class of mutants represents a biochemical defect that plays a common role in the repair of both UV-induced and chemically induced lesions in the DNA.

The study of mutant cell lines having defects in enzymatic pathways that repair damaged DNA is an important approach to understanding how cells recover from genetic injury and to elucidating the mechanisms of mutagenesis. Of the human mutations that are known to involve repair deficiencies, xeroderma pigmentosum (XP) has been the most intensively studied. XP fibroblasts and lymphoblasts are defective in the excision repair that operates on damage resulting from exposure to UV and many chemical mutagens and carcinogens (1, 2). The defects in XP appear to lie at or prior to the incision step (2-4) and may involve factors that play a role in enzymatic recognition of damaged DNA within chromatin (3, 4). Seven complementation classes of XP have been reported (5-8).

Recently, two techniques were introduced for isolating UV-sensitive mutant clones from the Chinese hamster ovary (CHO) cell line (9, 10). Assay of DNA repair replication indicated that these mutants, like XP mutants, have low or undetectable repair synthesis in response to UV (9, 10). These UV mutants of CHO cells, as well as alkylation-sensitive mutants (9), provide an alternative to human mutant strains for studying the role of DNA repair in genetic damage. CHO cells can be readily manipulated in a quantitative manner, and they may allow isolation of mutants having repair deficiencies not represented among human mutants. In the present study, we examined 44 UV mutants having pronounced sensitivity. We show here that they are genetically recessive and can be classified into four complemen-

tation groups on the basis of their responses to either UV or the chemical mutagen 7-bromomethylbenz(a)anthracene (7-Br-MeBA). These results show that different genetic defects can underlie the UV-sensitive phenotype and suggest the possibility of a genetic analysis of repair functions in CHO cells.

MATERIALS AND METHODS

Cells and Culture Conditions. The wild-type CHO lines, SC1 and AA8, and culturing techniques have been described (11); UV-sensitive clones were obtained from AA8 (9, 10). Mutants designated UV-24 and UV-41 correspond, respectively, to lines previously labeled as 60-21 (10) and 361-112-10b (12). Hybrids are designated by the prefix "Hy" and a suffix for the clone number. Doubling times of mutants and hybrids were 13-16 hr.

Exposure to UV. Cells were exposed to germicidal UV on plastic dishes as described (11).

Isolation of Drug-Resistant Lines. For hybridization, drug-resistance markers were introduced into certain lines. Thioguanine resistance was obtained at 12 μ M and ouabain resistance was obtained at 3 mM, using spontaneous mutants. Drugs were obtained from Sigma.

Cell Fusion and Isolation of Hybrid Clones. Fusion was performed as described (13), except that polyethylene glycol treatment was done $\approx$ 6 hr after cell inoculation into cluster dishes. After overnight incubation, cells were removed with trypsin and replated for selection of hybrids or for UV irradiation. Each hybrid isolation was accomplished by using one parental line that was thioguanine- and ouabain-resistant (indicated by the suffix "TOR") and selecting in hypoxanthine/aminopterin/thymidine medium (13) containing 1 mM ouabain (14). The frequencies of hybrid clones were at least 100 times higher than the frequency of resistant cells appearing in either parental culture under drug selection. Isolated clones were confirmed to be hybrids on the basis of their having approximately twice the normal cell volume and DNA content.

Rapid Complementation Analysis. Each pair of mutants was fused (13), removed with trypsin, and carefully dispersed as single cells at a density of 1×10^5 cells per 100-mm dish. After 4 hr of incubation for attachment, replicate dishes were irradiated with 5-J/m² UV. The dishes were incubated 10 days for colony formation; then the colonies were stained and counted. In some experiments, the fused cultures were treated for 1 hr with 0.25 μ M 7-BrMeBA (courtesy of J. Mazrimas, Lawrence Livermore National Laboratory) using cell suspensions of $1 \times$

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Abbreviations: CHO, Chinese hamster ovary; XP, xeroderma pigmentosum; 7-BrMeBA, 7-bromomethylbenz(a)anthracene.

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10^5 per ml. The cells were then plated into dishes for colony formation at 2×10^5 cells per dish.

RESULTS

Rapid Screening for Complementation Classes. Working initially under the assumption (validated below) that the mutation in each of the CHO UV-sensitive mutants was recessive, we devised a rapid procedure (see *Materials and Methods*) that allowed preliminary assignment of the mutants to complementation classes. The procedure tests for complementation on the basis of the frequency of cells in a mixed fused culture that are resistant to a challenge dose of UV (e.g., 5 J/m^2) that is highly lethal to the mutants but only slightly toxic to wild-type cells. The results are summarized in Fig. 1 along with sample data from four representative mutants that produced clear-cut responses. (Eleven of 55 mutants originally isolated could not be analyzed because of high background frequencies of UV-resistant cells in the self-cross control dishes—i.e., frequencies $> 2 \times 10^{-4}$.) Each of the four prototype mutants had previously been shown to have no detectable repair replication after UV exposure [UV-5 and UV-20 (9), UV-24 (10), and UV-41 (12)]. The self-cross cultures of these mutants show very low survival—i.e., $1-2 \times 10^{-5}$. Similar results were seen with mixed unfused cultures (shaded boxes in Fig. 1). After fusion, each pairwise cross produced frequencies of survivors 25–50 times higher than the controls, strongly suggestive of complementation. Each of 44 clones tested yielded a low frequency of colonies in the cross that identifies its complementation class, and each yielded a high frequency (> 40 colonies per dish) when crossed with one or more of the other three standard mutants. As indicated, 37 out of 44 mutants were in class 2.

Tests for Recessiveness of the UV-Sensitivity Mutations. We tested explicitly for the recessiveness of the mutations in the four presumptive classes. Hybrid clones were isolated as described in *Materials and Methods* and then analyzed (Fig. 2).

	Class 1 (4) UV-5	Class 2 (37) UV-20	Class 3 (2) UV-24	Class 4 (1) UV-41
UV-5	0,1,2	127,137, 126	52,38, 41	71,82, 79
UV-20	1,0,0	0,0,3	120,116, 110	61,57, 79
UV-24	0,0,1	1,1,1	2,2,2	38,52, 43
UV-41	2,2,3	0,1,0	0,1,1	0,1,2

FIG. 1. Summary of complementation screening results illustrated by one mutant from each of four classes. Cultures containing pairs of mutants were fused in each row-column combination (or not fused in the case of the control cultures shown by the shaded region) and assayed for the frequency of UV-resistant cells as described in *Materials and Methods*. Numbers in each box are colony counts per 1×10^6 on triplicate dishes for a mutant pair or self-cross. The numbers of mutant clones assigned to each class are given in parentheses.

FIG. 2. UV survival curves of parental and hybrid clones. (Left) (---), mutants UV-5 and UV-20 (redrawn from ref. 10), (.....), parental line AA8 (based on data in Fig. 3 Left); Δ , SC1(TOR); ∇ , (Hy AA8 $\times$ AA8); $\circ$ and $\bullet$, [Hy UV5 $\times$ SC1(TOR)–1] and [Hy UV5 $\times$ SC1(TOR)–3], respectively; $\square$ and $\blacksquare$, [Hy UV20 $\times$ SC1(TOR)–1] and [Hy UV20 $\times$ SC1(TOR)–2], respectively; (Right) ∇ , UV-24; ∇ , UV-41; (.....), AA8 as on Left; $\circ$ and $\bullet$, [Hy UV24 $\times$ SC1(TOR)–3] and [Hy UV24 $\times$ SC1(TOR)–4], respectively; $\blacksquare$ and $\square$, [Hy UV41 $\times$ (SC1(TOR)–1)] and [Hy UV41 $\times$ SC1(TOR)–2], respectively. Error bars are SEMs for colony counts on replicate (three or four) dishes; when not shown, errors fall within the symbols. Plating efficiencies were 60–90%.

Tetraploid (hybrid) AA8 cells were twice as UV resistant as AA8, suggesting that increased ploidy reduces UV sensitivity (see *Note Added in Proof*). Although tetraploid strains of UV mutants were not constructed, the low survival frequencies of the self-crosses (see Fig. 1) imply that such tetraploids would not exhibit resistance comparable with that of AA8. UV survival curves were obtained for the parental lines and for hybrids formed between a wild-type derivative, SC1(TOR), and each of the four representative mutants. For each cross, duplicate hybrid clones were measured and found to be similar to AA8 in their responses (see Fig. 2). Thus, each of the four representative UV-sensitivity mutations appears to behave as incompletely recessive under these conditions of expression in tetraploid cells.

Complementation as Shown by Survival Curves of Hybrid Cells. Having demonstrated that each of the four mutant strains shown in Fig. 1 behaved as a recessive phenotype, we next tested directly for complementation between each mutant pair. This was done by forming hybrids between mutants and measuring their UV survival. These data for hybrids from each of the six different pair combinations, along with the responses of AA8 and the drug-resistant parental mutant clones, are shown in Fig. 3, and the data for selected curves from Figs. 2 and 3 in a reduced form are summarized in Table 1. $D_q (= D_0 \times \ln n)$ was calculated to provide a single parameter for the UV resistance of each line.

Most hybrids had D_q values in the range 3.3–4.8 and were, therefore, more nearly like AA8 than the UV mutants in sensitivity. The D_q of mutants ranged from 0.27 to 0.92 (the possible influence of the drug-resistance markers on UV sensitivity is unclear). Some variation in response was also seen between

FIG. 3. Demonstration of complementation between pairs of mutants representing four classes. (Left) Δ — Δ , AA8, the wild-type parental line; $\square$, UV-24(TOR); $\bullet$ and $\circ$, [Hy UV5 $\times$ UV24(TOR)-1] and [Hy UV5 $\times$ UV24(TOR)-2], respectively; $\blacksquare$ and $\square$, [Hy UV20 $\times$ UV24(TOR)-1] and [Hy UV20 $\times$ UV24(TOR)-2], respectively. (Middle) (.....), AA8 from data in Left; $\square$, UV-5(TOR); $\blacksquare$ and $\square$, [Hy UV41 $\times$ UV5(TOR)-1] and [Hy UV41 $\times$ UV5(TOR)-3], respectively; $\bullet$ and $\circ$, [Hy UV41 $\times$ UV24(TOR)-2] and [Hy UV41 $\times$ UV24(TOR)-3], respectively. (Right) (.....), AA8 from data in Left; $\square$, UV-41(TOR); $\circ$ and $\bullet$, [Hy UV20 $\times$ UV5(TOR)-1] and [Hy UV20 $\times$ UV5(TOR)-2], respectively; $\square$ and $\blacksquare$, [Hy UV20 $\times$ UV41(TOR)-2] and [Hy UV20 $\times$ UV41(TOR)-3], respectively. Plating efficiencies were 33–71% for hybrids and 74–90% for parental lines. Repeated symbols represent independent experiments, and error bars are as in Fig. 2.

certain duplicate hybrid clones; this may be due to the greater chromosome instability of hybrids (15). At any rate, it is clear from the overall results that the relative resistance of the hybrids indicates complementation in each case.

Four Complementation Classes on the Basis of Response to 7-BrMeBA. Using the four mutants (UV-5, UV-20, UV-24, and UV-41) that defined four UV complementation classes (see Fig. 1), we performed tests after treatment with 7-BrMeBA. Using the rapid procedure (see *Materials and Methods*), we obtained the results shown in Table 2. Each of the pairwise crosses of mutants produced a high frequency of surviving cells as compared with the frequencies in the self-crosses. Thus, with this agent, the complementation classes are the same as in the case of UV treatment.

DISCUSSION

The results presented here show that mutants of CHO cells having similar phenotypes with respect to UV and 7-BrMeBA sensitivity, as well as DNA repair replication, can be placed into four genetic complementation classes of recessive mutations. This demonstration of genetic heterogeneity is significant in establishing that a variety of mutations affecting nucleotide excision repair can be obtained in a cell line that is being widely used for mutagenesis studies (16–20). The fact that the four prototype mutants show the same complementation classes for 7-BrMeBA treatment as for UV treatment strengthens the hypothesis that the function that is defective in each mutant has a specific role in a sequence of reactions that repair a broad spectrum of damage. Thus, we suggest that each complementation

class represents a different defect in one or more steps of the major UV-damage repair pathway.

We have shown that complementation analysis of CHO mutants having strong UV sensitivity can be performed using rapid procedures in which the end point is cell survival. This approach differs from the analyses of XP mutants in which complementation was based on the amount of unscheduled DNA synthesis measured by autoradiography or other means (5–8, 21).

Our data on hybrid cells containing two UV repair mutations (see Fig. 3 and Table 1) suggest that the relevant gene products determining UV sensitivity are not produced in excess in wild-type AA8 cells. Otherwise, the hybrids should have the same survival parameters as tetraploid AA8 cells, instead of being more sensitive. The four prototype mutants presented here may be fully deficient for the relevant functions because each mutant had no detectable repair activity (9, 10, 12). As discussed previously (22, 23), the relative ease of isolation of such recessive mutant phenotypes may result from partial functional hemizygosity throughout the CHO genome.

In summary, our results provide indirect, but good, genetic evidence that CHO cells possess substantial DNA repair capacity for damage resulting from UV or certain chemicals. Results with mutagens, other than 7-BrMeBA, that produce bulky DNA adducts [e.g., 4-nitroquinoline-1-oxide (10), the tryptophan pyrrolisate Trp-P-2, and 2-acetylaminofluorene (unpublished observations)] indicate that the UV mutants are hypersensitive with respect to killing by these chemicals also. In this regard, the CHO mutants behave like XP mutants (1). The results of Adair *et al.* (24), as well as our own recent results, in-

Table 1. UV-survival-curve parameters for wild-type, mutant, and hybrid cells

Cell strain	D_{37}	D_0	Extrapolation number (n)	D_q
AA8	7.7	2.0	18	5.8
SC1	8.2	2.0	24	6.4
SC1(TOR)	9.3	2.5	14	6.6
UV-5	1.0	0.4	4	0.55
UV-5(TOR)	1.2	0.4	10	0.92
UV-20	1.0	0.4	4	0.55
UV-24	0.70	0.25	3	0.27
UV-24(TOR)	0.70	0.2	10	0.46
UV-41	0.70	0.25	3	0.27
UV-41(TOR)	1.2	0.4	8	0.83
Hy AA8 × AA8	15.6	3.6	26	11.7
Hy UV20 × UV5(TOR)–1, –2*	6.6, 6.4	2.0, 1.9	11, 10	4.8, 4.4
Hy UV5 × UV24(TOR)–1, –2*	6.4, 5.5	1.7, 1.4	15, 18	4.6, 4.0
Hy UV41 × UV5(TOR)–1, –3*	6.6, 5.3	2.0, 1.8	10, 7	4.6, 3.5
Hy UV20 × UV24(TOR)–1, –2*	6.7, 4.4	2.0, 1.7	9, 7	4.4, 3.3
Hy UV20 × UV41(TOR)–2, –3*	4.2, 5.0	1.9, 1.8	3, 5	2.1, 2.9
Hy UV41 × UV24(TOR)–2, –3*	6.2, 5.4	1.5, 1.7	20, 9	4.5, 3.7

Values of D_{37} , D_0 , and n were obtained by visual curve fitting of the data points in Figs. 2 and 3. D_{37} , dose required to reduce survival to 0.37; D_0 , dose increment that results in a decrement in survival to 0.37 on the exponential portion of the curve; n , intercept on the ordinate obtained by back-extrapolating the exponential portion of the curve; D_q , intercept on the abscissa resulting from back-extrapolating the exponential portion of the curve. D_q was calculated from the relation $D_q = D_0 \ln n$ and confirmed graphically.

* Duplicate clones.

dicare that additional complementation classes also exist for UV sensitivity. These UV mutants, as well as CHO mutants that are hypersensitive to certain alkylating agents (9), can provide model systems that may be helpful in determining how DNA repair influences mutagenesis. The considerable degree of hypersensitivity of the mutants, combined with their specificities for certain chemical classes, suggests that these cell strains should also be useful in screening for mutagens of environmental concern.

Note Added in Proof. To further assess the effect of ploidy on UV sensitivity in the absence of drug-resistance markers, tetraploid clones of AA8 and UV-5 were induced by a 16-hr exposure to Colcemid at 0.06 $\mu\text{g}/\text{ml}$. A tetraploid clone of AA8 had a D_q of 5.5 (which is comparable with the value for AA8 itself in Table 1), and two tetraploid clones of UV-5 had D_q values of 0.77 and 0.88, which fall in between the values for UV-5 and UV-5(TOR) in Table 1. These results argue that ploidy does not markedly alter UV sensitivity and that the results for the hybrid Hy AA8 × AA8 may be atypical and are not just the result of the increase in ploidy.

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Table 2. Complementation of DNA repair deficiencies after treatment with 7-BrMeBA

	UV-5	UV-20	UV-24	UV-41
UV-5	28, 33	185, 184	208, 203	229, 207
UV-20		26, 32	207, 198	151, 126
UV-24			24, 14	198, 197
UV-41				5, 25

Values are colony counts on duplicate dishes after treatment with 7-BrMeBA as described in *Materials and Methods*.

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