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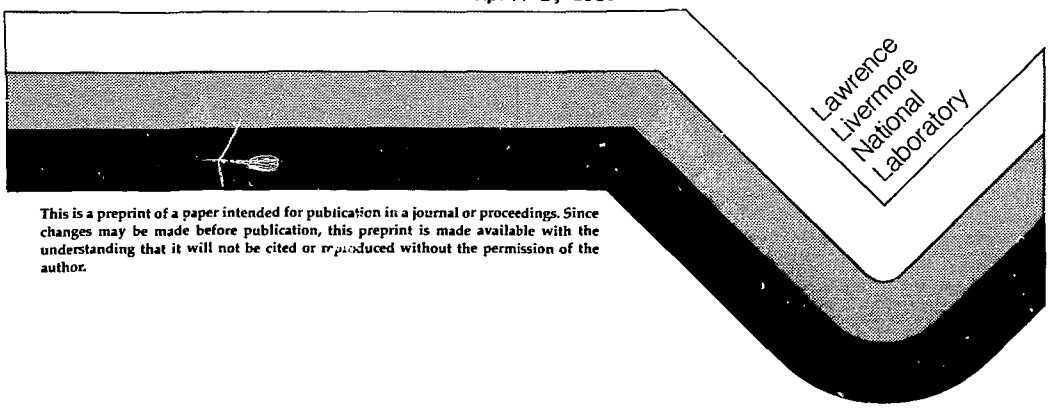

DISCLAIMER

RECENT PROGRESS WITH THE DNA REPAIR MUTANTS OF CHINESE HAMSTER OVARY CELLS

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SUMMARY

Repair deficient mutants of Chinese hamster ovary (CHO) cells are being used to identify human genes that correct the repair defects and to study mechanisms of DNA repair and mutagenesis. Five independent tertiary DNA transformants were obtained from the EM9 mutant, which is noted for its very high sister-chromatid exchange frequencies (SCE). In these clones a human DNA sequence was identified that correlated with the resistance of the cells to CldUrd. After Eco RI digestion, Southern transfer, and hybridization of transformant DNAs with the BLUR-8 Alu family sequence, a common fragment of 25-30 kb was present. Since the DNA molecules used to produce these transformants were sheared to < 50 kb in size, the correcting gene should be small enough to clone in a cosmid vector.

Using drug-resistance markers to select for hybrids after fusion, we have done complementation experiments with UV-sensitive mutants and have identified a sixth complementation group, line UV61. Additionally, CHO mutants UV27-1 and MMC-2, isolated in other laboratories, were found to belong to UV Group 3, which is represented by line UV24.

To study the behavior of transfected DNA molecules in repair-deficient cells, we treated plasmid pSV2gpt with either UV radiation or cis-diamminedichloroplatinum(II) (cis-DDP) and introduced the damaged DNA into normal CHO cells (AA8) and mutants UV4 and UV5. Unrepaired damage to the plasmid was indicated by loss of colony-forming ability of the transfected cells in selective medium containing mycophenolic acid. With UV damage, the differential survival of the cell lines was similar to that seen when whole cells are treated with UV. However, with cis-DDP damage, mutant UV4 did not exhibit the extreme hypersensitivity (50-fold) that occurs when cells are treated. This result suggests that UV4 cells may be able to repair cross-links in transfected DNA.

INTRODUCTION

Our laboratory has been characterizing DNA repair mutants of Chinese hamster ovary (CHO) cells as a tool for studying mechanisms of genetic change and mutagenesis (Thompson, 1985; Thompson & Hoy, 1986; Thompson et al. 1986). It is evident that repair mutants of rodent cells provide an important approach for isolating human (and rodent) genes involved in repair pathways. Westerveld et al. (1984) recently cloned a human gene that corrects UV-sensitive mutants belonging to UV complementation Group 2 described by Thompson et al. (1981). Efforts are underway in our laboratory and others to isolate genes that correct additional mutants that have sufficient hypersensitivity to provide an efficient selection system.

Earlier our laboratory identified five complementation groups of UV mutants (Thompson et al. 1981; Thompson & Carrano, 1983), all of which involve a defect in the incision step of repair (Thompson et al. 1982a). These mutants, therefore, have properties quite similar to those of xeroderma pigmentosum (XP) cells (Friedberg, Ehmann, & Williams, 1979; Cleaver, 1983). Mutants from each of the groups show a stable phenotype in response to 5-azacytidine treatment, suggesting that they did not arise by gene inactivation associated with methylation (Jeggo & Holiday, 1986). We have obtained evidence from chromosome mapping studies that the five complementation groups represent at least four different human genes. In hybrid cells, the mutation of line UV20 (Group 2) was corrected by a gene on human chromosome 19 (Thompson et al. 1985; Rubin et al. 1985). Mutant UV5 (Group 1) also appears to be corrected by human chromosome 19, but the mutants of Groups 3, 4, and 5 show correction by chromosomes 2, 16, 13, respectively (L. Thompson, M. Siciliano, & A. Carrano, unpublished data). Correction by

different chromosomes indicates that different human genes are involved. It is conceivable that the two complementation groups corrected by chromosome 19 are due to interallelic complementation of a single gene. However, this possibility seems unlikely because the mutants in Groups 1 and 2 consistently show very different phenotypes in terms of their sensitivity to DNA cross-linking agents (Hoy, Thompson, Mooney, & Salazar, 1985). The fact that two of the UV repair genes are linked on chromosome 19 could have functional significance, especially if they are close together. The isolation of the UV5-complementing gene is needed to give the appropriate DNA probes to determine the linkage. Gene transfer experiments in progress indicate that the human gene that corrects UV5 is functional in purified DNA and small enough to clone in a cosmid vector (unpublished data).

We are also proceeding to clone a human gene that corrects the EM9 mutant line, which is noted for its greatly elevated frequency of sister chromatid exchange (SCE), defective repair of strand breaks, and reduced rate of DNA maturation when BrdUrd is in the template strand (Thompson et al. 1982b; Dillehay, Thompson, Minkler, & Carrano, 1983). The SCEs in EM9 cells result primarily from the BrdUrd incorporation that is used in the standard SCE protocol (Pinkel, Thompson, Gray, & Vanderlaan, 1985). All the enzymes involved in DNA metabolism that have been examined in EM9 cells were found to be normal (for references see Thompson et al. 1985).

In hybrid cells, the EM9 defect is corrected by human chromosome 19 (Siciliano, Carrano, & Thompson, 1986). DNA isolated from one of these resistant hybrids was also shown to correct EM9 in the DNA transfection procedure (Thompson et al. 1985). The data presented here on recent DNA transformants gives our progress toward isolating the transferred human gene.

We hope to use this gene to determine what gene product is defective in EM9 cells and to use this product to study the mechanism of SCE formation.

In addition, we present the results of recent efforts to identify new UV complementation groups of CHO cells. A sixth group has been found with line UV61. We also describe the behavior of mutants UV5 (Group 1) and UV4 (Group 2) in response to transfected plasmid DNA that has been damaged with UV or cis-diamminedichloroplatinum(II) (cis-DDP).

MATERIALS AND METHODS

Culture conditions

Stock cell lines were grown in suspension as described earlier (Thompson et al. 1980, 1982a, 1982b) in α-MEM medium supplemented with 10% fetal bovine serum (K.C. Biological, Lenexa, KA). Medium contained 100 units/ml of penicillin and 100 µg/ml of streptomycin sulfate. Stocks were renewed every 3 months from material in liquid nitrogen and periodically tested for mycoplasma. The mutant lines were checked about once a month for sensitivity. Plasticware for monolayer culture and colony formation was from Corning.

Cell fusion complementation tests

For fusion, 2×10^6 cells (1:1 ratio of each type) were inoculated into a 6 cm dish and allowed several hours for attachment. Cells were exposed for 60 sec to 2 ml of a solution containing 45% polyethylene glycol 1000 (Baker Chemical Co.) and 10% dimethyl sulfoxide (DMSO) in medium. The cells were rinsed twice with 10% DMSO in medium and incubated in normal medium for ~24 h for hybrid cell formation. Cells were then trypsinized, counted, and plated at 2×10^5 cells per 10 cm dish. After 4 h dishes were rinsed

with phosphate-buffered saline and irradiated with far UV as described by Thompson et al. (1980). For colony formation, cells were then incubated 10 days in normal medium or medium containing 1 mM ouabain (Sigma) plus hypoxanthine (74 uM), amethopterin (550 nM), and thymidine (41 uM). Colonies around the periphery of the dish, where shielding occurs during irradiation, were not counted.

Transfection of EM9 with sheared DNA

DNA from the secondary transformant 9TT3 (Thompson et al. 1986) was sheared so that almost all the molecules were below 50 kb. This was done by passing the DNA four times through a 30 gauge needle. Sheared genomic DNA was combined with pSV2gpt DNA in a 1:1 ratio by weight. Calcium phosphate precipitates were prepared as described by Corsaro & Pearson (1981). To each dish of 2 x 10^6 EM9 cells containing 10 ml of medium, 40 ug of DNA in 1 ml was added. Sixty dishes were exposed to DNA for 20 h. Dishes were rinsed with 10 ml of medium and given 30 ml of fresh medium for a 48 h incubation for expression. Each dish was trypsinized and replated into 3 replicates with 30 ml of medium containing both MAXTA supplements and CldUrd as described by Thompson et al. (1985) for selection of gpt-positive, repair-proficient cells. On days 4 and 8, 15 ml of fresh medium was added, and only the CldUrd selection was continued. Eleven to 15 days after plating, five colonies were isolated and grown to mass culture under CldUrd selection.

Transfer of pSV2gpt DNA treated with UV radiation or cis-DDP

For exposure to UV radiation, pSV2gpt DNA was diluted into a solution containing 0.25 M $CaCl_2$, 1 mM Tris-HCl (pH 8), and 10 mM NaCl; 0.5 ml was irradiated in a 35 mm plastic dish. Three ug of precipitated DNA (see above) was added in a volume of 0.5 ml to each 10 cm dish containing 2 x 10^6

cells. A lower volume of precipitate was used here, compared to the case of genomic DNA, to reduce toxicity. The precipitate was left on the cells for 16-20 h. Then the dishes were rinsed, given fresh medium, and incubated for 24 h for expression of gpt function. Cells were trypsinized and replated at 1-2 x 10^6 cells per 10 cm dish in MAXTA-supplemented medium (Thompson et al. 1985) containing 2.5 times the standard concentration of glutamine. Three replicate dishes were plated for each dose point, and duplicate untreated controls were used in each experiment. Plating efficiency was determined at each dose by plating 300 cells into 3 replicate dishes, and these values (0.7-0.95) were used to calculate the frequency of MAXTA resistant colonies per viable cell plated. MAXTA selection dishes were incubated 12-14 days, and plating efficiency dishes were incubated 7-9 days.

For exposure to cis-DDP, the DNA in 0.4 ml of 10 mM Tris buffer at pH 8 was combined with cis-DDP (100 ug/ml in 10 mM Tris) and incubated at 37° C for 1 h. The DNA in 0.3 M sodium acetate (pH 5) was precipitated with ethanol at -70° C, then redissolved in 10 mM Tris, 10 mM EDTA, pH 7.4, for calcium phosphate precipitation. Two ug of DNA in 1 ml was added to each 10 cm dish containing 4 x 10^6 cells in 10 ml of medium, and the cells were incubated at 37° C for 4 h. The medium was aspirated gently, and 2.5 ml of 20% glycerol in medium (v:v) at room temperature was added to the side of the dish. After 1 min, the glycerol was removed, and the dishes were rinsed 3 times with serum-free medium and given 20 ml of growth medium. After 24 h incubation the cells were plated for MAXTA selection as described above for UV-treated DNA.

Molecular Hybridization

DNAs were digested with restriction enzymes, separated by electrophoresis, and transferred from agarose gels to nitrocellulose filters by the method of

Southern (1975). Prehybridization of filters was done at 65° C with 25 ml of a solution containing 5x SSC (1x SSC is 150 mM NaCl, 15 mM sodium citrate), 50 µg/ml of denatured salmon sperm DNA, and 0.1% each of Ficoll, bovine serum albumin (BSA), polyvinylpyrrolidone (PVP), and sodium dodecylsulfate (SDS). Hybridization was carried out for 18 h in 20 ml of a solution consisting of the prehybridization ingredients plus 10% dextran sulfate and the probe DNA, which contained 4.6 x 10^6 cpm in ~300 ng of the Alu family BLUR-8 sequence (Deininger, 1981) isolated as a Bam HI fragment. The probe was labeled with α-^{32}P-dCTP by nick translation. Filter washes were done as follows: First in 2x SSC + 0.1% each of Ficoll, BSA, PVP, SDS (once for 5 min, 3 times for 15 min each); second in 1x SSC + 0.1% each of SDS, Ficoll, BSA, and PVP (four times for 30 min each); third in 1x SSC at room temperature. The filter was exposed to Kodak X-OMAT AR5 film with an intensifying screen (Cronex Lightning-Plus, DuPont) for ~3 days.

RESULTS AND DISCUSSION

Progress toward isolating a human DNA repair gene that corrects the EM9 mutation

Earlier, we introduced into EM9 cells human DNA sequences that normalized the SCE level and restored resistance to mutagens (Thompson et al. 1985). It is interesting to note that, unlike SCEs, chromosomal aberrations induced by BrdUrd remained slightly elevated in the primary transformants (Thompson et al. 1985). This result suggested that the transfected human gene was unable to fully correct all aspects of the biochemical defect in EM9.

Our strategy has been to identify the correcting human gene in the background of EM9 hamster DNA on the basis of the linkage of the gene to the

human Alu-family repetitive sequences. These repetitive sequences are associated with most genes (Schmid & Jelinek, 1982). The initial transfection was done with high molecular weight DNA (>160 kb) isolated from hybrid cells, which were derived by fusing EM9 cells with normal human lymphocytes. A 3.8 kb human Eco RI restriction fragment, which was present in 6/6 primary transformants (Thompson et al. 1985), looked promising as being part of the repair gene, and this sequence was isolated from a cosmid library of DNA from one of the primary transformants (see below). However, this fragment was later found to be absent in 3/6 secondary transformants (Thompson et al. 1986). To determine whether the repair gene was small enough to clone in a cosmid vector, a DNA transfer was performed using sheared DNA. DNA from the secondary transformant 9TT3 was sheared such that most of the molecules were 25-50 kb in size. This DNA was coprecipitated with pSV2gpt DNA and used to treat 1.2×10^8 EM9 cells. Five independent colonies were obtained that were able to grow in medium containing both MAXTA and ClduRd, implying that cotransfer of functional gpt and repair genes had occurred.

During the course of these experiments the resolution of the human restriction fragments on Southern blots was improved by using the 300 bp Alu family sequence (BLUR-8) as the probe and by optimizing the hybridization conditions. (The analysis of the primary clones was done using total human DNA, and the presence of large human restriction fragments could not be discerned in the high molecular weight region of the blots).

The DNAs of the five tertiary clones were analyzed for the presence of human sequences (Figure 1). The prominent feature of these Eco RI digested DNAs was the presence of a band corresponding to a human fragment of 25-30 kb in each of the transformants. The restriction fragment at 3.8 kb in the lane

of 9TT3 donor DNA was not present in any of the tertiary clones. This result implies that the 3.8 kb fragment was a sequence flanking the complementing gene and was lost when the DNA was sheared. No bands were visible in the control lane of CHO DNA (from mutant UV135). The important large common fragment has similar intensity in each of the five independent transformants, suggesting that it is present as a single copy in each cell line. Lane 1 of Fig. 1, which illustrates the detection of human repetitive sequences, contains the recombinant cosmid pH9T12-1 present at a level equivalent to five copies per cell. This cosmid clone contains an insert of about 40 kb of human DNA, which includes the 3.8 kb fragment seen in the primary transformants.

As an additional control, two of the lanes in Fig. 1 contained UV135 DNA to which was added the equivalent of 1x or 10x copies per cell of the pSV2gpt DNA. Because of the cotransfer procedure used, all the transformants contain pSV2gpt. Since the 300 bp BLUR-8 sequence was isolated from pBR322, which has homology with pSV2gpt, we wanted to ensure that the BLUR-8 probe was not contaminated with vector sequences. No bands were evident in these two control lanes. Because these transformants were made from DNA <50 kb, the human fragment at ~30 kb probably contains at least a part of the correcting gene. The gene may extend beyond this fragment since none of the transformants recovered show evidence of breakage of this fragment. The gene should be small enough to clone in a cosmid vector, which can accept inserts up to about 45 kb. Efforts toward reaching this objective are underway.

Line UV61 represents a sixth complementation group for UV sensitivity

Preliminary tests on the mutant line UV61 [previously designated 6-56-37 by Busch (1980)] were performed by the rapid complementation procedure described earlier (Thompson et al. 1981). These results suggested that this

mutant might complement the 5 existing groups (Thompson et al. 1981; Thompson & Carrano 1983) but were equivocal because the UV sensitivity of UV61 is less than that of the other mutants. As shown in Figure 2, the UV fluence required to cause a given level of killing of UV5 cells is only about 60% as much as that required to give the same killing of UV61. The D_{37} values for UV5, UV61, and parental AA8 cells are 2.2, 3.8, and 10.6 J/m^2, respectively. It is of interest to note that these D_{37} values of UV5 and AA8 are somewhat higher than our previously published values (Thompson et al. 1980; Thompson et al. 1981). We attribute these differences to changes in culture conditions, possibly the use of dialyzed serum in earlier experiments.

Drug resistance markers were used to make the complementation tests more efficient. This approach is illustrated by the data in Table 1 for the mutants UV4 and UV41, which have been assigned to complementation Groups 2 and 4, respectively (Thompson et al. 1981). The use of lines having both thioguanine and ouabain resistance (shown by the designation TOR) allows one to select for hybrids without having a selectable marker on the fusion partners. By selecting for hybrids after UV treatment, the background of colonies from surviving parental cells is eliminated. As shown in the last line of Table 1, when UV4 was fused with UV41-TOR, the frequency of hybrids was 0.0012 in the absence of UV irradiation. After exposure to 6 J/m^2, about 50% of these hybrids survived, indicating that they were relatively resistant although not as resistant as AA8 cells. When UV41 was crossed with its derivative line UV41-TOR, no UV resistant hybrids were formed, which was the expected result. Also in the three self-cross controls, there were no detectable UV resistant colonies, again, as expected.

Similarly, UV61 was fused with mutants from each of the five complementation groups that had the "TOR" phenotype, as shown in Table 2. The lines used were derivatives of UV5, UV20, UV24, UV41, and UV135, which belong to Groups 1 through 5, respectively. These mutants were all isolated from the AA8 parental line. Mutant UV27-1, which belongs to Group 3 (see below), has a different origin. In each cross of UV61 with a TOR line, a high frequency of UV resistant hybrids was formed. These frequencies were in the same range as that seen with the complementing pair of mutants shown in Table 1. In each of the self crosses in Table 2, no colonies were seen. These results indicate that UV61 complements each of the first five groups.

We showed above that the UV sensitivity of UV61 differs from that of the other mutants shown in Table 2. It is interesting to note that the biochemical defect in UV61 also appears to differ. Groups 1 through 5 have defective incision after UV treatment (Thompson et al. 1982a), but UV61 has normal unscheduled DNA synthesis (D. Bootsma, personal communication), implying a functional incision step.

Mutant UV27-1 belongs to Complementation Group 3

Clone "27-1" was isolated by Wood & Burki (1982). (We added the "UV" prefix to indicate that it belongs to the collection of UV sensitive lines). Based on the data in Table 3, we have assigned UV27-1 to Group 3, which contains UV24. In each cross with the TOR lines several hundred colonies were obtained, except with UV24-TOR. (We previously reported [International Conference on Mechanisms of Antimutagenesis and Anticarcinogenesis, Lawrence, KA, Oct. 6-10, 1985] that UV24 complemented UV27-1, but this result was in error due to mislabeling of a frozen stock.) Another mutant line MMC-2, which

was isolated by Robson, Harris, & Hickson (1985), was also found to belong to Group 3 (results not shown).

Survival of plasmid pSV2gpt in normal and UV-sensitive lines

The plasmid pSV2gpt provides a convenient system for evaluating the repair of damaged DNA molecules introduced by the calcium phosphate precipitation procedure. In MAXTA selection medium, which includes mycophenolic acid, the bacterial gpt gene serves as a dominant selectable marker (Mulligan & Berg, 1981). Loss of gpt function can be determined by measuring the survival of transfected cells in MAXTA medium. One advantage of this approach to studying repair is that the damage is localized to the target DNA sequence.

In the present study we were interested in the question of whether the UV repair mutants would exhibit a repair-deficient phenotype when the plasmid was damaged with mutagens and transfected into the cells. Cells that can repair damage that would otherwise inactivate the plasmid, should express a functional gpt gene and form colonies in selective medium. Thus, frequencies of transformation were determined as a function of the dose of DNA-damaging agent to which the plasmid was exposed.

As seen in Figure 3, when the plasmid was treated with UV radiation, it was 3 to 4 times more resistant when transfected in the normal CHO cells (AA8) as compared with the repair deficient lines UV5 and UV4 (Groups 1 and 2, respectively). These two mutants, which have similar survival response to UV (Thompson et al. 1980; Busch, Cleaver, & Glaser, 1980), also showed the same response with irradiated plasmid. The differential survival between the normal and mutant cells was slightly less than that obtained with irradiated cells. Perhaps the repair of transfected plasmid DNA is less efficient than genomic DNA. At low UV fluence we observed a stimulation of transformation of

about 2-fold in the normal CHO cells, but the UV-sensitive lines showed no evidence of this effect. Overall, our results with irradiated plasmid differ in several respects from those seen with normal and XP human cell lines. The normal human cells showed higher levels of stimulation by UV, and the same behavior was seen with XP cells (Spivak, Ganesan, & Hanawalt, 1984; van Duin et al. 1985). In both studies the XP cells showed transformation frequencies that were as high as those obtained with normal cells, suggesting that in the plasmid transformation assay the XP (Group A) cells could repair UV damage. However, with damaged viral DNA a repair defect has been seen in XP cells. Using a plaque assay for host cell reactivation of adenovirus 2, Day (1974) consistently found XP lines to have a more sensitive response to UV irradiated virus than did normal human cells.

In a second set of transfection experiments we used the compound cis-diamminedichloroplatinum(II) (cis-DDP), which, like UV, produces bulky adducts but also produces DNA cross-links. This compound has been shown to produce DNA intrastrand cross-links as well as low, but potentially toxic, levels of interstrand cross-links (Plooy et al. 1985) in CHO cells. Under our conditions of treatment of the plasmid a very high percentage of the molecules should have interstrand cross-links (Poll et al. 1984). The pattern of responses with the three cell lines was similar to that seen with UV except the mutants showed less difference compared with AA8 (Figure 4). UV5 cells had a response that was about 2 times more sensitive than that of AA8 cells, and UV4 cells had a 3-fold more sensitive response. This result for UV5 is similar to the differential sensitivity seen with cis-DDP treated cells in a cytotoxicity assay (Hoy et al. 1985). However, the behavior of UV4 contrasts sharply with our finding that UV4 cells were 50x more sensitive than AA8 in

the cytotoxicity assay (Hoy et al. 1985). Since UV4 cells were also very sensitive to many other agents known to produce DNA interstrand cross-links, the results obtained with cis-DDP treated plasmid were unexpected.

These results suggests that cross-links might not be nearly as toxic to UV4 when introduced in the plasmid as when present in the genomic DNA molecules. One interpretation is that in UV4 cells cross-links in naked DNA molecules can be repaired rapidly and efficiently compared with DNA in nucleosomes. For example, evidence has been presented that many of the mutations in the UV excision repair pathway in human cells act at the level of chromatin rather than unprotected DNA (Mortelmans et al. 1976; Kano & Fujiwara, 1983). In addition, the critical unhooking event for cross-links may have much faster kinetics than the removal of pyrimidine dimers (Reid & Walker, 1969) and occur before DNA integration. Our results with UV4 are analogous to those seen with Fanconi's anemia (FA) cells, which are characteristically very hypersensitive to killing by cross-linking agents (Ishida & Buchwald, 1982). Poll et al. (1984) found FA cells to be very sensitive to killing by cis-DDP, but in a host cell reactivation assay in which SV40 DNA was treated with cis-DDP, the FA cells showed a normal response. Alternatively, the unexpected results with UV4 might be due to a different spectrum of lesions formed in vitro versus in vivo. Clearly much remains to be learned about the role of chromatin and other factors in the nucleotide excision repair process in both rodent and human cells.

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FIGURE LEGENDS

Fig. 1: Autoradiogram of a Southern transfer of DNA and hybridization to detect human sequences in tertiary transformants of mutant EM9. DNA from the secondary transformant 9TT3 was sheared to 50 kb and used to produce the five transformants designated 9TTT1, 9TTT2, 9TTT3, 9TTT4, and 9TTT5. DNAs were digested with Eco RI, subjected to electrophoresis in a 0.8% agarose gel, transferred to nitrocellulose, and probed with nick-translated BLUR-8 Alu-family sequence. The common fragment at ~25 kb in the five transformants probably contains repair gene sequences. The lanes containing UV135 DNA are negative controls (see text), and the lane containing DNA from the cosmid pH9T12-1 is a positive control. The position of the molecular weight markers (lambda phage DNA intact and digested with Hind III) takes into account the curvature of the gel. See Materials and Methods for hybridization conditions.

Fig. 2: UV survival curves of parental AA8 and mutants UV5 and UV61. Cells were irradiated at a density of 2×10^6 cells per 10 cm dish, trypsinized, and plated at varying inocula for colony formation. Error bars are standard errors of the means for values from 2 or 3 experiments. Average plating efficiencies for UV5, UV61, and AA8 were 0.86, 0.88, and 0.90, respectively. Symbols: (Δ), AA8; (○), UV61; (□), UV5.

Fig. 3: Relative frequency of MAXTA resistant colonies of AA8, UV4, and UV5 cells transfected with UV irradiated pSV2gpt DNA. The absolute frequencies of MAXTA resistant colonies in the absence of UV damage were 5-18 x 10^{-5}, 3 x 10^{-5}, and 8-18 x 10^{-5} for AA8, UV4, and UV5, respectively. Error bars show standard errors of the mean for repeat experiments of AA8 and UV5. For the AA8 data an exponential best fit was done for the points between 400 and 2600 J/m^2 and the remainder of the curve was drawn by eye. For UV5, a linear best fit with a line going through a survival of 1.0 at zero dose was performed. Symbols: (○), AA8; (△), UV4; (□), UV5.

Fig. 4. Relative frequency of MAXTA resistant colonies of AA8, UV4, and UV5 cells transfected with pSV2gpt DNA treated with cis-DDP. The absolute frequencies of MAXTA resistant colonies in the absence of cis-DDP were 19-23 x 10^{-5}, 14 x 10^{-5}, and 13-20 x 10^{-5} for AA8, UV4, and UV5, respectively. Error bars show standard errors of the mean for repeat experiments. For each cell line an exponential best fit was obtained with a line going through a survival of 1.0 at zero dose. Plasmid DNA was exposed to cis-DDP for 1 hr at 37° C. Symbols: (□), AA8; (△), UV4; (○), UV5.

Table 1.

Illustration of complementation tests using drug resistance markers with mutants from Groups 2 and 4

Cross	No. colonies (0 J/m^2)	No. colonies (6 J/m^2)
UV4 x UV4	4,4,3	0,0,0
UV41 x UV41	4,2,-	0,0,0
UV41-TOR x UV41-TOR	0,0,0	0,0,0
UV41 x UV41-TOR	83,79,101	0,0,0
UV4 x UV41-TOR	244,226,242	111,103,121

Each number is the colony count on a replicate dish seeded with 2 x 10^5 cells and incubated with 1 mM ouabain + HAT ingredients for 10 days (see Materials and Methods).

Table 2.

Line UV61 complements mutants from UV Groups 1 through 5

Cross	Avg. no. colonies (6 J/m^2)	Avg. no. colonies (8 J/m^2)
UV61 x UV5-TOR	287	167
UV61 x UV20-TOR	120	121
UV61 x UV24-TOR	175	161
UV61 x UV41-TOR	76	86
UV61 x UV135-TOR	274	175
UV61 x UV27-1-TOR	478	414[a]
UV61 self cross	0	0
UV5-TOR self cross	0	0
UV20-TOR self cross	0	0
UV24-TOR self cross	0	0
UV41-TOR self cross	0	0
UV135-TOR self cross	0	0
UV27-1-TOR self cross	0	0

The data represent 2 experiments. Each value is the average number of colonies on 3 replicate dishes seeded with 2 x 10^5 cells. Dishes were incubated in medium containing 1 mM ouabain + HAT.

[a]Dishes seeded with 1 x 10^5 cells.

Table 3.

UV27-1 belongs to complementation group 3

Cross	Avg. no. colonies ($8\ J/m^2$)
UV27-1 x UV5-TOR	381
UV27-1 x UV20-TOR	178
UV27-1 x UV24-TOR	3,1[a]
UV27-1 x UV41-TOR	200
UV27-1 x UV135-TOR	342
all self crosses	0

Each value is the average of colonies on 3 replicate dishes. Dishes seeded with 2×10^5 cells were incubated in medium containing 1 mM ouabain + HAT.

[a] Values from two different experiments.

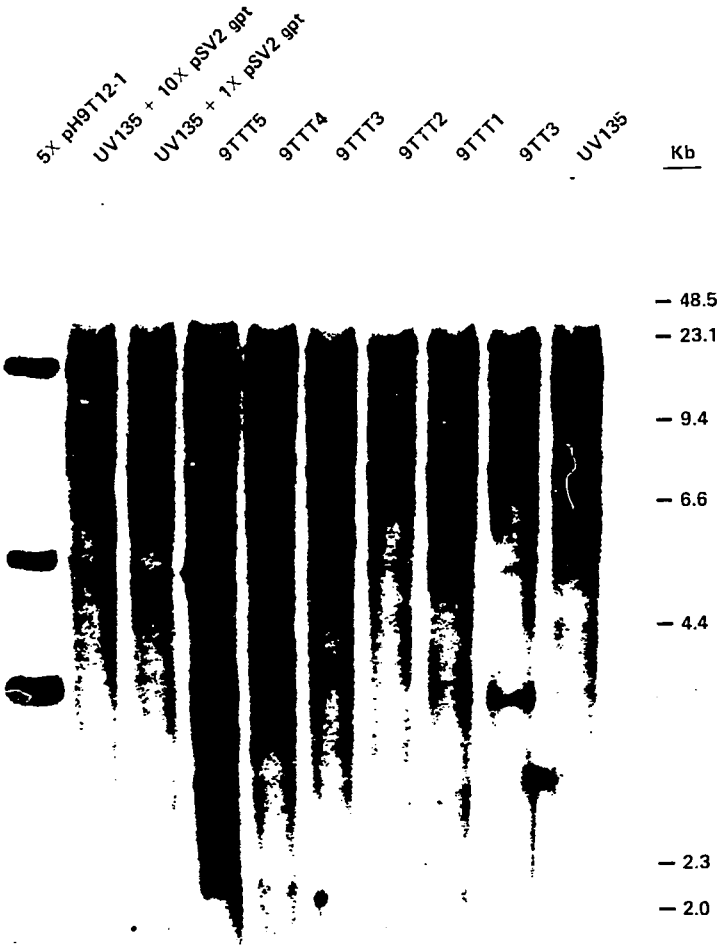
5X pH9T12-1
UV135 + 10X pSV2 gpt
UV135 + 1X pSV2 gpt
9TTT5
9TTT4
9TTT3
9TTT2
9TTT1
9TT3
UV135
Kb
48.5
23.1
9.4
6.6
4.4
2.3
2.0

Fig. 1

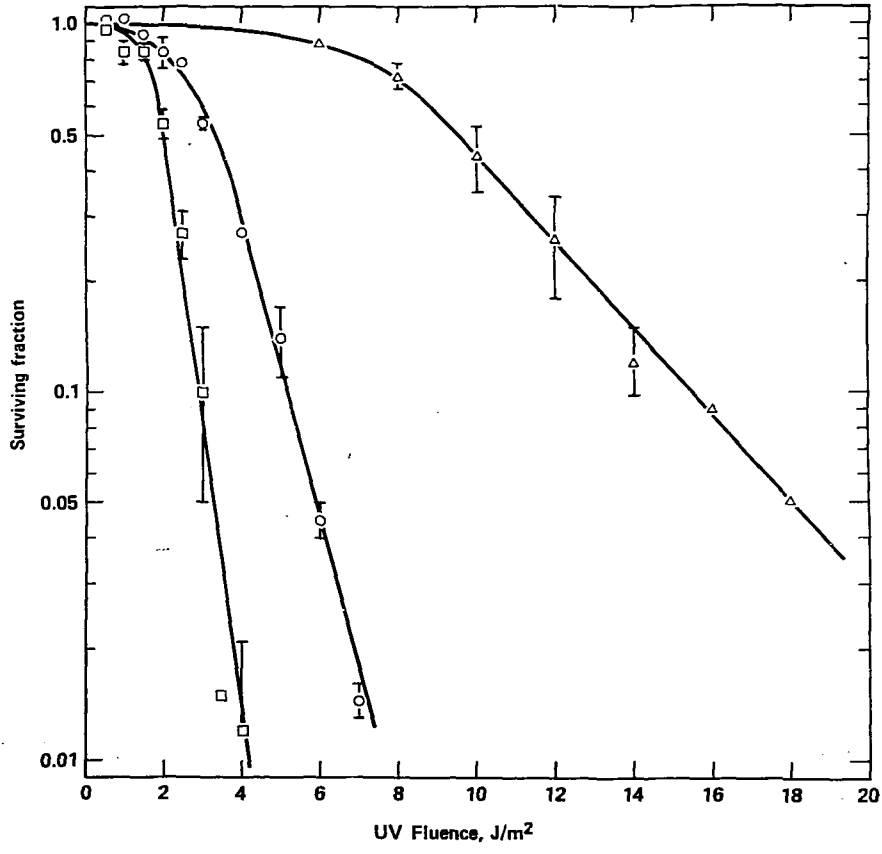
1.0
0.5
0.1
0.05
0.01
Surviving fraction
0
2
4
6
8
10
12
14
16
18
20
UV Fluence, J/m²

Fig. 2

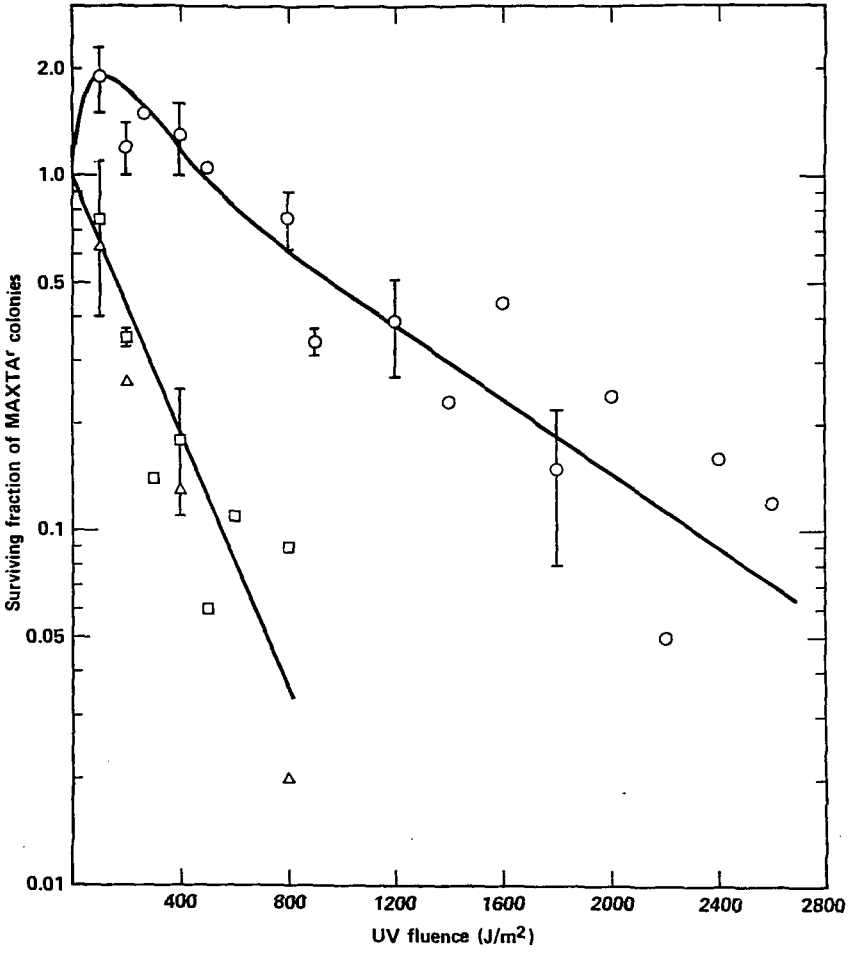
2.0
1.0
0.5
0.1
0.05
0.01
Surviving fraction of MAXTA^r colonies
400
800
1200
1600
2000
2400
2800
UV fluence (J/m^2)

Fig. 3

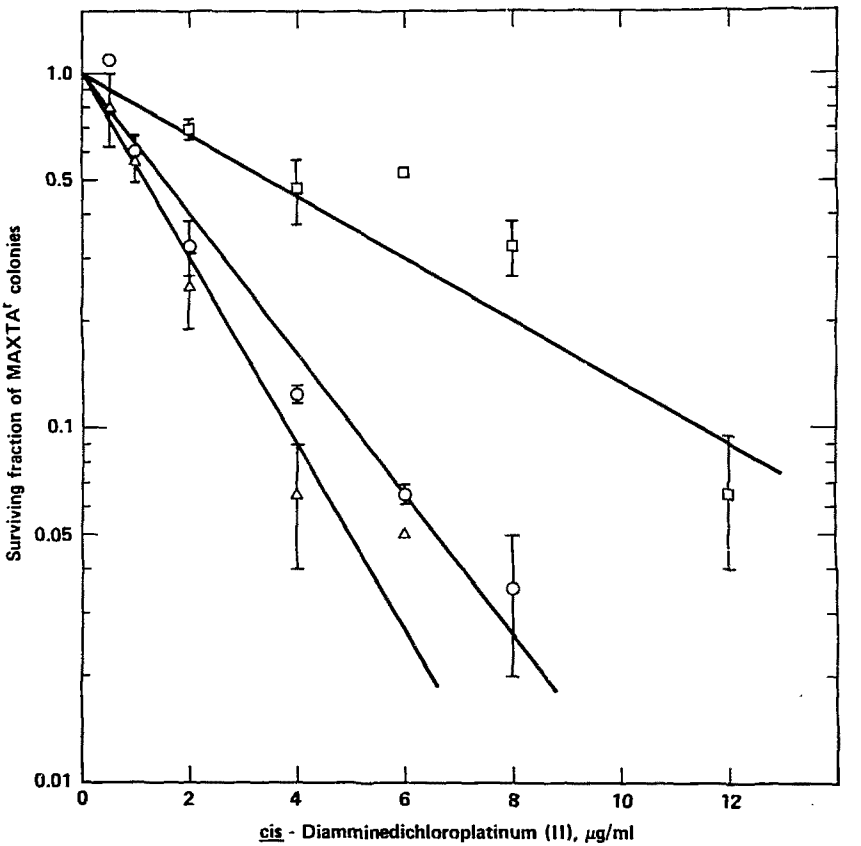
1.0
0.5
0.1
0.05
0.01
0
2
4
6
8
10
12
Surviving fraction of MAXTA^r colonies
cis - Diamminedichloroplatinum (II), μg/ml

Fig. 4