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Increased UV resistance in xeroderma pigmentosum group A cells after transformation with a human genomic DNA clone

(competition hybridization/DNA repair/complementation/DNA damage)

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ABSTRACT **Xeroderma pigmentosum (XP) is an autosomal recessive disease in which the major clinical manifestation is a 2000-fold enhanced probability of developing sunlight-induced skin tumors, and the molecular basis for the disease is a defective DNA excision repair system. To clone the gene defective in XP complementation group A (XP-A), cDNA clones were isolated by a competition hybridization strategy in which the corresponding mRNAs were more abundant in cells of the obligately heterozygous parents relative to cells of the homozygous proband affected with the disease. In this report, a human genomic DNA clone that contains this cDNA was transformed into two independent homozygous XP-A cell lines, and these transformants displayed partial restoration of resistance to the killing effects of UV irradiation. The abundance of mRNA corresponding to this cDNA appears to correlate well with the observed UV cell survival. The results of unscheduled DNA synthesis after UV exposure indicate that the transformed cells are repair proficient relative to that of the control XP-A cells. However, using this same genomic DNA, transformation of an XP-F cell line did not confer any enhancement of UV survival or promote unscheduled DNA synthesis after UV exposure.**

The nucleotide excision pathway is a major DNA repair mechanism in mammalian cells responsible for the removal of DNA lesions such as bulky chemical adducts or pyrimidine dimers generated by UV irradiation (1). This pathway is important for the maintenance of cellular genetic integrity throughout the life span of the organism and is defective in xeroderma pigmentosum (XP), a rare autosomal disorder. The resulting inability to repair DNA lesions gives rise to the extreme sensitivity of affected individuals to the deleterious effects of sunlight and to their predisposition to develop tumors on exposed parts of the skin (2–5). Of the eight complementation groups, the loss of repair capacity is most severe in XP complementation group A (XP-A) (6). The identification of the enzymes and genes that are defective in XP should facilitate the elucidation of the molecular defect in XP and allow insight into the normal mechanism of DNA damage recognition and repair synthesis.

In a system analogous to the XP complementation groups, Thompson and collaborators (7–11) have shown the genetic diversity of laboratory-induced mutants of Chinese hamster ovary (CHO) cells with eight complementation groups. However, despite intensive studies, there is still no overlap between these two mammalian DNA repair systems. To date, human genomic clones designated as ERCC-1 through ERCC-6 have been isolated that can correct the DNA repair defect by using the strategy of genomic transformation in some of the CHO mutant cell lines (7–11), but cloning strategies that rely on similar methodologies to clone normal human genes into any of the XP complementation groups have not yet yielded any defined genes.

Tanaka *et al.* (12) have reported the molecular cloning of a mouse DNA repair gene that complements XP-A cells but not XP-C, -D, -F, or -G cells and Arrand *et al.* (13) have cloned a mouse DNA repair gene that complements XP-D cell lines but not an XP-A cell line. However, in neither case has the normal human gene been identified.

To address this research area, we have used a differential competition hybridization technique to identify and characterize cDNA clones representing mRNAs that are more abundant in cells of the obligately XP heterozygous parents when compared to levels found in a cell line derived from the affected XP-A homozygous child (14). The design of this competition hybridization strategy was based on the observation that, when HeLa cell poly(A)$^+$ RNA, ≈700 nucleotides long, was microinjected into an XP-A cell line and the cells were challenged with UV, these cells were transiently capable of repairing the DNA damage as measured by enhanced unscheduled DNA synthesis (UDS) (15). In this study we demonstrate that the human genomic DNA sequences corresponding to our cDNA clones are capable of partially restoring the UV resistance to two independent XP-A cell lines but not to an XP-F cell line.

MATERIALS AND METHODS

Human Cells. All XP human cell lines used throughout this study were obtained from the Human Genetic Mutant Cell Repository (Camden, NJ). Human breast tumor cells, MCF-7, were obtained from Craig R. Fairchild (National Cancer Institute). The simian virus 40 (SV40)-transformed human lung cell line WI38 VA13, subline 2RA, was obtained from the American Type Culture Collection.

Cosmid Library Screening. A human cosmid expression library, pCV108, containing the selectable genetic marker for G418 resistance (SV2-neo), the SV40 early promoter with the 72- and 21-base-pair repeats, and the SV40 origin of replication was kindly provided by Y. F. Lau (University of California, San Francisco) (16). This library was screened by using the nick-translated 0.7-kilobase (kb) *Sac* I–*Sac* I fragment of the cDNA insert from pEMKR clone (14). This cDNA represents an mRNA that is present in lower abundance in the child's XP-A cell line relative to the mother's cell line. The screening procedure for cosmids of potential interest was performed essentially as described by Grunstein and Hogness (17) except that Whatman 541 paper was used to lift the colonies. The yield in this screening was 5 positive signals out of 500,000 colonies.

Cosmid DNA Isolation. Cosmid DNAs were isolated using an alkaline lysis technique and purified over a pZ523 column (5 Prime → 3 Prime, Inc.) as suggested by the supplier.

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Abbreviations: XP, xeroderma pigmentosum; UDS, unscheduled DNA synthesis; SV40, simian virus 40.

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Endonuclease restriction enzyme analyses of the cosmid DNAs were performed and the results indicated that these sequences are overlapping (data not shown).

Cell Transformation. Five cosmid DNAs were used to transform the SV40-immortalized XP-A cell line GM4429. This cell line is a derivative of the child's XP-A cell line (GM5509), which was originally used to clone the cDNA by a competition hybridization strategy (14).

Transformation of this cell line was performed using the calcium precipitation method (18). Stable cell transformants were selected by growing cells in medium containing G418 sulfate. After 4 weeks of growth, drug-resistant colonies were treated with trypsin and either cloned at limiting dilution in 96-well plates or plated into 60-mm Petri dishes and irradiated at 0, 2, 3, or 4 J/m^2. For the limiting dilution method, cells grew in 16 of the 96 wells. Eleven of those clones were expanded and subjected to UV-resistance screening assays. Six of the 11 clones displayed enhanced UV resistance, and clone 2-0-F_6 is an example of that selection strategy. By using the UV-selection cloning procedure, foci that grew in the Petri dishes exposed to 4 J/m^2 were isolated using a cloning cylinder technique. Of the 7 clones that were expanded by this procedure and subsequently assayed for enhanced UV resistance, 4 displayed a significant increase in UV survival relative to the child's cells. Clone 2-4-A_1 is an example of cells derived by that selection strategy.

Other SV40-transformed cell lines, XP-A (GM4312) and XP-F (GM8437), were transformed using a cationic liposome-mediated transformation technique (19). After transformation, cells were grown for 2 weeks with G418 selection and then drug-resistant colonies were cloned using the cloning cylinder technique. These clones were expanded and screened for UV resistance using the UV-gradient assay as described below. Three of the 5 cosmid 2-transformed XP-A cell lines (GM4312) displayed enhanced UV resistance but none of the 20 cosmid 2-transformed XP-F cell lines (GM8437) showed enhanced UV resistance. These clones, 2-0-A_2, 2-0-A_4, and 2-0-F_{13}, were selected for further analysis using the colony-forming-ability assay as described below.

Analysis of Cosmid 2 DNA. The circular cosmid 2 DNA was linearized by *Cla* I digestion, yielding 25-kb and 14-kb fragments. The smaller fragment was composed of a 6-kb genomic insert and an 8-kb portion of the pCV108 vector. For restriction enzyme mapping of the 25-kb fragment, the *Cla* I-digested cosmid 2 DNA was further subjected to a partial digestion with a variety of restriction enzymes. DNAs from these partial digests were blotted onto GeneScreen*Plus* (New England Nuclear) and probed with a ^{32}P-labeled oligonucleotide, 5′-GCTTTAATGCGGTAGTTTAT-3′. This sequence represents a fragment of pBR322 between the *Cla* I and *Bam*HI sites of the 25-kb fragment. All DNA fragments containing this sequence were identified by autoradiography, and the restriction sites of the corresponding enzymes were determined from these data. Another oligonucleotide, 5′-TTCTCATGTTTGACAGCTTA-3′, representing an upstream sequence of the *Cla* I site of pBR322, was used as a probe to map the other 6-kb genomic insert.

Colony-Forming-Ability Assay. Cells were grown in 60-mm Petri dishes in triplicate on Dulbecco's modified Eagle's medium (DMEM) supplemented with 20% (vol/vol) fetal calf serum and G418 (450 μg/ml). After incubation for 3 hr to allow the cells to attach, the medium was aspirated and replaced with 1 ml of isotonic phosphate-buffered saline (PBS). The cells were UV-irradiated (Spectroline model EF-16; Spectronics, Westbury, NY) at 254 nm with dosages ranging from 0 to 5 J/m^2. After UV irradiation, the PBS was aspirated and replaced with G418-containing medium, and the cells were incubated in the dark to avoid any possible photoreactivation repair. After 10 days, the resulting colonies were stained with methylene blue and those containing 50 cells or more were counted. The D_{37} value of the shoulder of the survival curve was calculated from the UV dose required to reduce the survival from a point on the curve to 0.37 ($1/e$) of the survival at that point.

Qualitative Evaluation of Cellular UV Resistance: A UV-Gradient Assay. Various numbers of repair-proficient and -deficient cells were grown in 150-mm Petri dishes, as described above. For each cell line to be tested, 500, 5000, 50,000 and 500,000 cells were plated per dish. After attachment, the medium was removed and replaced with PBS, and cultures were prepared for a gradient UV dose. The plates that contained the cells were covered with a sterile cover plate beneath a UV lamp and a UV step gradient of 0, 0.5, 1.0, 2.0, and 4.0 J/m^2 was given to the cells by moving the cover plate stepwise through various times of irradiation. After 10 days, the Petri dishes were stained with methylene blue and photographed.

UDS. Cells were grown as monolayers in Petri dishes containing coverslips until they were confluent. Cells were washed with PBS and challenged with a UV dosage of 4 J/m^2. The cells were then incubated with DMEM supplemented with 20% fetal calf serum containing [^{3}H]thymidine (10 μCi/ml) at 37°C for 3 hr. Coverslips were then washed with PBS, fixed with ethanol/acetic acid [3:1 (vol/vol)], and treated with 5% (wt/vol) trichloroacetic acid for 15 min at 4°C to remove the acid-soluble material. Coverslips were then dipped in Kodak NTB-2 microautoradiography emulsion. The films were developed after a 7-day exposure and subsequently stained with Giemsa. The lightly labeled nuclei resulting from UDS were randomly scored and the distribution of number of grains per nucleus was analyzed. The background number of silver grains that were not associated with cells was subtracted from the reported values.

Quantitation of mRNAs. RNAs from each cell line were purified as described (14) and denatured by incubation for 1 hr at 50°C in 50% (vol/vol) deionized formamide/6% (vol/vol) formaldehyde. After chilling the samples on ice, 10, 3, 1, 0.3, and 0.1 μg were blotted onto GeneScreen*Plus* filter paper. RNA samples were adsorbed onto the filter for 30 min without suction, followed by 30 sec of filtration with a slight vacuum. The filter was subsequently baked at 80°C to remove the formaldehyde and hybridized with a 23-mer oligonucleotide labeled with ^{32}P by polynucleotide kinase. This oligonucleotide is derived from part of the cDNA fragment as reported (14). The hybridization was performed at 30°C using the buffers recommended by the GeneScreen*Plus* supplier. The filters were sequentially washed as follows: twice with 2× SSC at room temperature, twice with 2× SSC/1% SDS at 35°C, and twice with 0.1× SSC at room temperature. (1× SSC = 0.15 M NaCl/0.015 M sodium citrate, pH 7.0). Each wash was carried out for 30 min. The filters were autoradiographed on XAR-5 x-ray film and scanned with an integrating densitometer.

RESULTS AND DISCUSSION

Previously a competition hybridization strategy had been used to identify a human mRNA that was more abundant in cells of the obligate heterozygotic XP-A parents relative to that found in cells from the homozygotic XP-A child (14). Human genomic clones that contain DNA coding for that unique mRNA were isolated from a cosmid expression library (16). The 108HL cosmid library was screened by using a nick-translated probe (0.7-kb *Sac* I–*Sac* I fragment of the cDNA insert from the pEMKR clone). Five overlapping cosmid clones were isolated and individually used to transform the homozygous XP-A cell line GM4429 by using the calcium phosphate precipitation method (18). Cells were selected first for G418 resistance and then assayed for enhanced UV resistance. The results of a UV-resistance

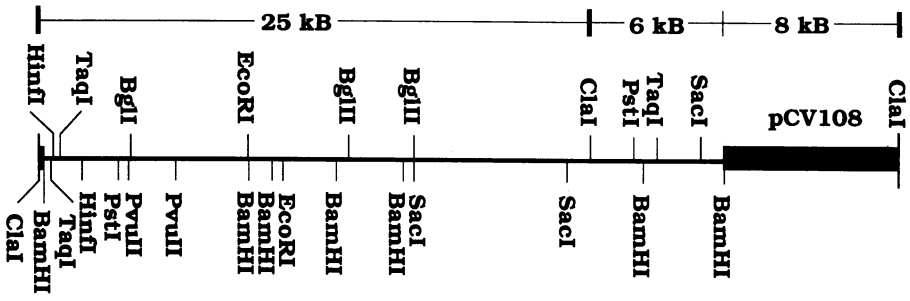

FIG. 1. Restriction map of the genomic insert of cosmid 2.

screening assay showed that one of the cosmid clones (cosmid 2) conferred enhanced UV resistance to a subset of the G418-resistant cells, whereas the other four cosmids conferred only G418 resistance and no enhanced UV resistance (data not shown). These results suggested that four of the genomic DNA inserts did not entirely cover all of the exons or regulatory regions of this gene. Since only cosmid 2 conferred enhanced UV resistance, further efforts were concentrated on this DNA. The restriction map of this cosmid revealed that the length of the genomic insert was ≈31 kb (Fig. 1). Transformation of two XP-A cell lines (GM4429 and GM4312) and one XP-F cell line (GM8437) was repeated using cosmid 2 as the only source of exogenous DNA. Table 1 shows a comparison of the transformation efficiency using the calcium phosphate precipitation method versus the cationic liposome-mediated method in producing G418-resistant cells. The yield of drug-resistant cells was ≈5 times higher using the liposome-mediated transformation procedure. Also when using cosmid 2, the percentage of cells that acquired both G418 and UV resistance (see below) was ≈50% of all drug-resistant cells and this ratio was independent of the transformation procedure (Table 1).

To obtain populations of UV-resistant XP-A cells that arose from the expansion of a single cell, two basic methods of selection were applied: (*i*) G418 selection, cloning by limiting dilution propagation, and then UV challenge and (*ii*) G418 selection, UV challenge, and then colony isolation. Clones derived by the first method were designated as 2-0 clones (indicating that they were derived from cosmid 2 without selection by UV) and clones derived by the second method were designated as 2–4 isolates (indicating they were derived from transformation with cosmid 2 and were selected with UV at 4 J/m^2. Both XP-A and XP-F recipient cell lines and all their transformants were used for the UV-gradient assay. To serve as control cells, the repair-proficient VA13 cell line and the fibroblasts of the XP-A mother (GM5510) and child (GM5509) were used in the same assay. The result of these experiments demonstrated that there was an enhancement of UV survival for only the XP-A transformants and not the XP-F (photographs not shown).

To obtain quantitative UV survival curves for all clones, cells were irradiated with UV at dosages ranging from 0 to 5 J/m^2. The survival curves of the heterozygous cell line from the mother (GM5510) and the homozygous cell line from the XP-A-affected child (GM5509) are shown in Fig. 2*A*. Comparisons between several of the transformants and those of the repair-defective recipient cell line are shown in Fig. 2 *B–D*. The presence of a positive shoulder in the initial portion of the survival curve indicates that post-UV DNA repair had occurred in the G418-resistant transformants (20). The extent of the repair capacity of all cell lines was estimated from the D_{37} value of the shoulder. The survival curve of the SV40-containing cell line from the child (GM4429) had a D_{37} value of 0.7 J/m^2, without any shoulder, whereas the cell line from the child (GM5509) not containing SV40 had a D_{37} value of 0.6 J/m^2. These results are in good agreement with the findings of Andrews *et al.* (21) using the XP12BE cell line (a synonymous designation of GM5509). These results indicate that the 2-4-A_1 clone has a D_{37} value of 1.5, which is 2.1- to 2.5-fold higher than the D_{37} value of either GM4429 or GM5509. Clone 2-0-F_6 had a D_{37} value of 1.0 J/m^2 with no apparent shoulder. The D_{37} value of the heterozygous cell line from the mother (GM5510) was 4 J/m^2. Thus, the D_{37} values for these transformants suggest that DNA repair was clearly enhanced but to a degree significantly lower than that of the heterozygous cell line from the mother.

In other experiments, the XP-A cell line (GM4312) was transformed by liposome-mediated transformation and G418-resistant foci were cloned and expanded. As an initial screening, five of the G418-resistant transformants were assayed for their approximate level of UV resistance using the UV gradient assay. Of the five clones tested, three of them (2-0-A_2, 2-0-A_4, and 2-0-A_5) displayed significantly enhanced UV survival up to 4 J/m^2. UV survival data were collected for two of these clones (2-0-A_2 and 2-0-A_4) and both clones displayed significantly enhanced UV resistance (D_{37} = 1.0 and 0.9, respectively) (Fig. 2*C*). The D_{37} value of the recipient cell line was 0.2 J/m^2 and this value is very similar to the data reported by Painter (22), Takebe *et al.* (23), and Goldstein (24). The ratio of the D_{37} values for the transformants versus the recipient child was 4.5–5.0, thus suggesting a very substantial enhancement of UV resistance.

Since cosmid 2 was able to confer enhanced UV survival to two XP-A cell lines, it was important to test whether this cosmid could confer enhanced survival to a different XP complementation group. For these experiments an XP-F cell line (GM8437) was chosen. GM8437 was transformed with cosmid 2 and G418-resistant foci were grown and expanded. Although the transformation of cosmid 2 into the XP-F cell line (GM8437) resulted in G418-resistant cells, when the UV

Table 1. Comparison of transformation techniques that resulted in UV- and G418-resistant clones

Recipient cell line	Transformation technique	Frequency		Cloning procedure	Further characterized Neo^R and UV^R clones
		Neo^R	UV^R/Neo^R		
GM4429 (XP-A)	CPT	3.3×10^{-6}	6/11 (55%)	LDT	2-0-F_6
GM4429 (XP-A)	CPT	3.3×10^{-6}	4/7 (57%)	CCT	2-4-A_1
GM4312 (XP-A)	LMT	1.2×10^{-5}	3/5 (60%)	CCT	2-0-A_2 and 2-0-A_4
GM8437 (XP-F)	LMT	1.75×10^{-5}	0/20 (0%)	CCT	2-0-F_{13}

CPT, calcium phosphate precipitation transformation; LMT, liposome-mediated transformation; LDT, limiting dilution technique; CCT, cloning cylinder technique; Neo^R, neomycin resistance; UV^R, UV resistance.

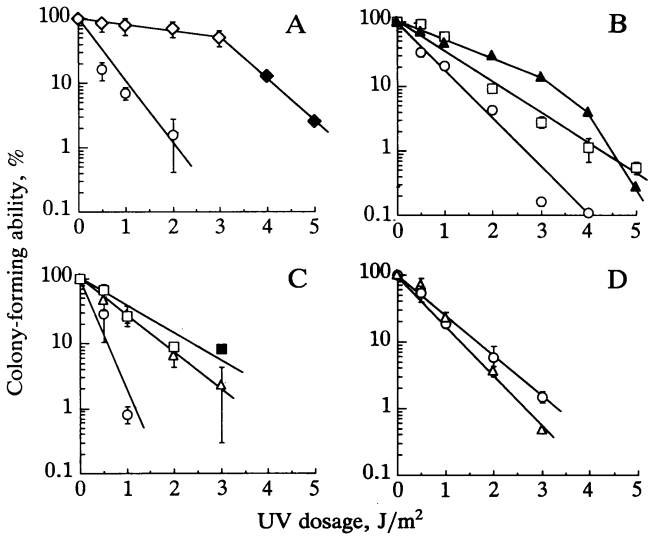

FIG. 2. UV survival curves obtained by colony-forming ability after UV irradiation. (*A*) Heterozygous fibroblast from the mother GM5510 (◇, ◆) compared to the homozygous fibroblast from the XP-A-affected child GM5509 (○). These cells are not transformed with SV40. (*B*) XP-A recipient GM4429 (○) compared to the transformants 2-4-A_1 (▲) and 2-0-F_6 (□). (*C*) XP-A recipient cell line GM4312 (○) compared to the transformants 2-0-A_2 (□, ■) and 2-0-A_4 (△). (*D*) XP-F recipient cell line GM8437 (○) compared to the transformant 2-0-F_{13} (△). Cell lines used in *B–D* are SV40-transformed. Open symbols represent the average of three to six determinations and SDs (error bars) are given; however, in some cases, the size of the symbol was greater than the size of the error bars and thus the error bars do not show. Solid symbols represent the average of two experiments.

resistance of 20 individual transformants was assessed by the UV gradient assay, none of the clones displayed enhanced UV resistance. UV-survival data for the recipient cell line and one of the transformants (2-0-F_{13}) were obtained (Table 1 and Fig. 2*D*). This demonstrates that cosmid 2 can complement XP-A but not XP-F cell lines. The D_{37} value of 0.7 J/m² for this XP-F cell line is consistent with the value reported by Yagi and Takebe (25).

The enhanced UV resistance of the two XP-A cell lines was related to enhanced DNA repair activity by using an UDS assay. UDS was performed on the XP-A and XP-F recipient cell lines and their transformants. Control experiments with cell lines from the child (GM5509) and mother (GM5510) were also performed. These results, which are displayed in the histogram in Fig. 3, show the enhanced UDS activities of the transformants 2-4-A_1 and 2-0-F_6, as well as 2-0-A_2 and 2-0-A_4, which were derived from XP-A GM4429 and GM4312, respectively. When these enhancements were compared to the heterozygous GM5510 cell line, the average number of grains obtained from 100 nuclei of each cell line showed that transformants 2-4-A_1 and 2-0-F_6 have significantly higher number of grains, whereas transformants 2-0-A_2 and 2-0-A_4 have lower numbers. It should be noted that although these UDS results suggest that repair levels are near normal values, all of these transformants are less UV-resistant relative to GM5510, as assayed by colony-forming ability and D_{37} values (Fig. 2). Since the UDS assay is only a measure of the first 3 hr of repair synthesis, it is not clear whether pyrimidine dimers, 6-4 lesions, or both are being actively removed from these genomes. However, in this regard, these results are in agreement with the results of microscopic immunofluorescence quantitation of thymidine dimers that remain in the nucleus at 24 hr after UV exposure. These assays used monoclonal antibodies and were performed in collaboration with R. A. Baan and L. Roza at the TNO Medical Biological Laboratory (Rijswijk, The Netherlands) (to be published elsewhere). In addition, the difference between the UDS assay and survival may be exaggerated due to the fact that the UV-resistant transformants are SV40 transformed and do not exhibit contact inhibition and thus major populations of the transformants were in S phase of the cell cycle and could not be scored in the UDS assay. The intensities of UDS for both XP-A GM4429 and GM4312 as well as XP-F GM8437 and its cosmid 2-transformant 2-0-F_{13} were not substantially different than the intensity of the child's fibroblast GM5509 (UDS data and histograms not shown).

Fig. 4 shows correlation between the enhanced UV survival of the transformants and the expression of a gene from cosmid 2, as measured by a hybridization slot blot analysis. Total RNA from the UV-resistant transformants, 2-4-A_1 and 2-0-F_6, the repair-deficient XP-A recipient cell line, the repair-proficient cell line from the mother, and an unrelated repair-proficient human breast carcinoma cell line, MCF-7, were used in this analysis. The gene-specific RNA was probed with a labeled 25-base oligonucleotide probe whose sequence represents a portion of our cDNA clone (14). The message abundance for this gene in the transformants was intermediate between the XP-A recipient cell line (GM4429) and the repair-proficient MCF-7 cell line (Fig. 4*B*). Fig. 4*A* shows that the level of expression in the mother's (GM5510) cell line was substantially higher than that of the child's (GM5509) cell line.

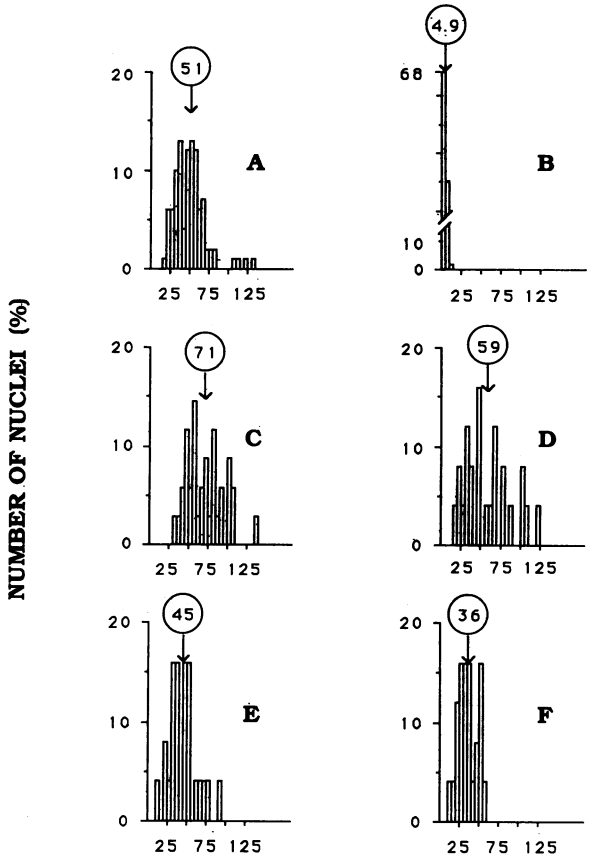

FIG. 3. Histogram of the distribution of silver grains over nuclei resulting from the incorporation of [^{3}H]thymidine during UDS after UV irradiation. Cosmid 2-transformed XP-A cells of GM4429 (2-4-A_1 and 2-0-F_6) and GM4312 (2-0-A_2 and 2-0-A_4) are compared with the control fibroblasts from the mother and child. The histograms appear as follows. (*A*) Mother (GM5510). (*B*) Child (GM5509). (*C*) 2-4-A_1. (*D*) 2-0-F_6. (*E*) 2-0-A_2. (*F*) 2-0-A_4. Arrows show mean number of grains per nucleus, calculated from 100 nuclei.

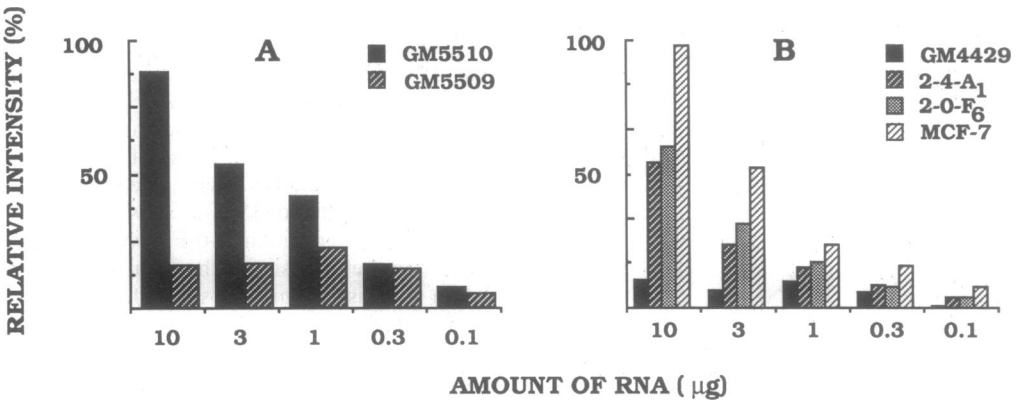

FIG. 4. Quantitation of the mRNA levels for the gene related to the XP-A defect in GM5509 using slot blot analyses. (*A*) Comparison of RNA from the heterozygous cell line from the mother (GM5510) to the homozygous cell line from the XP-A child (GM5509). The blots were probed with a 25-mer oligonucleotide derived from part of the XP-A-related cDNA (17). These sequences were more abundant in the mother's than in the child's cells. Same analysis using the RNA from the UV-resistant transformants, 2-4-A_1 and 2-0-F_6, compared to the recipient cell line (GM4429, containing SV40 DNA). A breast carcinoma cell line (MCF-7) was also used as an independent positive control.

This result is consistent with the level of enhanced UV survival of these cells as shown in Fig. 2 *A* and *B*.

The data concerning the enhancement of UV survival in these clones and the enhanced expression of a gene present on this cosmid isolate suggest that the reduction in a specific mRNA whose gene is carried on cosmid 2 is involved in the enhanced UV sensitivity in this XP-A cell line. This observation is consistent with findings reported by Tanaka *et al.* (12). The product of this gene may be necessary to initiate the excision repair pathway. However, we do not know whether cosmid 2 contains the complete regulatory region(s) for the proper expression of this message. The inability to completely restore UV resistance of two XP-A cell lines to the parental levels may reflect inadequate amounts of the complementing gene product and may be due to lowered levels of gene transcripts as shown in the data from blot analyses. This cosmid clearly did not complement XP-F cell line, a result that was expected based on the genetics that define a complementation group and differences in the DNA repair characteristics among the various groups. Since many XP patients display similar phenotypic expression of the disease, this indicates that a large protein complex is responsible for the multisteps in the excision repair of UV damage in the DNA. An abnormality in a component within this protein complex could alter the activity of the whole complex. It is then possible that one of these components is encoded by the 31-kb insert of cosmid 2.

Thus our data demonstrate that introduction of a specific fragment of the human genome into two XP-A cell lines can partially complement the severe UV sensitivity of these cells. The genetic information found within this DNA may code for the protein that appears to be defective in initiating excision repair in XP-A cells. It is also possible that we have identified a gene that enhances or restores the efficient expression of genes involved in the initiation of the excision repair process.

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