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Molecular Cloning and Chromosomal Localization of DNA Sequences Associated with a Human DNA Repair Gene

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The genes and gene products involved in the mammalian DNA repair processes have yet to be identified. Toward this end we made use of a number of DNA repair-proficient transformants that were generated after transfection of DNA from repair-proficient human cells into a mutant hamster line that is defective in the initial incision step of the excision repair process. In this report, biochemical evidence is presented that demonstrates that these transformants are repair proficient. In addition, we describe the molecular identification and cloning of unique DNA sequences closely associated with the transfected human DNA repair gene and demonstrate the presence of homologous DNA sequences in human cells and in the repair-proficient DNA transformants. The chromosomal location of these sequences was determined by using a panel of rodent-human somatic cell hybrids. Both unique DNA sequences were found to be on human chromosome 19.

Procaryotic and eucaryotic cells contain a number of DNA repair processes that are able to respond to damage to cellular DNA (13). One of these repair pathways, the excision-repair pathway, is involved in the repair of DNA damaged by UV light or cross-linking agents. In procaryotes, the genes and gene products that control these processes are beginning to be understood in detail. In eucaryotes, however, attempts are just being made to identify the genes involved.

The importance of intact repair systems in humans is demonstrated by several hereditary disorders which are associated with defective DNA repair capacities. These include xeroderma pigmentosum, ataxia telangiectasia, and Fanconi's anemia, all of which are associated with an increased sensitivity to DNA-damaging agents and a predisposition to cancer (12). These diseases demonstrate considerable genetic heterogeneity, since at least seven different complementation groups of xeroderma pigmentosum, all defective in the initial incision step of the excision-repair pathway, have been identified (10, 36). Two different complementation groups in Fanconi's anemia (35; M. Buchwald, personal communication) and five in ataxia telangiectasia (20) have also been identified. These findings suggest that the human DNA repair processes are complex, with a number of different genes controlling the repair pathways.

A number of different UV-sensitive mutants have been generated from established cell lines (5, 15, 23, 25, 27, 30, 34). One of these mutant lines, UV-20, isolated from a parental Chinese hamster ovary (CHO) cell line, is sensitive to a number of other DNA-damaging agents, including the DNA cross-linking agent mitomycin C (MM-C) (29). Similar to xeroderma pigmentosum, the genetic diversity associated with the initial incision step is demonstrated by the fact that UV-20 represents one of five complementation groups (29), all defective in the initial incision step (28).

To understand the genetic organization of the human DNA repair system, we have been attempting to identify and characterize genes involved in this process. In a previous communication (22), we described the isolation of DNA repair-proficient transformants generated by transfecting DNA from a human repair-proficient cell line into CHO mutant UV-20 (22). The transformants exhibited resistance to both UV and MM-C, similar to the repair-proficient CHO wild-type cell line AA8-4, demonstrating that these cell lines were phenotypically repair proficient (22). After genomic blot analysis, independent transformants exhibited a common set of human-specific restriction fragments associated with the transfected DNA repair gene (22). In the work described here, we present biochemical evidence that the transformants are repair proficient. In addition, we describe the molecular cloning of DNA fragments associated with this human DNA repair gene, demonstrate the presence of homologous DNA sequences in human cells and repair-proficient DNA transformants, and localize these sequences to human chromosome 19.

MATERIALS AND METHODS

Cell lines and preparation of cellular DNA. The cell lines used in this study included the human repair-proficient cell line HeLa, the parental CHO wild-type repair-proficient line AA8-4, the CHO repair-deficient mutant cell line UV-20, and two primary repair-proficient DNA transformants, X25 and X38, and their corresponding secondary transformants, X25-37 and X38-69. All of these lines were maintained in culture in alpha medium (26) supplemented with antibiotics and 10% fetal calf serum. Human leukocytes were isolated after Hypaque-Ficoll density centrifugation of peripheral blood samples as previously described (4). Cellular DNA was prepared after extraction with organic solvents as previously described (22).

Alkaline elution. Cultures used for alkaline elution studies were grown in alpha medium lacking nucleosides but supplemented with 10% dialyzed fetal calf serum. Before elution, cells were maintained in growth medium containing ^{14}C-labeled thymidine (10 nM; 59 mCi/mmol) for 20 h followed by 2 h of growth in fresh medium lacking labeled thymidine. Cells were then incubated for 15 min in growth medium containing 100 μM cytosine arabinoside (araC),

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centrifuged, and suspended at a concentration of 2×10^5 cells per ml in phosphate-buffered saline containing 100 μM araC (4°C), and then 10^6 cells were immediately irradiated with 6 J/m^2 of UV light. After irradiation, cells were either maintained at 4°C or incubated for various periods at 37°C in growth medium containing 100 μM araC. At the end of the incubation, cells were centrifuged and suspended in phosphate-buffered saline containing 100 μM araC (4°C). Cells (10^6 per sample) were loaded by suction onto a polycarbonate filter (pore size, 2.0 μm; Nucleopore, Pleasanton, Calif.) and then eluted with 2% tetrapropylammonium hydroxide–20 mM EDTA–0.1% sodium dodecyl sulfate (SDS) (pH 12.1). Elution was performed essentially as described previously (16), and fractions were collected every 1.5 h with a pump rate of 0.03 ml/min. Radioactivity was determined by liquid scintillation counting.

Gel electrophoresis and blot hybridization. Cellular DNA (25 μg) was digested overnight, ethanol precipitated, suspended in buffer (4% Ficoll, 0.12 M EDTA, 0.5% SDS, 0.012 M bromophenol blue), and electrophoresed overnight on a 0.8% agarose gel. After treatment with alkaline and neutralization buffers, the gel was transferred (24) to a nitrocellulose filter for 2.5 days. The filter was then baked for 2 h at 80°C and prehybridized at 42°C overnight in 50% formamide–5× SSC(1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–5× Denhardt solution (8)–0.02 M sodium phosphate (pH 6.5)–200 μg of denatured salmon testes DNA per ml. Hybridization with ^{32}P-labeled hD1UV (10^6 cpm/ml) was conducted in the same solution supplemented with 10% dextran sulfate overnight at 42°C. After hybridization, filters were washed twice at room temperature for 5 min and twice at 50°C for 30 min in 2× SSC plus 0.1% SDS followed by two washes at 65°C in 0.1× SSC plus 0.1% SDS. After air drying, filters were exposed overnight to a Kodak XAR-5 film with one Cronex Lightning-Plus intensifying screen at −70°C.

Construction and screening of genomic library λX25-37. A genomic library (19) of the secondary DNA repair-proficient transformant X25-37 was constructed by ligating 1.25 μg of cellular DNA, digested to completion with the restriction enzyme *Eco*RI, to 10 μg of purified *Eco*RI arms of the bacteriophage cloning vector λgtWES.λB (31). DNA was ligated with T4 DNA ligase overnight at 15°C and then for 2 days at 4°C. The recombinant DNA was packaged in vitro (1) to form 1.1×10^6 viable phage particles. Bacteriophage vector DNA was prepared by the method ascribed to B. Seed (18). The sonicated extract and freeze-thaw lysate packaging reagents were prepared essentially as described by Hohn and co-workers (14, 19).

Recombinant phage (10^6) were plated on *Escherichia coli* DP50 *supF* at a density of 50,000 plaques per dish (22 by 22 cm) and screened by the procedure of Benton and Davis (2). After treatment with alkaline and neutralization buffers, 20 pairs of duplicate filters were baked for 2 h at 80°C and then prehybridized at 65°C for 6 h in 5× Denhardt solution (8)–5× SSPE (1× SSPE is 0.18 M NaCl, 10 mM $NaPO_4$[pH 7.7], 1 mM EDTA)–0.1% SDS–100 μg of denatured salmon sperm DNA per ml. Hybridization with ^{32}P-labeled human cellular DNA (specific activity, $>10^8$ cpm/μg), prepared by nick translation (21), was carried out in the same solution at a concentration of 10^5 cpm/ml overnight at 65°C. After hybridization, filters were washed twice for 1 h at 65°C in 1× SSC plus 0.1% SDS followed by one wash at the same temperature in 0.3× SSC plus 0.1% SDS. After air drying, filters were exposed to Kodak XAR-5 film with one Cronex Lightning-Plus intensifying screen at −70°C for periods up to 2 weeks. Hybridizing plaques were picked, replated, and rescreened twice to identify true positives. They were subsequently amplified for DNA preparation.

Subcloning of DNA sequences. DNA fragments were isolated by preparative gel electrophoresis and electroelution (9). Restriction endonuclease digestions were performed overnight under conditions specified by the supplier (Boehringer Mannheim Biochemicals, Indianapolis, Ind.; New England Biolabs, Beverly, Mass.; or Bethesda Research Laboratories, Gaithersburg, Md.) at a concentration of 2 to 4 U per μg of DNA. Reactions with phage T4 DNA ligase (New England Biolabs) were conducted overnight at 15°C at a concentration of 1,000 U per μg of DNA in the recommended ligation buffer. All plasmids were transfected into *E. coli* HB101 (3) by a calcium chloride procedure described previously (17). Reactions with calf intestinal alkaline phosphatase (Bethesda Research Laboratories) were performed for 1 h at 37°C as described previously (18). Bacteriophage and plasmid DNAs were prepared by CsCl density gradient centrifugation.

Chromosome mapping. The chromosomal location of the DNA sequences associated with the human DNA repair gene was determined by gel electrophoresis and blot hybridization analysis of DNA obtained from a series of rodent-human somatic cell hybrids segregating human chromosomes. Construction of these hybrids has been described previously (6, 33). Hybrids belonging to series A, L or LT, and W were derived from fusions of human cells with mouse A9 (HPRT$^-$), mouse LTK$^-$, or Chinese hamster line wg3H (HPRT$^-$) cells, respectively. Hybrid AHA-11a was provided by F. Ruddle and was characterized as described previously (33). Hybrid cell clones were grown in mass culture and harvested for DNA preparation and for chromosome analysis at the same cell passage. Determination of human chromosome constitution was performed by detailed karyotyping of a minimum of 25 cells after trypsin-Giemsa banding (11). In most instances the presence or absence of human chromosome 19 was confirmed by genomic blot hybridization analysis with DNA probe p3-10, independently assigned to chromosome 19 (S. Naylor and M. Skolnick, personal communication).

RESULTS

Repair proficiency of MM-C-resistant UV-20 transformants. To determine whether the DNA transformants selected for MM-C resistance were DNA repair proficient, alkaline elution studies were carried out after irradiation with UV. Figure 1 illustrates the results of a study conducted with CHO wild-type line AA8-4, CHO mutant UV-20, and two primary and secondary transformants. Cultures were incubated for 0, 8, 16, or 30 min at 37°C after irradiation. As the incubation time increased, the DNA eluted progressively faster in both wild-type and transformant cultures (Fig. 1). These results demonstrate the accumulation of DNA strand breaks due to the incision step of the excision-repair process and the inhibition of polymerization due to the presence of araC (7). Although there were differences in the rate of elution with the wild-type and transformant cell lines, the alkaline elution rates for these cells were in marked contrast to those of UV-20, which exhibited only a slight increase in the rate of elution. Thus, MM-C-resistant UV-20 transformants, like wild-type CHO cells but in contrast to mutant UV-20, are proficient in the initial incision step of the excision-repair pathway.

Screening of bacteriophage library λX25-37. To isolate and characterize DNA sequences associated with the repair gene transferred into UV-20 transformants, we constructed a phage library with genomic DNA from secondary trans-

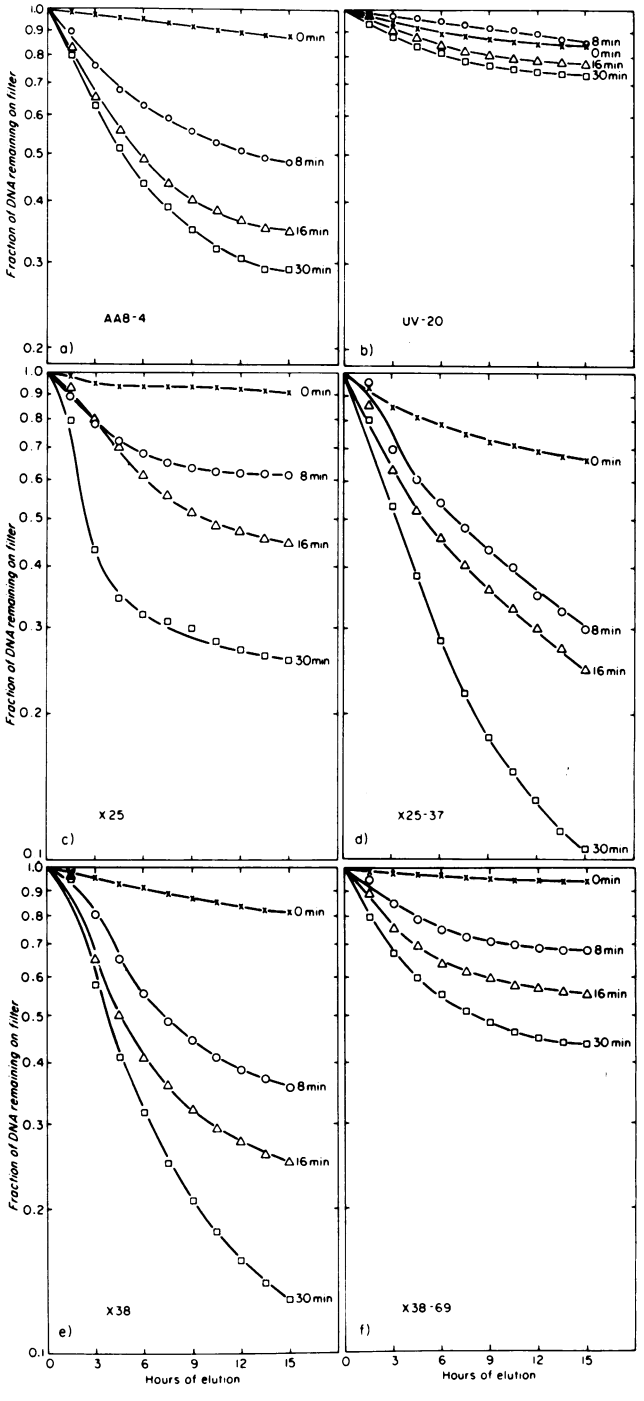

FIG. 1. Alkaline elution of DNA from CHO wild-type strain AA8-4, DNA repair-deficient mutant UV-20 and DNA repair-proficient transformants X25, X25-37, X38, and X38-69 after UV irradiation (6 J/m^2). After exposure to UV light, the survival levels of AA8-4 and of all of the transformants were essentially 100%, whereas that of UV-20 was ca. 1% (22). The amount of DNA remaining on the filter was determined by measuring the fraction of radioactivity eluted.

formant X25-37. Figure 2 is a flow diagram illustrating the identification and construction of a number of bacteriophages and plasmids that were used in the study. Briefly, large DNA fragments known to be of human origin were isolated by screening, with a probe specific for human DNA, a genomic library constructed with DNA extracted from the repair-proficient DNA transformant X25-37. Phage recombinants that contained inserts of human origin were identified in this manner. The inserts were then examined by restriction enzyme and blot-hybridization analysis (24) to identify single-copy subfragments that did not hybridize to a human repetitive DNA probe.

Two phage-containing inserts that strongly hybridized to a human cellular DNA probe were identified. One of these phages, λh1UV, contained an *Eco*RI insert of 4.6 kilobase pairs (kbp) as well as a second *Eco*RI insert of 1.5 kbp that did not hybridize to human-specific DNA. This smaller fragment was probably ligated to and packaged along with the 4.6-kbp *Eco*RI fragment during construction of the bacteriophage library and is likely of hamster origin. The second phage, λh2UV, contained an 8.6-kbp *Eco*RI insert. The 4.6- and 8.6-kbp *Eco*RI inserts were identical in size to *Eco*RI restriction fragments observed in all four of the DNA repair-proficient transformants in genomic blot analysis with a human-specific DNA probe (22).

Identification of single-copy subfragments. To isolate DNA fragments containing nonrepetitive unique sequences, the h1UV and h2UV inserts were each subcloned into plasmid pBR322. These plasmids were designated ph1UV and ph2UV, respectively, and were subjected to restriction endonuclease digestion and blot hybridization analysis with nick-translated total human DNA as a probe. We were thus able to identify a subfragment by ethidium bromide staining that did not hybridize to the radioactive probe. A 0.9-kbp *Pvu*II-*Sph*I fragment derived from h1UV (labeled hD1UV) that did not hybridize to the human DNA probe was identified. As shown below, however, this DNA segment does appear to contain some repetitive sequences. A similar analysis with DNA fragment h2UV identified a 0.32-kbp *Pvu*II-*Pvu*II subfragment (labeled hD2UV) that did not hybridize to the human-specific repetitive DNA probe. These fragments, designated phD1UV and phD2UV, were subcloned into plasmid pBR322 and used as probes in genomic blot analysis. Restriction endonuclease maps for DNA fragments h1UV and h2UV, with identification of the subfragments containing the unique sequences, are shown in Fig. 3 and 4, respectively.

Genomic blot hybridization analysis. To determine whether fragments hD1UV and hD2UV were closely associated with the DNA repair gene, DNA from human peripheral blood leukocytes, HeLa cells, CHO wild-type cell line AA8-4, CHO DNA repair-deficient mutant cell line UV-20, and primary DNA repair-proficient transformants X25 and X38 and their corresponding secondary transformants X25-37 and X38-69 were examined by the genomic blot procedure, with the cloned subfragments as probes.

DNA sequence hD1UV (0.9 kbp), derived from the 4.6-kbp *Eco*RI restriction fragment in phage λh1UV, hybridized to a 4.6-kbp *Eco*RI DNA fragment from HeLa cells, human peripheral blood leukocytes, and the four DNA repair-proficient transformants (Fig. 3) but failed to hybridize to wild-type (AA8-4) or mutant (UV-20) CHO DNA. This probe also hybridized to a relatively high-molecular-weight *Hin*dIII fragment (ca. 24 kbp) in the two human lines and in three of the transformants (Fig. 3). For the fourth transformant, X25-37, the probe hybridized to two smaller *Hin*dIII fragments (17 and 13 kbp). A smaller *Hin*dIII fragment of 4.9 kbp can also be identified in the X25 cell line. The apparent hybridization to other restriction fragments in HeLa and human leukocyte DNAs was the result of the presence of some human repetitive sequences along with the single-copy sequence in plasmid phD1UV.

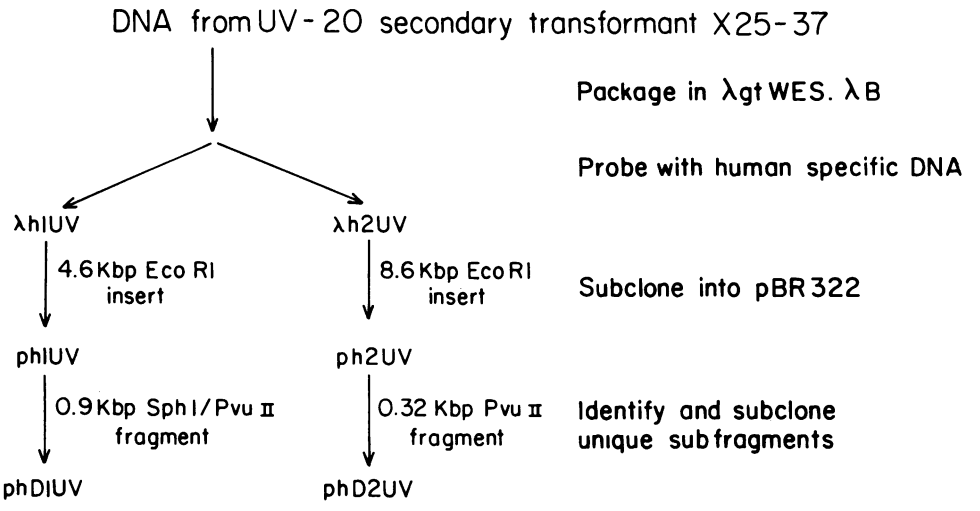

FIG. 2. Schematic representation of the experimental protocol used to identify unique sequences associated with the human DNA repair gene. A genomic library of *Eco*RI-digested DNA from the UV-20 secondary transformant X25-37 was constructed in vector λgtWES.λB as described in the text. Two clones, λh1UV and λh2UV, were identified by screening the library with a total human DNA probe. The inserts in these clones, 4.6 and 8.6 kbp, respectively, were subcloned into plasmid vector pBR322. Plasmid phD1UV was constructed by ligating *Sph*I-*Pvu*II-digested pBR322 and the 0.9-kbp *Sph*I-*Pvu*II fragment of ph1UV. The plasmid phD2UV was constructed by ligating the 0.32-kbp *Pvu*II-*Pvu*II fragment of ph2UV to phosphatase-treated, *Pvu*II-digested pBR322.

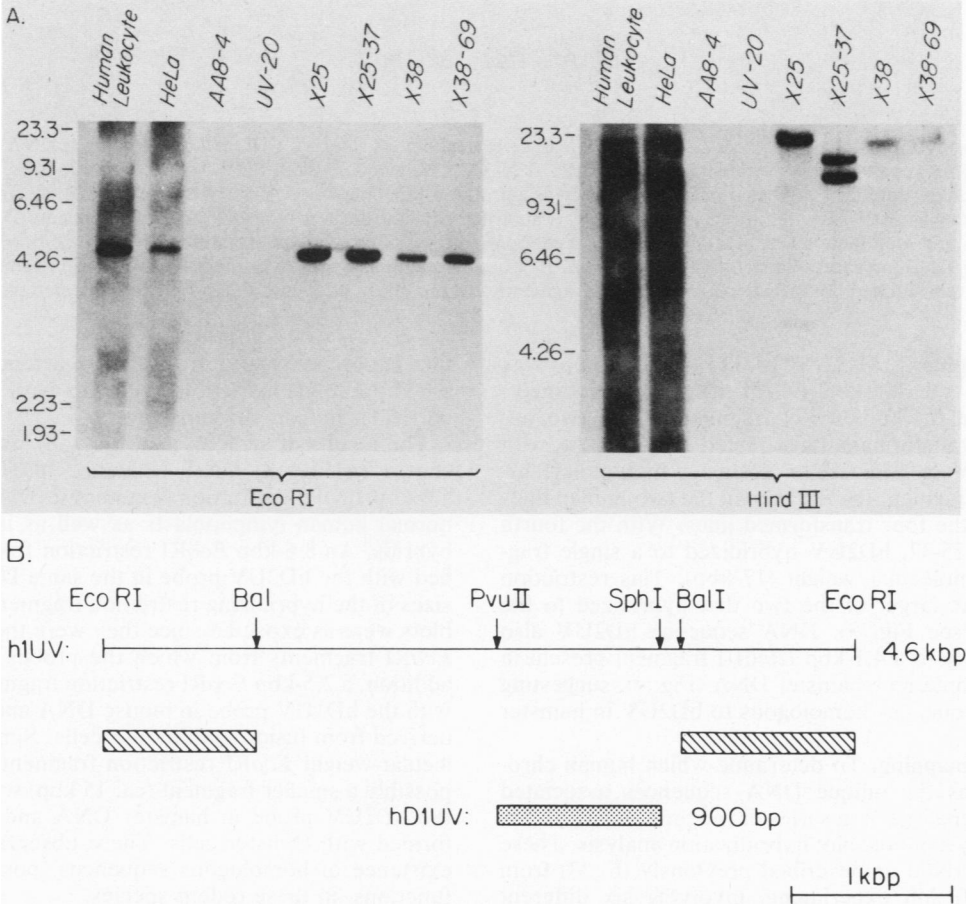

FIG. 3. (A) Gel electrophoresis and blot hybridization analysis of DNA from human cells (leukocytes, HeLa), CHO wild-type AA8-4, DNA repair-deficient mutant UV-20, and DNA repair-proficient transformed lines X25, X25-37, X38, and X38-69 to the hD1UV probe. Southern gel analysis was performed as described in the text. Numbers to the left indicate DNA markers in kilobases based on λ DNA markers. (B) Restriction endonuclease map of DNA fragment h1UV and identification of hD1UV, a subfragment that hybridized weakly to human DNA probe. The restriction map and hD1UV identification were deduced from restriction enzyme digestion and blot hybridization analysis with ^{32}P-labeled total human DNA as a probe. Restriction fragments that hybridized with the total human DNA probe are indicated by the cross-hatched boxes, whereas the restriction fragment that hybridized only weakly to this probe is indicated with the dotted boxes.

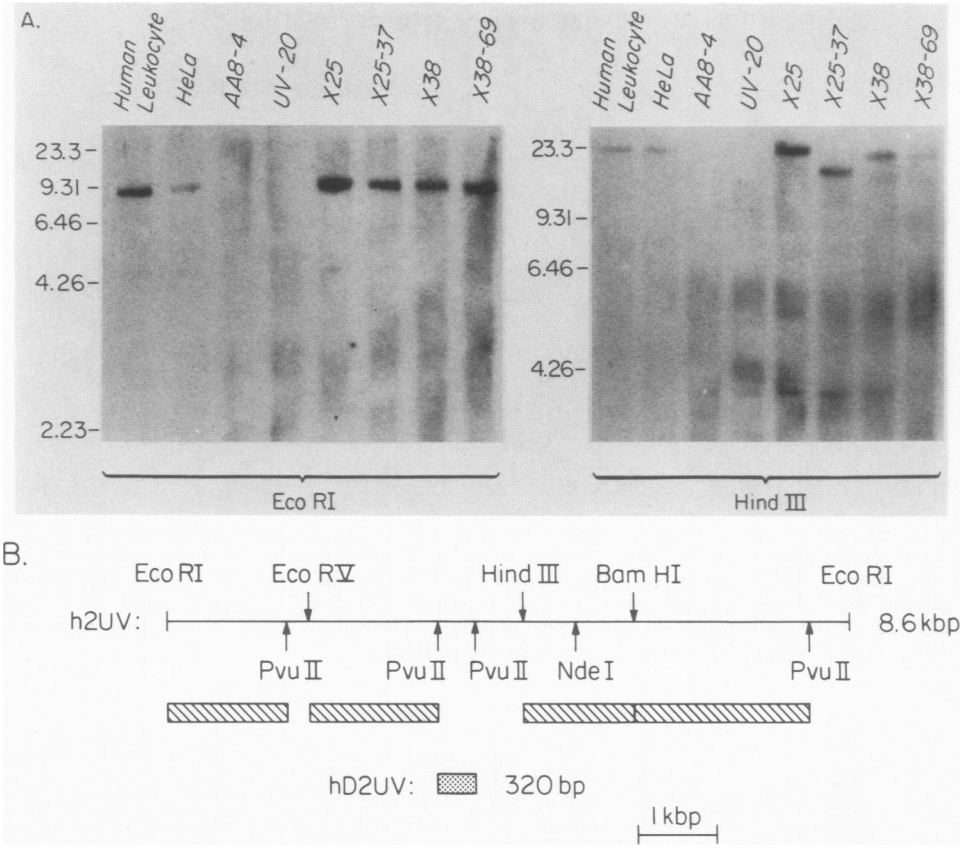

FIG. 4. (A) Blot-hybridization analysis of DNA from human cells (leukocytes, HeLa), CHO wild-type AA8-4, DNA repair deficient-mutant UV-20, and DNA repair-proficient transformed lines X25, X25-37, X38, and X38-69 to hD2UV. The conditions were identical to those described in the text except that ^{32}P-labeled h2DUV was used as a probe and the final two washes were at 50°C. (B) Restriction endonuclease map of DNA fragment h2UV and identification of hD2UV, a subfragment that did not hybridize to human-specific DNA. The restriction endonuclease map and identification of hD2UV were deduced from restriction enzyme digests and blot hybridization analysis with either ^{32}P-labeled human DNA or subfragment hD2UV as probe. Restriction fragments that hybridized with the total human DNA probe are indicated by the cross-hatched boxes, whereas restriction fragments that did not hybridize to this probe are indicated with the dotted boxes.

DNA subfragment hD2UV (0.32 kbp), which was subcloned from the 8.6-kbp *Eco*RI restriction fragment, hybridized to an 8.6-kbp *Eco*RI fragment in the two human and four transformant lines tested (Fig. 4). As with hD1UV, hD2UV hybridized to a single, high-molecular-weight *Hin*dIII fragment (ca. 24 kbp) in the two human lines and in three of the four transformed lines. With the fourth transformant, X25-37, hD2UV hybridized to a single fragment of lower molecular weight (17 kbp). This restriction fragment was the larger of the two that hybridized to the hD1UV probe (see Fig. 3). DNA sequence hD2UV also hybridized weakly to a 4.1-kbp *Hin*dIII fragment present in DNA samples containing hamster DNA (Fig. 4), suggesting that there are sequences homologous to hD2UV in hamster DNA.

Chromosome mapping. To determine which human chromosome contains the unique DNA sequences associated with the DNA repair gene, a series of rodent-human hybrids was examined by genomic blot hybridization analysis. These hybrids were derived as described previously (6, 33) from eight separate fusion experiments involving six different human diploid fibroblast strains and three different rodent cell lines. Each hybrid contained a full set of hamster or mouse chromosomes but only a partial and varying complement of different combinations of human chromosomes (Table 1). Thus, they could be used in DNA blot hybridization experiments to correlate the existence of the 4.6- and 8.6-kbp *Eco*RI restriction fragments with the presence of a particular human chromosome.

The results of such an analysis with six of the hybrids is shown in Fig. 5. DNA sequence hD1UV recognized a 4.6-kbp *Eco*RI restriction fragment in DNA extracted from normal human lymphoblasts as well as in three of the six hybrids. An 8.6-kbp *Eco*RI restriction fragment was identified with the hD2UV probe in the same DNA samples. The sizes of the hybridizing restriction fragments in the genomic blots were as expected since they were the same size as the *Eco*RI fragments from which the probes were derived. In addition, a 2.5-kbp *Eco*RI restriction fragment was identified with the hD1UV probe in mouse DNA and in those hybrids derived from fusion with mouse cells. Similarly, a high-molecular-weight *Eco*RI restriction fragment (ca. 25 kbp) and possibly a smaller fragment (ca. 15 kbp) were observed with the hD2UV probe in hamster DNA and in those hybrids formed with hamster cells. These observations suggest the existence of homologous sequences, possibly with similar functions, in these rodent species.

Seventeen hybrids were examined in this fashion. The results, (Table 1) indicate that sequences homologous to the two probes segregated only with chromosome 19, with no discordances. In all cases, both probes segregated together and with another DNA probe, p3-10, a sequence previously

TABLE 1. Distribution of human chromosomes in rodent-human somatic cell hybrids segregating sequences homologous to the DNA repair gene[a]

Interspecific hybrid	Hybridization with DNA repair gene probe:		Presence of human chromosome:[b]																						
	hD1UV	hD2UV	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X
A23-1A	+	+	+	−	+	−	−	−	+	+	−	+	+	+	−	+	+	+	−	−	+	+	−	−	+
A48-1G[c]	−	−	−	−	−	−	+	−	+	−	−	+	+	−	−	−	−	−	−	+	−	+	+	−	+
A48-5Bβ[c]	−	−	−	−	+	−	−	−	+	−	−	−	+	−	−	−	−	−	−	+	−	+	+	−	+
A48-5Ea	−	−	−	−	+	+	+	−	+	‡	−	+	*[d]	+	−	+	−	+	−	+	−	−	+	−	+
A54-8a	+	+	+	+	+	−	+	−	−	−	−	*[d]	+	−	−	−	+	−	−	+	+	−	+	−	+
AHA-11a	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+
L23-1B	−	−	+	−	+	−	+	+	+	−	−	−	−	+	‡	+	−	+	+	−	−	+	+	−	−
L23-4B-5C-2	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	+	+	−	+	‡	−	−
L23-4B-5C-12	−	−	−	−	−	−	+	−	−	−	−	−	+	−	−	−	−	−	+	+	−	−	+	−	−
LT23-1C1	+ (w)[e]	+ (w)[e]	−	−	−	−	−	−	−	−	−	−	−	−	+	+	−	−	+	−	‡	+	−	−	+
LT23-4B	+	+	−	+	−	+	+	+	+	−	−	+	+	−	+	+	−	−	+	+	+	+	+	−	‡
LT23-4C	+	+	+	+	−	+	+	+	+	−	−	+	+	−	+	+	+	−	+	+	+	+	+	−	+
W4-3A[c]	+	+	−	−	−	+	−	−	+	−	−	−	−	−	−	+	−	−	−	−	+	−	−	−	+
W19-3B	+	+	−	‡	+	−	−	−	−	−	+	−	−	−	+	+	−	+	−	−	+	−	+	+	+
W19-4B	−	−	−	−	−	‡	−	+	−	−	+	−	−	+	−	+	+	−	−	−	−	−	+	−	+
W44-14A	+	+	+	−	+	+	−	−	−	−	+	−	+	‡	−	+	−	+	−	−	+	−	−	−	+
W48-12B	+	+	−	−	+	+	−	−	−	−	−	−	−	+	−	‡	−	−	−	−	+	−	−	+	+
No. of informative clones			17	17	17	17	17	17	17	17	17	16	16	17	17	17	17	17	17	17	17	17	17	17	17
No. of discordant clones			6	5	7	6	11	9	9	9	8	7	7	9	6	4	7	8	9	11	0	9	12	7	5
% Discordant clones			35	29	41	35	65	53	53	53	47	44	41	53	35	24	41	47	53	65	0	53	71	41	29

[a] Construction of these hybrids has been described previously (6,33).

[b] Symbols: +, presence of that chromosome in at least 30% of the metaphase cells examined; ‡, presence of that chromosome in 10 to 30% of the metaphase cells examined. For determination of discordancy, a ‡ was scored as positive.

[c] For hybrids A48-1G (X/11 translocation), A48-5Bβ (X/11 translocation), and W4-3A (X/14 translocation), both products of the translocation were present in the hybrid, and each chromosome was scored as +.

[d] An asterisk indicates that the hybrid was involved in a rearrangement in the human parental cells and in the hybrid and was not scored.

[e] Weak hybridization signal after genomic blot analysis.

mapped to chromosome 19 (S. Naylor and M. Skolnick, personal communication). This synteny between hD1UV and hD2UV was as expected from the data presented in Fig. 3 and 4, indicating that these two fragments hybridized to the same *Hin*dIII fragments and hence are within ca. 24 kbp of each other in genomic DNA. All other human chromosomes were excluded by this analysis; the X chromosome and all autosomes other than chromosome 19 were discordant in at least four of the hybrids examined (Table 1). The Y chromosome was excluded because human female parental cells were used for most of the hybrids and because DNA from a mouse-human hybrid containing a human Y as its only human chromosome (33) did not hybridize with the two probes (data not shown). The high percentage of discordancy exhibited by all chromosomes other than chromosome 19 thus permits assignment of this human DNA repair gene to chromosome 19.

DISCUSSION

In an earlier communication (22), we demonstrated the DNA-mediated transfer of a repair-proficient phenotype from repair-proficient human cells to the repair-deficient UV-20 cell line. We showed that such transfer was associated with the presence of specific human DNA sequences in primary and secondary transformants. Earlier studies (28) had shown the UV-20 cell line to be deficient in the incision step of DNA repair. The alkaline elution studies reported here confirm the lack of incision in the UV-20 line and demonstrate that the phenotypic expression of UV light and MM-C resistance in primary and secondary DNA transformants is associated with return of the incision capability. Differences in alkaline elution patterns in the various primary and secondary transformants may reflect differences in the level of gene expression in independent cell clones after DNA transfer. These observations provide further evidence that the human gene transferred into UV-20 encodes either the regulatory or structural information necessary to restore DNA repair proficiency to this mutant hamster cell line.

We also describe here the isolation of two genomic clones containing unique sequences that are closely associated with this human DNA repair gene. Subclones containing these unique sequences identify two distinct *Eco*RI restriction fragments in DNA isolated from human cells as well as from repair-proficient CHO transformants. However, the two probes both identified the same 24-kbp *Hin*dIII fragment in human cells as well as in three of the four transformants, indicating that these two sequences are very closely linked (within 24 kbp) in human cellular DNA. Both probes also hybridized to the same 17-kbp *Hin*dIII fragment in the fourth transformant, X25-37. A smaller, 13-kbp *Hin*dIII fragment was also identified by probe hD1UV in this cell line. The fact that the corresponding primary transformant from which this line was generated, X25, demonstrated a single 24-kbp fragment, suggests the rearrangement of at least 7 kbp of transfected DNA during generation of the secondary transformant.

The fact that independently derived secondary DNA transformants contain the same human sequences suggests that both cell lines were rendered DNA repair proficient as a result of transfection with the same human gene. This result

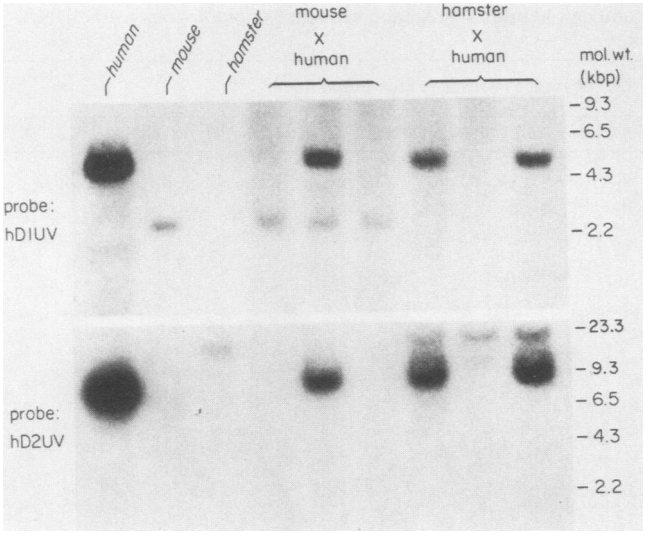

FIG. 5. Gel electrophoresis and blot hybridization analysis with the hD1UV and hD2UV probes. High-molecular-weight DNA was digested to completion with the restriction enzyme *Eco*RI and subjected to electrophoresis in 0.8% agarose. After treatment with alkaline and neutralization buffers, the DNA was transferred overnight to a nitrocellulose filter (24). The filters were then baked, prehybridized, and hybridized as described previously (22). Hybridization was carried out with ^{32}P-labeled hD1UV or hD2UV. The final wash for filters hybridized to hD1UV was 0.1× SSC plus 0.1% SDS at 65°C for 1 h, whereas those hybridized to hD2UV were washed in the same conditions except that the temperature was lowered to 50°C. The three leftmost lanes contain DNA from normal human, mouse, and hamster cells, respectively. The middle three lanes contain DNA from hybrids formed from fusion between human and mouse cells. The three rightmost lanes contain DNA from hybrids formed from fusion between human and Chinese hamster cells. The fifth, seventh, and ninth lanes from the left are positive for human chromosome 19, whereas the fourth, sixth, and eighth lanes from the left are negative for human chromosome 19 as determined by karyotype analysis and genomic blot analysis with DNA probe p3-10, independently assigned to chromosome 19 (S. Naylor and M. Skolnick, personal communication).

argues against the possibility that different human genes were able to complement the single genetic defect in the repair-deficient hamster mutant UV-20.

Neither the 4.6- nor the 8.6-kbp *Eco*RI fragments detected by phD1UV or phD2UV are present in MM-C-resistant DNA transformants of another hamster cell mutant, UV-41 (unpublished observations). This cell mutant is also UV and MM-C sensitive but falls into a different complementation group than UV-20 (29). Thus, the gene transferred into the repair-proficient UV-20 transformant does not appear to be nonspecifically transferred into CHO cells after DNA-mediated gene transfer, nor is transfer of the human gene that complements the defect in UV-20 necessary to complement the mutation in UV-41.

Using the unique DNA probes associated with the DNA repair gene and a series of rodent-human somatic cell hybrids that segregate human chromosomes, we localized this gene to chromosome 19. Another approach to mapping genes correlates the loss from hybrids formed between a mutant rodent cell and a normal human cell of a particular phenotype or gene product with the segregation of specific human chromosomes. Using this approach, L. H. Thompson (personal communication) has demonstrated in hybrids formed between the UV-20 hamster mutant cell line and a human cell an association between resistance to killing by DNA-damaging agents and the presence of human chromosome 19, thus confirming the present map assignment. Segregation analysis has also allowed the assignment of a presumably different human DNA repair gene that complements the defect in UV-light-sensitive mouse cells to human chromosome 13 (15).

Recently, Westerveld et al. (32) have independently described the molecular cloning of a human gene capable of complementing the repair defect in a CHO mutant cell line 43-3B that falls into the same complementation group (group 2) as UV-20. We have no biological or molecular evidence that the DNA segments obtained after transfection into UV-20 and described here are identical or related to those that complement the defect in 43-3B. It will be of interest to determine whether these sequences are structurally related and syntenic.

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ADDENDUM IN PROOF

Since submission of this manuscript, we have scored an additional eight independent hybrid clones for the presence of the human repair gene DNA. Chromosome 19 is concordant in all 25 hybrids examined. All other human chromosomes were discordant in at least seven hybrids.

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