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Molecular Cloning of the Human DNA Excision Repair Gene *ERCC-6*

C. TROELSTRA,[1] H. ODIJK,[1] J. DE WIT,[1] A. WESTERVELD,[2] L. H. THOMPSON,[3] D. BOOTSMA,[1] AND J. H. J. HOEIJMAKERS[1]*

Department of Cell Biology and Genetics, Erasmus University Rotterdam, P.O. Box 1738, 3000 DR Rotterdam,[1] and Institute of Antropogenetics, Academic Medical Centre, 1105 AZ Amsterdam,[2] The Netherlands, and Biomedical Sciences Division, Lawrence Livermore National Laboratory, Livermore, California 94550[3]

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The UV-sensitive, nucleotide excision repair-deficient Chinese hamster mutant cell line UV61 was used to identify and clone a correcting human gene, *ERCC-6*. UV61, belonging to rodent complementation group 6, is only moderately UV sensitive in comparison with mutant lines in groups 1 to 5. It harbors a deficiency in the repair of UV-induced cyclobutane pyrimidine dimers but permits apparently normal repair of (6–4) photoproducts. Genomic (HeLa) DNA transfections of UV61 resulted, with a very low efficiency, in six primary and four secondary UV-resistant transformants having regained wild-type UV survival. Southern blot analysis revealed that five primary and only one secondary transformant retained human sequences. The latter line was used to clone the entire 115-kb human insert. Coinheritance analysis demonstrated that five of the other transformants harbored a 100-kb segment of the cloned human insert. Since it is extremely unlikely that six transformants all retain the same stretch of human DNA by coincidence, we conclude that the *ERCC-6* gene resides within this region and probably covers most of it. The large size of the gene explains the extremely low transfection frequency and makes the gene one of the largest cloned by genomic DNA transfection. Four transformants did not retain the correcting *ERCC-6* gene and presumably have reverted to the UV-resistant phenotype. One of these appeared to have amplified an endogenous, mutated CHO *ERCC-6* allele, indicating that the UV61 mutation is leaky and can be overcome by gene amplification.

To counteract the deleterious consequences of DNA injury, an intricate network of DNA repair systems has evolved (for a review of DNA repair in general, see reference 10). One of the major, universal repair processes is the nucleotide excision repair pathway, which is molecularly best defined in *Escherichia coli* (10, 13, 21, 24). This system removes a broad category of DNA lesions having a very dissimilar structure, such as UV-induced cyclobutane pyrimidine dimers and (6–4) photoproducts, as well as bulky chemical adducts and DNA cross-links. To elucidate the molecular mechanism of the mammalian excision repair pathway, several nucleotide excision repair-deficient mutants are available. Cell lines from patients with the human hereditary disease xeroderma pigmentosum (XP) are one example (for a review, see reference 5). Extensive genetic heterogeneity has been demonstrated among XP patients. Cell fusion experiments have identified at least seven excision-deficient XP complementation groups (2, 9, 17). In addition to XP cells, a set of UV-sensitive, nucleotide excision repair-deficient rodent (mainly Chinese hamster) mutant cell lines have been generated in the laboratory. Eight genetic complementation groups have been described (32, 37, 45; for recent reviews, see references 3, 6, and 30). The cell fusion experiments performed between some of the CHO mutants and XP fibroblasts did not reveal any overlap between these two classes of repair mutants (28, 35). This suggests that a considerable biochemical complexity underlies the nucleotide excision repair process in mammalian cells, for which the molecular mechanism is largely unknown.

CHO mutants of various complementation groups have been successfully used to isolate the correcting human *ERCC* (excision repair cross-complementing rodent repair deficiency) genes, *ERCC-1*, *ERCC-2*, and *ERCC-3*, complementing the excision defects of mutants of groups 1 (44), 2 (41), and 3 (43), respectively. The first five groups display a similar, high degree of UV sensitivity and are deficient in the incision step of the excision repair process (31–33). In contrast, the two mutants making up group 6, CHO mutant UV61 (3) and mouse lymphoma mutant US46 (27, 37), are only moderately sensitive to UV exposure, and UV61 is partially deficient in the incision of damaged DNA (27, 36). Furthermore, mutant UV61 is remarkable in harboring a specific deficiency in the repair of cyclobutane pyrimidine dimers and bulky chemical adducts, but permitting apparently normal repair of (6–4) photoproducts (34). This phenotype suggests that the gene product affected in this mutant is involved in the repair of cyclobutane dimers and bulky adducts but not in the repair of (6–4) lesions. Alternatively, it is possible that the mutation in the UV61 protein alters the affinity of the repair complex for different types of damage. The alteration would then be such that the rate of removal of cyclobutane dimers and bulky adducts was notably diminished, whereas repair of (6–4) photoproducts was not significantly affected. In both hypotheses the UV61 (*ERCC-6*) polypeptide plays an important role in repair of specific types of DNA injury.

To obtain insight into the function of this particular protein in the DNA excision repair process, we have cloned and

* Corresponding author.

partially characterized the human gene correcting the mutation in UV61.

MATERIALS AND METHODS

Cell culture. The CHO mutant cell line UV61 used in this study was isolated by Busch et al. (3) and assigned to complementation group 6 (36). Its repair-competent, parental cell line is the CHO line AA8 (3). All cells were grown in 1:1 F10-Dulbecco minimal essential medium supplemented with antibiotics and 8% fetal calf serum.

DNA transfection. High-molecular-size HeLa DNA (ca. 200 to 300 kb), isolated as described previously (16), was either partially cleaved to an average fragment size of 50 to 60 kb and ligated to a dominant marker molecule before transfection or directly used for cotransfection with a dominant marker. For transfection of ligated DNA, HeLa DNA partially digested with *Pst*I or *Mbo*I was ligated to *Pst*I-digested pSV3gptH (44) or *Bam*HI-digested pMCS (14), respectively, in a molar ratio of 1:3, by using T4 DNA ligase (GIBCO/Bethesda Research Laboratories, Gaithersburg, Md.). Ligations were tested on agarose gels.

For transfections, 5×10^5 UV61 cells were seeded in 100-mm Petri dishes 1 day before DNA transfection. The high-molecular-size DNA, together with one of the two dominant marker molecules mentioned, was transfected into UV61 cells by a modification of the calcium phosphate precipitation technique of Graham and Van der Eb (12). A 20-μg sample of DNA (20 μg of ligated DNA or 15 μg of HeLa DNA plus 5 μg of circular pSV3gptH DNA) was applied to each petri dish. Following a 16-h exposure to the DNA, cells were treated for 30 min with 10% dimethyl sulfoxide and grown for 24 h on nonselective medium to allow expression of the transfected marker. Thereafter, the medium was changed to selective medium. When selecting for expression of the pMCS dominant marker, G418 (GIBCO Ltd., Paisley, Scotland) was added to the F10-Dulbecco minimal essential medium described above (concentration, 750 μg/ml during the first 3 days of selection and 800 μg/ml thereafter). Selection for pSV3gptH was done by adding aminopterin (0.2 μg/ml), thymidine (5 μg/ml), xanthine (10 μg/ml), hypoxanthine (15 μg/ml), mycophenolic acid (20 μg/ml), and deoxycytidine (2.3 μg/ml) to F10-Dulbecco minimal essential medium. The selection medium was refreshed every 3 to 4 days.

The same procedure was followed for secondary transfections. DNA of a primary transformant (PT) was used with or without addition of dominant marker pRSVneo (11) (20 μg of PT DNA plus 2 μg of pRSVneo in cotransfection).

UV selection and UV survival. After the appearance of G418- or mycophenolic acid-resistant colonies (within 10 to 12 days after transfection), the cells were trypsinized. UV selection was started 16 to 20 h after trypsinization. Cells were exposed three times to 8.4 J/m^2 (Philips TUV low-pressure mercury tube, 15 W, 0.6 J/m^2/s; predominantly 254 nm), with 24-h intervals. UV-resistant colonies were isolated, grown in selective medium, and characterized with respect to UV resistance and human DNA content by Southern blot hybridization.

For determination of UV sensitivity, cells were plated at densities varying from 2×10^2 to 2×10^4 cells per 60-mm petri dish, depending on the cell line and the UV dose. Cells were irradiated 1 day after plating. A series of dishes was irradiated for each cell line, each dish receiving a single dose (three dishes per UV dose). Clones were counted 6 to 7 days after UV irradiation, and relative cloning efficiencies were determined.

TABLE 1. Repair characteristics of mutant UV61

Characteristic	Result for UV61[a]
Sensitivity to DNA-damaging agents[b]	
UV	ca. 2.7×[c]
7-BrMeBA[d]	ca. 3×[c]
Rate of incision	Normal[e]
Removal of (6–4) photoproducts	Normal
Removal of dimers	Deficient
Removal of bulky adducts	Deficient
UDS[f]	ca. 70%[e]
UV-induced mutagenesis	ca. 5×[c]
Postreplication recovery	Normal

[a] Data from reference 33.
[b] At D_{10}.
[c] Compared with that of the wild type.
[d] 7-BrMeBA, 7-Bromomethylbenz[*a*]anthracene.
[e] Measured first at 2 h after UV irradiation.
[f] Own unpublished results.

Southern blot hybridization. Digestion of DNA with restriction enzymes, gel electrophoresis, and hybridizations were performed by using routine procedures as described by Maniatis et al. (19). Southern blotting to Zeta probe blotting membranes (Bio-Rad, Richmond, Calif.) was performed by using alkaline transfer (22), as described by the manufacturer.

Library construction and screening. High-molecular-size DNA was partially digested with *Mbo*I and size fractionated on a sucrose gradient. The 15- to 25-kb fraction was ligated to the *Bam*HI sites of lambda EMBL-3 phage arms (Stratagene, La Jolla, Calif.), packaged as described previously (25), and then used to infect *E. coli* LE392 cells. A total of 3×10^6 phage were plated, and replica filters were prepared as described previously (19).

The filters were hybridized with radioactively labeled human Cot-1 DNA. Hybridizing plaques were plated again for a second round of screening. Single plaques were grown in liquid culture, and DNA was purified as described previously (19).

Overlapping phages were identified by using restriction enzyme site mapping and the restriction fingerprinting technique as described by Coulson et al. (7). Enzymes used in the latter procedure were *Sau*3A and *Hin*dIII.

RESULTS

Generation of repair-proficient PTs. The main characteristics of mutant UV61 are summarized in Table 1. In view of the moderate UV sensitivity of UV61 cells, it was necessary to develop an optimal selection protocol for repair-proficient transformants. In reconstruction experiments wild-type and UV61 cells were mixed in various ratios and different selection protocols were tested. The deduced UV selection procedure is described in Materials and Methods.

Two strategies were followed simultaneously to generate PTs. One approach, transfection of ligated DNA (partially digested HeLa DNA ligated in vitro to dominant marker molecules), increases the chance that a dominant marker copy is near the repair gene. This permits secondary transfection of the gene linked to a dominant marker copy and facilitates gene identification and cloning. The cotransfection, on the other hand, is more efficient for large genes.

Transfection of ligated DNA to more than 3.6×10^8 cells resulted in two PTs that survived the UV selection (PT-3 and PT-4). Cotransfection of high-molecular-size HeLa DNA

TABLE 2. Transfection efficiencies of UV-resistant PTs and STs of UV61

Transfection	Approach[a]	No. of UV[r] transformants[b]	Transfection efficiency[c]
Primary	Ligation	2 (0)	1:150,000
Primary	Cotransfection	4 (4)	1:100,000 (1:100,000)
Secondary	Linking	0 (0)	
Secondary	Cotransfection	4 (2)	1:20,000 (1:40,000)

[a] For a detailed description of procedures, see Materials and Methods.
[b] Number of UV-resistant, dominant marker-containing transformants isolated; the number after subtraction of revertants is given in parentheses.
[c] Transfection efficiencies given as UV-resistant transformants per dominant marker-expressing transformant. Efficiencies after correction for the presence of revertants are given in parentheses.

and pSV3gptH yielded four PTs (PT-1, PT-2, PT-5, and PT-6) in experiments involving 6 × 10^8 UV61 cells. The transfection frequencies (i.e., number of UV-surviving transformants per dominant marker-containing transformants) were extremely low (Table 2).

Characterization of PTs. To assess the degree of repair proficiency of the transformants, we determined their UV survival. PT-1 had regained wild-type (AA8) UV resistance, and the other PTs were in the wild-type range too (Fig. 1).

The PTs were analyzed for the presence of human sequences by using human Cot-1 DNA or a cloned human Alu repeat (8) as probes. As expected for PTs, Southern blot analysis (Fig. 2) revealed the presence of considerable amounts of human DNA in PT-1, PT-2, PT-3, PT-5, and PT-6 (results for PT-5 and PT-6 not shown). Surprisingly, PT-4 did not contain any detectable human sequences. This could mean that PT-4 does not contain a human gene, but is in fact a dominant marker-containing revertant. In view of the large scale of the genomic DNA transfections, this possibility cannot be ruled out.

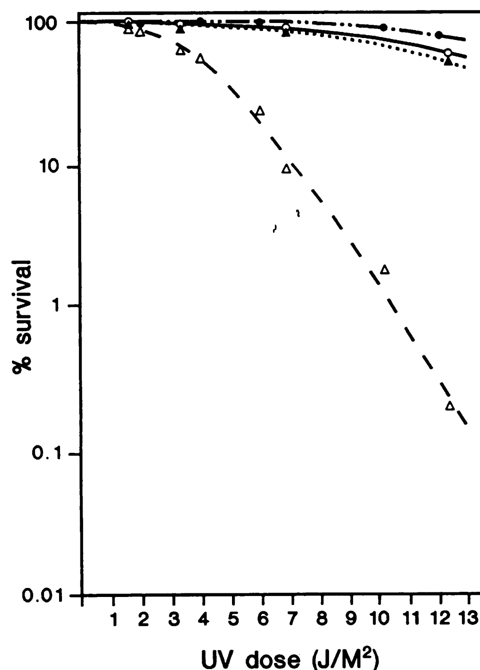

FIG. 1. UV survival curves of wild-type cell line AA8 (○), mutant UV61 (△), and UV61 transformants PT-1 (●) and ST-1 (▲).

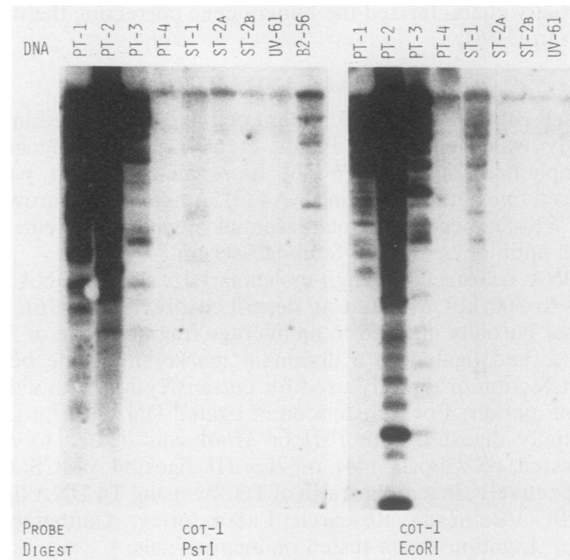

FIG. 2. Southern blot analysis of *Pst*I- (left) or *Eco*RI (right)-digested DNA (20 μg) from mutant UV61 and from UV61 PTs and STs after hybridization with ^{32}P-labeled human Cot-1 DNA as the probe. B2-56 (lane 9, left panel) is a secondary transformant of CHO mutant 27-1, containing the human *ERCC-3* gene (43), and served as a positive control.

In our selection protocol each petri dish with colonies of dominant marker-containing UV61 transformants (>100 cells per colony) was trypsinized prior to UV irradiation. When a transformant colony stably retains the correcting human *ERCC-6* gene, one expects that respreading the cells will result in many (>10 to 50) UV-resistant clones, assuming normal cloning efficiency and growth rate. For a revertant arising during dominant marker selection or, more likely, during UV selection, this number is expected to be much smaller. Table 3 summarizes our findings with respect to the number of UV-resistant clones after trypsinization and UV irradiation. It is striking that PT-4 falls in the second class (i.e., only one or a few UV-surviving colonies). This further suggests that PT-4 is a UV-resistant revertant.

Generation of repair-proficient STs. In secondary transfection experiments with DNA of PTs without addition of extra dominant-marker molecules, no clones with combined MPA and UV resistance or G418 and UV resistance were isolated from a total of about 9 × 10^8 transfected UV61 cells. This

TABLE 3. Characteristics of UV-resistant transformants of UV61

Transformant	No. of UV[r] clones/dish	Presence of human DNA
PT-1	10–50	++
PT-2	<3	++++
PT-3	10–50	++
PT-4	<3	–[a]
PT-5	10–50	++
PT-6	10–50	++++
ST-1	10–50	+
ST-2a	<3	–
ST-2b	<3	–
ST-5	5–10	–

[a] The minus sign indicates that no human DNA is detectable on Southern blots with human repeat (Cot-1, Alu) as the probe.

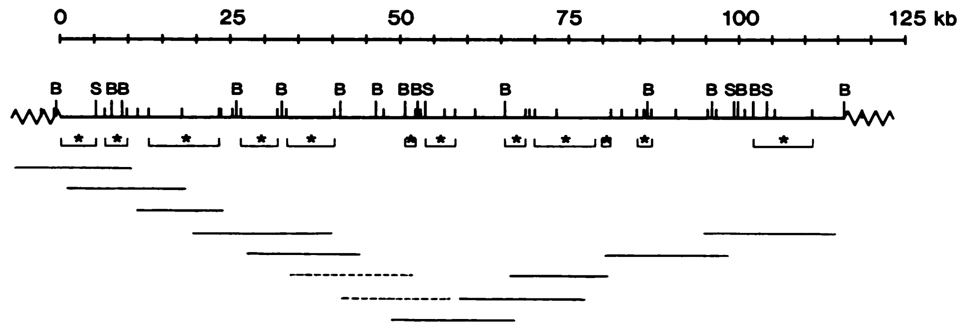

FIG. 3. Physical map of the human insert cloned from ST-1. Fragments of <300 bp have not been mapped. Indicated below the map are various overlapping lambda clones isolated from the library by screening with human Cot-1 DNA (for the sake of simplicity, only part of the lambda clones isolated and mapped are indicated). Symbols: *, repeat-containing fragments; ⊥, *Eco*RI; B, *Bam*HI; S, *Sal*I; —, human insert; ∿∿, flanking UV61 DNA; - - -, phages isolated after chromosomal walking.

result suggests that the distance between an integrated dominant-marker copy and the human gene conferring UV resistance, in the DNA of the PTs tested, is too large to be transferred simultaneously. Therefore, new dominant-marker molecules (pRSVneo) were added to PT DNAs for subsequent secondary transfection. This approach yielded four UV-resistant secondary transformants (STs) (after transfection of 4.0×10^8 UV61 cells), designated ST-1, ST-2a, ST-2b, and ST-5 (the numbers refer to the parental PTs; ST-2a and ST-2b were isolated from two independent dishes in one experiment with PT-2 as the donor DNA). Again, transfection efficiencies were very low (Table 2).

Characterization of STs. The UV survival of all STs was determined. ST-1 was corrected up to wild-type (AA8) and PT-1 UV resistance (Fig. 1). Similar results were obtained for the other STs.

Southern blot analysis with human Cot-1 DNA as the probe revealed the presence of human sequences only in the DNA of ST-1 (Fig. 2). For ST-2a and ST-2b (and ST-5 [not shown]) we were unable to detect any human repetitive DNA.

The absence of detectable human DNA in three of the four STs and in PT-4 can be explained in several ways. The transfected repair gene may contain two few repetitive sequences to be visualized by Southern blot hybridization with human Cot-1 DNA as the probe. Alternatively, the three empty STs (ST-2a, ST-2b, and ST-5) may simply be dominant marker-containing revertants. Two of these three STs (ST-2a and ST-2b) were from dishes with one or a few UV-resistant clones after trypsinization and UV selection (Table 3), as was PT-4 (see above). These results further suggest that ST-2a and ST-2b may be revertants.

Molecular cloning of the human insert. ST-1, which contains detectable human material, is from a dish with multiple UV-resistant clones after trypsinization and UV selection. Furthermore, ST-1 is derived from a PT (PT-1), also belonging to this category (Table 3). This information suggests that ST-1 is a bona fide transformant. Therefore, ST-1 DNA was chosen to construct a genomic library in lambda EMBL-3. The library (2.5×10^6 plaques; total complexity, 16 times the haploid genome) was screened with human Cot-1 DNA as the probe. Two hundred plaques containing human DNA sequences were identified; after a second round of screening pure cultures were grown from 50 plaques. DNA of these lambda recombinants was isolated and characterized by restriction mapping. The human and hamster parts were identified by hybridization with species-specific repeat probes.

Overlapping lambda clones were identified as described in Materials and Methods. A restriction site map of the inserts resulted in the identification of two nonoverlapping segments. Both segments harbored UV61 sequences at one end. Chromosomal walking was performed by using unique sequences from the human termini of both segments. Subsequently, phages were isolated that joined all contiguous parts, yielding a human insert with a total size of approximately 115 kb. A physical map is shown in Fig. 3. The phages isolated via chromosomal walking are indicated. These cover a part which contains very few repetitive sequences, explaining why these lambda recombinants had escaped detection in the first Cot-1 screening. In fact, the cloned region overall has relatively few repeats (Fig. 3).

Coinheritance analysis of independent transformants. A systematic search for coinheritance of the same human DNA segment in independent UV61 transformants was performed. This search was based on the notion that the human gene specifically conferring UV resistance to UV61 should be present in each genuine transformant. To this aim, 14 unique probes spread over the entire cloned human region were isolated and hybridized to genomic DNA digests of all UV-resistant PTs and STs obtained. The results are shown in Fig. 4A and summarized in Fig. 4B. Probe IV, for instance, does recognize the expected 6.4-kb *Eco*RI fragment in ST-1, from which the library was made. In addition, it hybridizes to a band of the same size in PT-1, PT-2, PT-5, PT-6, ST-5, and HeLa DNA. PT-3, PT-4, ST-2a, and ST-2b do not contain this human fragment. The same holds true for probes III through XIV, with the exception of probe VIII (Fig. 4). This indicates that the same contiguous region of at least 100 kb is present in 6 of 10 UV61 transformants.

Probes I and II are near the left UV61 border in ST-1 (Fig. 4). Probe I recognizes a rearranged *Eco*RI fragment in DNA of ST-1 compared with HeLa DNA, or, in the case of PT-2, PT-6, and ST-5, no fragment at all (Fig. 4). Probe II recognizes a 1.8-kb *Eco*RI fragment in ST-1 DNA, as in HeLa DNA; a rearranged fragment is detected in PT-2 (Fig. 4B). At the right-hand end the DNA of ST-1 is the first to diverge from the human genomic sequence and that of the other transformants (Fig. 4B). From these results, we deduce that the length of the human segment common to all transformants is approximately 100 kb.

The signals in the lanes with PT-1 and ST-1 are stronger than those in the other lanes (Fig. 4A and 5). Because both probe VII (Fig. 4A) and probe XV (Fig. 5) weakly cross-hybridize with hamster sequences, the endogenous bands (2.5 kb for probe VII in Fig. 4A [arrowhead]; the lower two

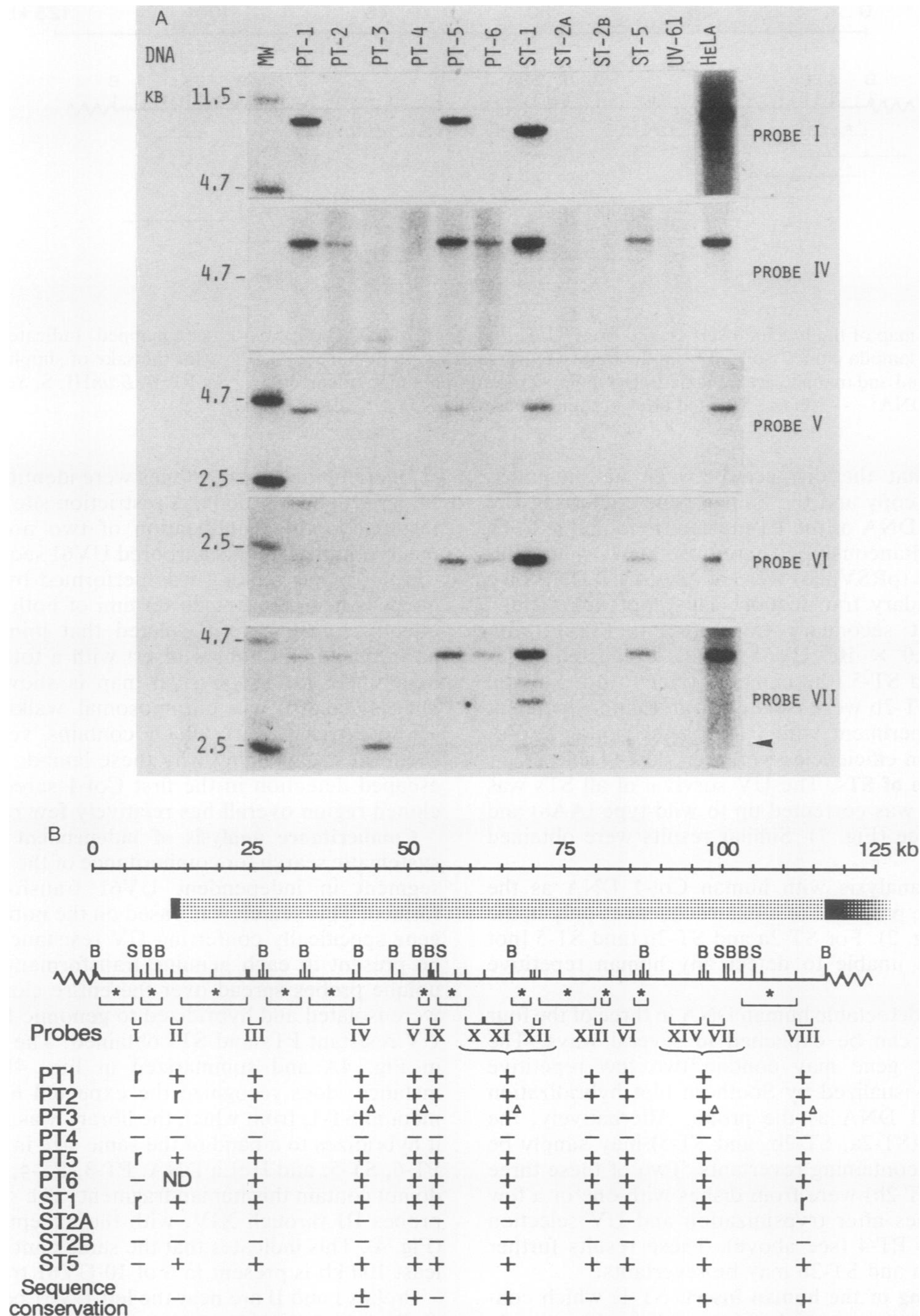

FIG. 4. Coinheritance analysis of all transformants for the retention of human sequences present in ST-1. (A) The same Southern blot of *Eco*RI-digested DNA from all primary and secondary UV61 transformants, mutant UV61, and HeLa cells was hybridized with different probes from the human region present in ST-1. Probes are indicated below the map in panel B. (B) Summary of coinheritance analysis. Symbols: +, same fragment hybridizing as in HeLa DNA; −, no fragment hybridizing; r, hybridizing fragment rearranged compared with HeLa DNA; $+^{\triangle}$, endogenous UV61 fragment hybridizing more strongly than in other transformants and UV61; ND, not determined. See the legend to Fig. 3 for explanations of other symbols.

hybridizing bands for probe XV in Fig. 5) serve as a convenient internal control for the amount of DNA loaded. Apparently, both PT-1 and ST-1 have a 5- to 10-fold amplification of the transfected, human region.

The amplification of this region in ST-1 is probably the reason that we were able to detect it by using human Cot-1 DNA as the probe on Southern blots of genomic DNA. ST-5, on the other hand, has only one copy (Fig. 4A and 5), which was not visible on our blots. Since this region contains relatively few repeats, the Southern blot hybridizations were clearly not sensitive enough to detect it as a single copy.

Presence of UV61 revertants. The coinheritance analysis revealed that PT-3, PT-4, ST-2a, and ST-2b all lack the entire human locus (Fig. 4B), which strongly suggests that these

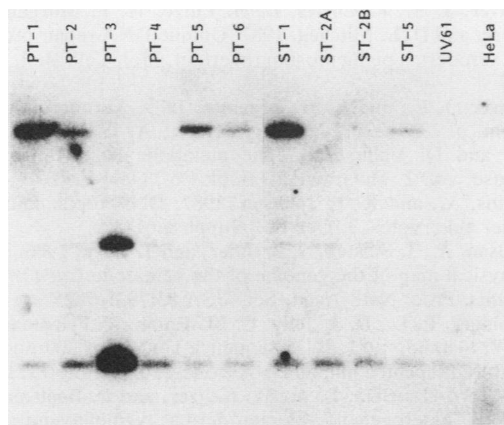

FIG. 5. Southern blot analysis of *Hin*dIII-digested DNA (20 μg) from HeLa cells, mutant UV61, and all PTs and STs with ^{32}P-labeled unique sequences from the region between probes VII and VIII on the map in Fig. 4B (probe XV).

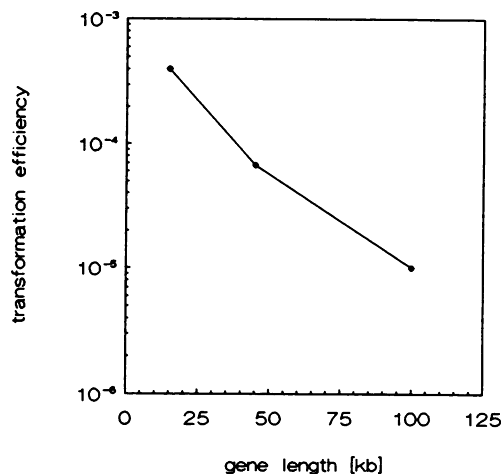

FIG. 6. Relationship between primary transfection efficiencies and gene size of *ERCC-1*, *ERCC-3*, and *ERCC-6*. Transfection efficiency is given as UV-resistant transformants per dominant marker-containing transformants. For *ERCC-1*, ligated genomic DNA (50 to 60 kb in size) was used.

are revertants. This agrees well with the fact that three of them (PT-4, ST-2a, and ST-2b) lack detectable human sequences (Fig. 2) and originated from a dish with a small number of UV-resistant clones after UV selection (Table 3). (One additional transformant, PT-2, also yielded a small number of UV-resistant clones, but nevertheless appeared to contain the common human segment (compare Table 3 and Fig. 4). This may be explained by initial instability of the transfected sequences, low cloning efficiency, and/or slow growth of this transformant.)

Various human genomic probes contained conserved sequences and cross-hybridized under normal stringency to Chinese hamster genomic sequences representing the hamster *ERCC-6* homolog (for example, probe VII in Fig. 4A [arrowhead] and probe XV in Fig. 5). The cross-hybridization to Chinese hamster DNA in the lane of PT-3 is considerably stronger than that in the other lanes, irrespective of the probe or digest. This indicates that PT-3 has amplified an endogenous, mutated allele at the CHO *ERCC-6* locus.

DISCUSSION

The evidence that we have indeed cloned *ERCC-6* is based on the coinheritance analysis of independent transformants for the retention of human sequences present in ST-1. This study showed that six transformants harbored the same human segment of 100 kb. The chance that this is due to coincidence is extremely small. When one assumes that every transformant integrates on the average 10^4 kb of exogenous sequences, as we have shown for several CHO lines (15), the likelihood that four independent PTs contain the same human fragment by coincidence is approximately 10^{-10}. Taking into account that two STs also possessed this region, this chance becomes vanishingly small. Two additional findings also strengthen the significance of the correlation between the cloned human integrate and the repair-proficient phenotype of the UV61 transformants: (i) the exceptionally large size of the common genomic fragment for transfection experiments and (ii) the observation that one of the transformants (PT-3) appeared to have amplified the CHO sequence cross-hybridizing to the human integrate. Although the gene is spread over too many lambda recombinants to permit direct transfection (of pooled clones) to UV61 cells, we conclude, on the basis of the arguments summarized above, that *ERCC-6* must be retained on the 100-kb common region. Hence this gene was cloned notwithstanding the moderate sensitivity of the mutant to UV, the extremely low transfection frequency, the low content of repetitive elements in the gene, and the occurrence of revertants.

The *ERCC-6* gene probably spans most of the cloned common area because during transfection double-strand breaks have been shown to occur on the average every 5 to 15 kb in various cell lines (15). Preliminary mapping of partial *ERCC-6* cDNA clones on the isolated genomic region confirms this proposition. A cDNA clone representing approximately 75% of the smallest *ERCC-6* mRNA already covers 80 kb of the cloned locus. As far as we know, *ERCC-6* is the largest gene to be cloned by using the genomic transfection approach. The frequent occurrence of double-strand breaks in transfected DNA molecules during the transformation process (15) strongly selects against large genes. This observation provides a reasonable explanation for the extremely low transfection efficiency encountered in the cloning of *ERCC-6* (Table 2), which is even more pronounced when we correct for the revertants among the UV61 transformants isolated. The transfection frequency (primary cotransfections) for *ERCC-6* is 1 in 100,000 dominant marker-containing UV61 transformants. This is 40-fold lower than the frequency of *ERCC-1* transformants (44) (gene size, 15 to 17 kb [39]) and 7-fold below that of the *ERCC-3* gene (approximate size, 45 kb [43]). These genes were isolated by us under comparable conditions. Hence a relationship exists between gene size and transfection frequency in the CHO lines used for these experiments (Fig. 6).

An interesting aspect concerns the identification of revertants. The occurrence of revertants has been described before in attempts to clone other repair genes (see, e.g., references 1, 4, 18, 23, and 26). For two simian virus 40-transformed fibroblasts isolated from XP patients and belonging to complementation group A, which have been used extensively as recipients for genomic DNA transfections, the recovery of partial and complete revertants has been reported by several groups (1, 23). One partial revertant has been characterized in detail and found to be proficient in (6-4) photoproduct excision, but affected in dimer

removal (4), a remarkable phenotype that resembles UV61 and a Chinese hamster mutant, VH-1, belonging to complementation group 2 (20, 46). The frequency of revertants seems to be enhanced when long-lasting UV selection protocols are used. This is one of the reasons why we have tried to minimize the number of UV exposures of UV61.

In none of the instances mentioned above has the nature of reversion been elucidated. The analysis of PT-3 in this study revealed that amplification of an endogenous, mutated Chinese hamster *ERCC-6* allele underlies the acquired UV resistance in this UV61 revertant. We have found recently that the amplification is also reflected at the mRNA level. These data suggest that the mutation in UV61 is leaky and that its effect can be overcome by increasing the copy number of the gene and mRNA, thereby raising the amount of (partly functional) gene product. This explanation would be compatible with a model in which the affinity of the *ERCC-6* protein for dimer lesions is lowered by the UV61 mutation and can be compensated for by larger quantities of the defective polypeptide. The molecular basis of the reversion in the other three UV61 revertants is unknown, but must involve other mechanisms than gene amplification.

ERCC-6 is the fourth of a set of human repair genes that have been cloned by using UV-sensitive, laboratory-induced CHO mutants. One of the most interesting features to emerge from the analysis of these genes is their high interspecies sequence conservation. The *ERCC-1* protein is homologous to the yeast RAD10 excision repair gene product and to parts of the *E. coli* UvrA and UvrC proteins (38, 40). The protein encoded by the *ERCC-2* gene, cloned by Weber et al. (41), possesses a high level of identity with the yeast RAD3 protein (42), which was shown by Sung et al. to specify a 5'-to-3' DNA helicase (29). Finally, the *ERCC-3* gene also is very strongly conserved, and a homologous yeast gene has been identified (M. H. M. Koken, G. Weeda, and J. H. J. Hoeijmakers, unpublished data). This gene was recently found to be involved in the human repair diseases XP and Cockayne's syndrome (G. Weeda, R. C. A. van Ham, W. Vermeulen, D. Bootsma, A. J. van der Eb, and J. H. J. Hoeijmakers, Cell, in press). Future analysis of *ERCC-6* should reveal the level of sequence conservation of this gene, its specific role in dimer repair, and whether it is implicated in one of the human repair disorders.

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