MOLECULAR AND CELLULAR BIOLOGY, June 1990, p. 2570–2581
0270-7306/90/062570-12$02.00/0

Vol. 10, No. 6

Molecular Cloning and Biological Characterization of the Human Excision Repair Gene *ERCC-3*

GEERT WEEDA,[1]* REINIER C. A. VAN HAM,[1] REMCO MASUREL,[1] ANDRIES WESTERVELD,[2]†
HANNY ODIJK,[2] JAN DE WIT,[2] DIRK BOOTSMA,[2] ALEX J. VAN DER EB,[1] AND JAN H. J. HOEIJMAKERS[2]

Laboratory for Molecular Carcinogenesis, Sylvius Laboratory, P.O. Box 9503, 2300 RA Leiden,[1] and Department of Cell Biology and Genetics, Erasmus University Rotterdam, 3000 DR Rotterdam,[2] The Netherlands

Received 1 September 1989/Accepted 3 January 1990

In this report we present the cloning, partial characterization, and preliminary studies of the biological activity of a human gene, designated *ERCC-3*, involved in early steps of the nucleotide excision repair pathway. The gene was cloned after genomic DNA transfection of human (HeLa) chromosomal DNA together with dominant marker pSV3gptH to the UV-sensitive, incision-defective Chinese hamster ovary (CHO) mutant 27-1. This mutant belongs to complementation group 3 of repair-deficient rodent mutants. After selection of UV-resistant primary and secondary 27-1 transformants, human sequences associated with the induced UV resistance were rescued in cosmids from the DNA of a secondary transformant by using a linked dominant marker copy and human repetitive DNA as probes. From coinheritance analysis of the *ERCC-3* region in independent transformants, we deduce that the gene has a size of 35 to 45 kilobases, of which one essential segment has so far been refractory to cloning. Conserved unique human sequences hybridizing to a 3.0-kilobase mRNA were used to isolate apparently full-length cDNA clones. Upon transfection to 27-1 cells, the *ERCC-3* cDNA, inserted in a mammalian expression vector, induced specific and (virtually) complete correction of the UV sensitivity and unscheduled DNA synthesis of mutants of complementation group 3 with very high efficiency. Mutant 27-1 is, unlike other mutants of complementation group 3, also very sensitive toward small alkylating agents. This unique property of the mutant is not corrected by introduction of the *ERCC-3* cDNA, indicating that it may be caused by an independent second mutation in another repair function. By hybridization to DNA of a human × rodent hybrid cell panel, the *ERCC-3* gene was assigned to chromosome 2, in agreement with data based on cell fusion (L. H. Thompson, A. V. Carrano, K. Sato, E. P. Salazar, B. F. White, S. A. Stewart, J. L. Minkler, and M. J. Siciliano, Somat. Cell. Mol. Genet. 13:539–551, 1987).

To cope with spontaneously and environmentally induced structural DNA alterations, all living organisms have acquired a complex network of repair pathways. One of the most important and best-characterized repair systems is the nucleotide excision repair pathway, which is involved in the removal of a wide range of lesions caused by very dissimilar agents such as UV-induced pyrimidine dimers and 6-4 photoproducts, bulky chemical adducts, and cross-links (see reference 11 for an extensive review on DNA repair). In *Escherichia coli* this process constitutes part of the SOS response and includes the concerted action of at least six proteins, including *uvrA*, *urvB*, and *urvC* for which the individual roles have been elucidated in great detail (for recent reviews see references 16 and 31). However, the mechanism of this pathway in eucaryotic cells is poorly understood. In *Saccharomyces cerevisiae*, mutant analysis and gene cloning have revealed the existence of at least 10 genetic loci, collectively designated the *RAD3* epistasis group, that are implicated in excision repair (12, 17). The RAD3 protein has been studied most extensively and was shown to possess a single-stranded DNA-dependent ATPase and helicase activity (35, 36). In mammals, two classes of mutant excision repair cell lines can be discerned. To the first belong cells of patients suffering from the autosomal, recessive disorder xeroderma pigmentosum (XP), which is characterized by extreme sensitivity of the skin to sunlight (UV) exposure and predisposition to skin cancer (5, 25); the second class consists of laboratory-induced repair mutants derived from rodent cell lines (6, 18). Cell fusion studies thus far have identified eight complementation groups in both categories of mutants (3, 4, 7, 40, 44). Several types of complementation tests have been performed to establish a link between these two classes of mutants, but until now no overlap has been found (34, 42). This implies that at least 8 but perhaps 16 or more genes control nucleotide excision in mammalian cells.

To unravel the molecular intricacies of this system, we set out to clone the genes involved in excision repair by genomic DNA transfection into repair-deficient mutants, selection of repair-proficient transformants, and retrieval of the correcting repair gene. The rodent cell lines and particularly some of the Chinese hamster ovary (CHO) cell repair mutants appear to be far more suitable as recipients for DNA-mediated gene transfer than simian virus 40 (SV40)-transformed XP fibroblasts (20). The isolation of two human DNA excision repair genes, *ERCC-1* and *ERCC-2* (excision repair cross-complement rodent repair deficiency), that are able to correct the defect in CHO complementation groups 1 and 2, respectively, has been reported (49, 51). Recently, Tanaka and co-workers described the cloning of the XP-A correcting gene after extensive transfection experiments to an SV40-transformed XP-A fibroblast cell line (37). The *ERCC-1* gene was found to be the human equivalent of the *S. cerevisiae* *RAD10* repair gene (45). In addition, this gene harbors two domains present in the *E. coli* excision repair genes *uvr*A (21) and *uvr*C (9). In this paper we describe the cloning and partial characterization of a third human nucleotide excision

* Corresponding author.
† Present address: Academical Medical Center, Department of Anthropogenetics, University of Amsterdam, 1105 AZ Amsterdam, The Netherlands.

TABLE 1. UV-sensitive CHO mutants and corresponding complementation groups that were used for transfection experiments

Mutant	Complementation group	Parental line	Reference
43-3B	1	CHO-9	52
UV5	2	AA8	40
27-1	3	CHO-9	52
UV24	3	AA8	40
UV47	4	AA8	40
UV135	5	AA8	38
UV61	6	AA8	39
VB11	7	V79	55

repair gene, *ERCC-3* (GenBank accession no. M31899), that complements the repair defect in the CHO mutants belonging to complementation group 3.

MATERIALS AND METHODS

Cell lines and culture conditions. The UV-sensitive mutants used in this study are summarized in Table 1. Cells were cultured in F10-Dulbecco minimal essential medium (DMEM) supplemented with 5% fetal calf serum and 5% newborn calf serum (GIBCO Laboratories), 100 μg of streptomycin per ml, and 100 U of penicillin per ml. Transformed cell lines containing the *E. coli gpt* gene were selected and cultured in XGPT medium containing F10-DMEM as above with aminopterin (0.2 μg/ml), thymidine (5 μg/ml), xanthine (10 μg/ml), mycophenolic acid (MPA; 25 μg/ml; GIBCO), hypoxanthine (15 μg/ml), and deoxycytidine (2.3 μg/ml). The selection medium was replenished every 3 to 5 days.

Transfection. CHO mutant 27-1 cells were plated 1 day before transfection at 10^6 cells per 10-cm dish. Cells were transfected with human genomic DNA by using a modification of the calcium phosphate precipitation technique (15). High-molecular-weight DNA from HeLa cells isolated as described previously (24) with an estimated length of 100 to 300 kilobases (kb) was partially cleaved to an average length of 35 to 50 kb with restriction endonuclease *Pst*I. The restricted DNA was ligated to a twofold molar excess of *Pst*I-linearized dominant marker pSV3gptH (51). Then 20 μg of DNA (vector plus HeLa DNA) was applied to each petri dish. The cells were exposed to the DNA for 16 to 20 h, shocked in 5 ml of phosphate-buffered saline containing 10% dimethyl sulfoxide for 1 to 2 min, rinsed twice with 10 ml of phosphate-buffered saline, and incubated in 10 ml of nonselecting medium for 48 h before the addition of XGPT medium to allow time for integration and expression of the transfected sequences (28). Routinely, we obtained 100 to 250 MPA-resistant clones per μg of pSV3gptH. Alternatively, cotransfection was done with 20 μg of HeLa genomic DNA (100 to 300 kb) and 2 μg of the dominant marker pSV3gptH. Secondary transfection was performed as described above, with the exception that DNA of primary transformants was used as the donor DNA, in the presence or absence of additional pSV3gptH. In the case of secondary transformant ST1-1, no pSV3gptH was added to the DNA of primary transformant PT-1. When cosmid, phage, or plasmid DNA was transfected, 2 μg of DNA was cotransfected with 1 μg of pSV3gptH together with 17 μg of carrier salmon sperm DNA. Selection for repair-proficient transformants was done as follows. After the appearance of MPA-resistant colonies (usually at approximately 2 weeks), each dish was trypsinized and the cells were reseeded into two dishes and UV irradiated (three times at 4.5 J/m^2) at 1-day intervals.

Restriction endonuclease digestion and Southern blot hybridization. Restriction endonuclease digestions were carried out as recommended by the manufacturer (Pharmacia Fine Chemicals). Chromosomal DNA was electrophoresed overnight on a 0.8% agarose gel. After treatment with alkali, the DNA was transferred to GeneScreen-plus (Dupont, NEN Research Products) filters. The filters were baked for 2 h at 80°C and prehybridized at 65°C in 5× SSPE (1× SSPE is 0.15 M NaCl plus 0.01 M NaH_2PO_4 plus 1 mM EDTA), 5× Denhardt solution, 5% dextran sulfate, 1% sodium dodecyl sulfate, and 100 μg of denatured salmon sperm DNA per ml. Hybridization with ^{32}P-labeled, nick-translated probes was conducted in the same solution. The filters were washed for 30 min in 3× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–0.1% sodium dodecyl sulfate at 65°C and twice in 1× SSC and 0.3× SSC at 65°C. Kodak XAR-5 film was exposed to the filters with an intensifying screen at −70°C. To isolate DNA fragments containing unique nonrepetitive sequences, the fragments were subcloned in M13 vectors and subjected to restriction enzyme digestion and Southern blot hybridization analysis with nick-translated, ^{32}P-labeled total human DNA as a probe. Inserted fragments that did not hybridize were selected. Subfragments still containing small amounts of repetitive sequences were reassociated with a large excess of total genomic DNA to screen out repetitive elements, essentially as described by Sealey et al. (33).

Construction and screening of genomic libraries. To construct a cosmid library of ST1-1, a partial size-fractionated *Mbo*I digest of ST1-1 DNA with an average fragment size of 40 to 50 kb was ligated to *Bam*HI-cleaved C2RB cosmid vector arms, packaged in vitro, and transduced into the bacterial host 1046, essentially as described earlier (2). This library was screened by colony filter hybridization (26) with total ^{32}P-labeled, nick-translated SV40 DNA or other genomic probes. A human genomic lambda library inserted in the EMBL-3 replacement vector (13) was kindly provided by G. Grosveld (Rotterdam). For isolation of subclones of the *ERCC-3* locus, genomic DNA of the secondary transformant ST1-1 was digested with *Eco*RI, size fractionated on a preparative agarose gel, and ligated to *Eco*RI-cleaved lambda pDJ14 arms (obtained from P. de Jong, Livermore). The ligated DNA was packaged in vitro and transduced into the bacterial host LE392. This library was screened by colony filter hybridization with human *cot-1* DNA as a probe. Filters were washed with 1× SSC–0.1% sodium dodecyl sulfate at 65°C and exposed to X-ray film as described above. For construction of subclones containing single-copy sequences, restriction fragments were isolated from low-melting-point agarose gels and ligated into convenient sites of plasmid pUC18 or cloned into phage vector M13mp18 or M13mp19. Bacteriophage and plasmid DNAs were purified by CsCl density-gradient centrifugation. All other DNA manipulations followed established protocols (26, 27).

Isolation of cDNA clones. A cDNA library made from a human SV40-transformed fibroblast was generously provided by H. Okayama (30). In this cDNA expression library, the cloned cDNA is under the control of the SV40 early promoter, which allows isolated cDNA clones to be used directly in transfection experiments with mammalian cells. The library was screened by colony filter hybridization with a 0.5-kb ^{32}P-labeled, nick-translated *Hha*I fragment derived from the *ERCC-3* gene.

Unscheduled DNA synthesis (UDS). Two days after cells were seeded in medium without MPA, they were exposed to

TABLE 2. Phenotype of CHO-9 mutant 27-1 (complementation group 3) compared with the wild type

Characteristic	Value for 27-1 relative to wild type[a]
Sensitivity to damaging agents[b]	
UV	6- to 7-fold
Mitomycin C	2-fold
X-rays, bleomycin	Same as wild type
Alkylating agents	
Ethylnitrosourea	5- to 6-fold[c]
Ethyl methanesulfonate	6- to 7-fold[c]
MMS	7- to 8-fold[c]
Rate of incision	<10%
Dimer removal	Deficient
UDS	5- to 20%
UV-induced mutagenesis	5- to 12-fold

[a] Data from reference 54.
[b] At D10, the dose required to reduce survival to 10%.
[c] Not a characteristic feature of complementation group 3 mutants.

UV light (16 J/m^2) and incubated in thymidine-free F10 medium supplemented with [^{3}H]thymidine (10 μCi/ml; specific activity, 50 Ci/mmol) and 5% dialyzed fetal calf serum. After fixation with Bouin solution, the preparations were processed for autoradiography (Kodak AR10 stripping film [48]), exposed for 1 week at 4°C, developed, and stained with Giemsa solution. For each preparation the number of grains per fixed square of 25 to 50 nuclei was counted.

RNA isolation and Northern (RNA) blot analysis. Total RNA was isolated by the LiCl-urea method, essentially as described by Auffray and Rougeon (1). The poly(A)$^+$ RNA fraction was purified by two passages over oligo(dT)-cellulose. Agarose gel electrophoresis, transfer onto nitrocellulose, and hybridization were performed as described by Schrier et al. (32).

Survival experiments. For UV survival experiments, exponentially growing cultures were trypsinized, and 10^2 to 10^4 cells were plated onto 6-cm dishes and left to attach for about 12 h. Then cells were rinsed with phosphate-buffered saline and exposed to UV light with a 254-nm low-pressure mercury, germicidal Philips TUV lamp with a fluence rate of 0.5 J/m^2. After cultivation in nonselective medium for 7 days, clones were fixed and stained with Coomassie brilliant blue. For methyl methanesulfonate (MMS) survival experiments, MMS was dissolved in phosphate-buffered saline and added to 5×10^6 cells in a volume of 10 ml. The medium was supplemented with 15% newborn calf serum, and the tubes were placed in a rotary shaker at 37°C for 1 h. After treatment, cells were washed three times with normal medium and plated onto 9-cm dishes.

Chromosomal localization. Southern blot analysis was done on DNA from a panel of 17 human × rodent (mouse or Chinese hamster) somatic cells hybrids. Three different Chinese hamster cell lines, designated A3, E36, and CHO, and the mouse cell line, Pg19, were used for the construction of the hybrids as previously described (8, 14).

RESULTS

Isolation of repair-proficient primary transformants. The phenotype of CHO-9 mutant 27-1 (generously donated by R. D. Wood, Imperial Cancer Research Fund, London) is summarized in Table 2. This mutant, like other members of complementation group 3, is very sensitive to UV irradiation, only slightly sensitive to cross-linking agents such as mitomycin C, and displays wild-type resistance to X rays and bleomycin. The rates of incision, dimer removal, and UDS indicate an impairment in early steps of the excision repair pathway. However, unlike other representatives of complementation group 3, mutant 27-1 was also very sensitive to the cytotoxic effect of alkylating agents. Since this may be due to an independent second mutation (see below), we decided to use UV as the sole agent for selecting transformants corrected with respect to the excision repair defect after DNA transfection. We used two strategies for isolation of the correcting human repair gene *ERCC-3*. In the first approach high-molecular-weight HeLa DNA was cleaved partially with *Pst*I to an average length of 35 to 50 kb, ligated to the *Pst*I-linearized, dominant selectable marker pSV3gptH, and transfected to 27-1 cells as described for the isolation of the *ERCC-1* gene by Westerveld et al. (51). The transfected cell populations were first subjected to selection for expression of the *gpt* marker (resistance to MPA-medium), followed by selection for UV resistance. More than 1.5×10^5 primary transformants were screened for enhanced UV survival, but only one UV-resistant clone (PT-3) was found. One of the possible explanations for this disappointing result was that the human repair gene is too large to be present as an intact gene in significant quantities in the partially digested DNA. Therefore, we decided as an alternative strategy to perform cotransfection of uncleaved, human chromosomal DNA (average size, 100 to 300 kb) with pSV3gptH instead of partial digestion and ligation in vitro. With this approach four UV-resistant primary transformants (designated PT1, PT2, PT4, and PT5) were isolated after screening 6.0×10^4 MPA-resistant clones (i.e., ~10-fold more than were found with the partially digested HeLa DNA). This transfection frequency is well above the reversion frequency of 27-1, which we determined in separate experiments to be 10^{-6} or less.

To assess the repair characteristics of the selected transformants, two repair endpoints were measured: UV survival and UDS. As shown by the UV survival curves (Fig. 1), PT-1 had regained nearly wild-type UV resistance. Similar results were obtained with other primary transformants (data not shown). Also with respect to UV-induced UDS, all transformants displayed repair levels similar to wild-type CHO cell repair levels (Table 3).

Southern blot analysis of genomic DNA digests of all primary transformants probed with human *cot-1* DNA indicated the presence of considerable amounts of human sequences, in addition to multiple copies of the dominant marker (see Fig. 3) (data not shown).

Secondary transformation. To examine whether the transfected repair gene happened to be close to a cotransfected dominant marker, we first tried linked secondary transfection with PT DNA without the addition of extra pSV3gptH. In this way, one UV-resistant secondary transformant (ST1-1) was obtained from a total of 150 MPA-resistant clones by using PT-1 DNA. This indicates physical linkage between the repair gene and at least one dominant marker copy in the DNA of this primary transformant. Furthermore, three secondary transformants (ST2-1, ST3-1, ST3-2) were generated after the addition of extra copies of the dominant marker to the DNA from PT-2 and PT-3, respectively. As for the primary transformants, UV survival and UV-induced UDS demonstrated that the secondary transformants had regained a repair-proficient phenotype (Fig. 1, Table 3; data shown for ST1-1 only).

To verify the presence of human sequences in the genome of secondary transformants, Southern blot analysis was carried out. *Eco*RI digests (Fig. 2) indicated that all second-

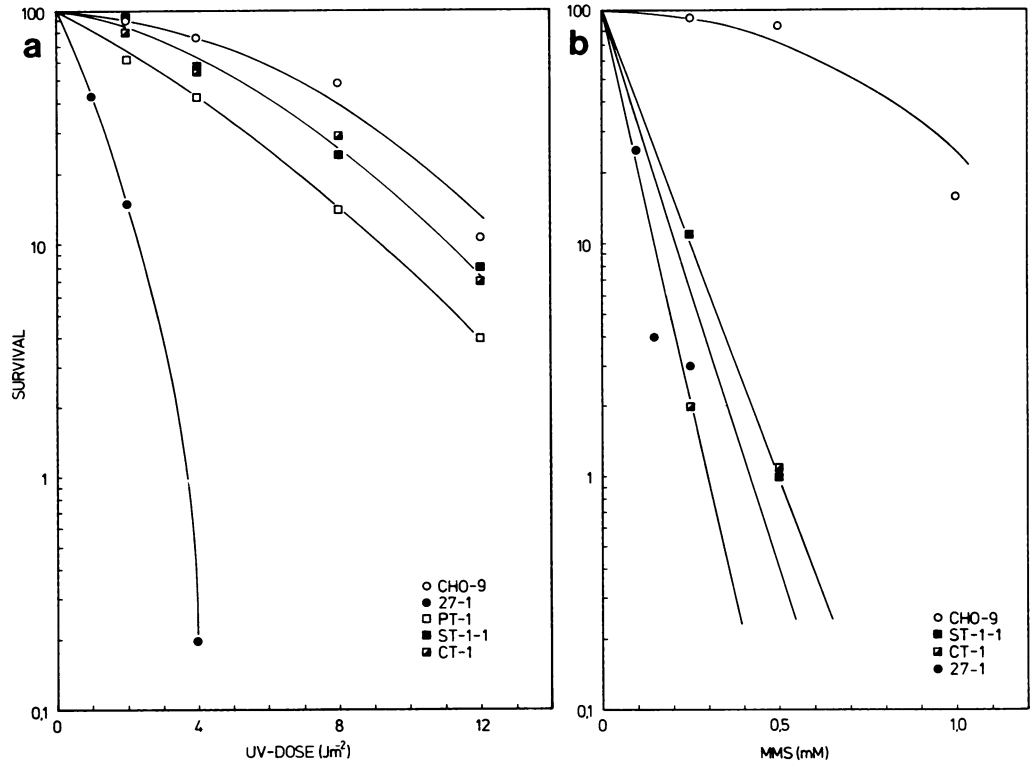

FIG. 1. UV (a) and MMS (b) survival of the wild-type parental cell line CHO-9, the UV-sensitive mutant 27-1, and the primary (PT-1), secondary (ST1-1), and pcD1 *ERCC-3* cDNA (CT-1) transformants. The curves show averages of two experiments. The MMS sensitivity of PT-1 is the same as that of ST1-1 and is not depicted separately.

ary transformants retained human DNA. More importantly, they appeared to share at least five human repeat-containing *Eco*RI fragments (indicated by arrows). Several other fragments were common between two of the three transformants (data for ST2-1 not shown). This indicates that independent transformants have "coinherited" the same human genomic segment. Probing the DNA of ST1-1 with pSV3gptH revealed that this secondary transformant harbored two *gpt* dominant marker copies (Fig. 3). Since these two copies had been transferred together with the human sequences responsible for the correction of the repair defect of 27-1, we expected that they would be located in the vicinity of the *ERCC-3* gene.

Cloning of genomic *ERCC-3* sequences. To identify cosmids from the DNA of ST1-1, which harbored the two pSV3gptH copies, more than 2×10^6 independent cosmids (i.e., 25 haploid genome equivalents) were screened with SV40 DNA as a probe. Fifty-five clones containing *Ecogpt* sequences were isolated. When the clones containing human sequences were transfected to 27-1 cells, no UV-resistant transformants were obtained. This indicates that the *ERCC-3* gene is

TABLE 3. UV-induced unscheduled DNA synthesis of 27-1 transformants[a]

Cell line[b]	No. of grains
HeLa	35 ± 3
27-1	10 ± 2
CHO	25 ± 3
PT-1	23 ± 3
ST1-1	25 ± 3
CT-1	25 ± 3

[a] For each cell line 25 nuclei were counted. The number of grains is given as mean ± standard error of the mean per fixed square of non-S-phase nuclei.
[b] PT1, Primary transformant; ST1-1, secondary transformant; CT-1, pcD1-transfected cell line. All other primary and secondary transformants gave very similar results.

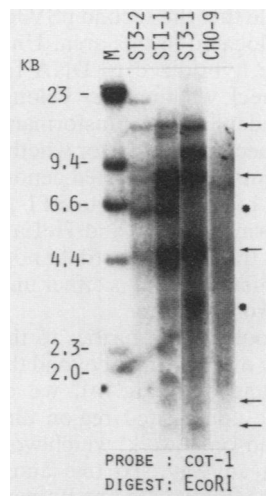

FIG. 2. Autoradiogram of Southern hybridizations (see Materials and Methods) of secondary genomic transformants (ST1-1, ST3-1, and ST3-2) and the wild-type parental line CHO-9. High-molecular-weight DNA (20 μg) was digested with *Eco*RI. The DNAs were electrophoresed and hybridized with a ^{32}P-labeled, nick-translated human *cot-1* probe. The filter was washed with 1× SSC–0.1% sodium dodecyl sulfate at 65°C. Arrows indicate the position of the common bands in each transformant. Asterisks indicate common bands in two of three transformants.

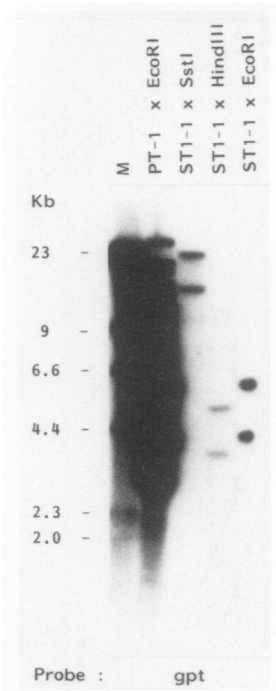

FIG. 3. Autoradiogram of Southern hybridization of primary (PT1) and secondary (ST1-1) transformants digested with the restriction enzymes indicated and hybridized with an *Ecogpt* probe. (The small *Sph*I fragment of pSV3gpt containing the *Ecogpt* gene and a part of the SV40 early region [51].)

either absent or not intact in the cloned human segments. Physical maps were prepared for the cloned human inserts of the cosmids (Fig. 4), and unique probes were isolated (data not shown). It appeared that only one of the two pSV3gptH copies was cloned. Comparison of the cloned insert with the genomic DNA of ST1-1 revealed that the region on the left of the *Ecogpt* marker was consistently rearranged in all clones (Fig. 4). It is possible that the second pSV3gptH copy (which was not cloned) is located in this area. Unique probes from the right part were hybridized to DNA from the panel of transformants to check whether they belonged to the region, common among independent transformants, in which the *ERCC-3* gene is expected to reside or whether they were part of another coincidently cotransfected genomic fragment. All probes appeared to hybridize only to ST1-1 DNA, its parental primary transformant PT-1, and HeLa DNA but not to other, independent transformants (data not shown). Hence, this transfected human segment is either unrelated to *ERCC-3* or too far away from the gene.

Since *ERCC-3* could reside further to the right or on the other side of the *gpt* marker (in or beyond the region that was found to be rearranged in Fig. 4), we decided to clone sequences from the coinherited region directly, instead of performing a chromosomal walk, which would be possible in only one direction anyway. To this aim, *Eco*RI-digested ST1-1 DNA was size fractionated by preparative agarose gel electrophoresis, and the 4.0- to 4.5-kb region that contained the common 4.3-kb human fragment (Fig. 2) was isolated and used for the construction of a lambda library in vector pDJ14. Four recombinant phages were identified that strongly and specifically hybridized to human *cot-1* DNA. A single-copy 0.5-kb *Hha*I subfragment (probe V, Fig. 4) from the insert was isolated and used as a probe for Southern blot hybridization to the panel of primary and secondary transformants. It hybridized with the expected 4.3-kb *Eco*RI fragment in all transfectants. This probe was subsequently used to identify homologous cosmids from the ST1-1 library and other (human) libraries. Only one cosmid clone (from the ST1-1 library, designated cos8) could be isolated. It contained human as well as CHO genomic sequences (Fig. 4). A chromosomal walk was performed, which yielded only clones in one direction (to the left side). This resulted in the isolation of three overlapping DNA clones, cos1 and cos2, and the lambda clone, F15. The physical map of the cloned regions is depicted in Fig. 4. Six unique probes from across the entire cloned human segment were utilized to define the DNA region common to independent primary and secondary transformants. Probe I, situated at the left end (Fig. 4), recognized a human fragment only in two independent secondary transformants (ST1-1, ST2-1) and their corresponding primary transformants but not in ST3-1 and ST3-2 (Fig. 5, probe I). This indicates that probe I is closely associated with but does not form part of the *ERCC-3* gene. When the same blot was hybridized with probe II, which is positioned approximately 13 kb to the right, ST3-1 and ST3-2 also became positive (Fig. 5, probe II). The region recognized by probes II to V was present unchanged compared with control DNA in all four secondary transformants (Fig. 5). Even the rightmost probe (probe VI), just before the junction with CHO DNA in cos8, hybridized to all transformants, indicating that is was still part of the common region. In cos8, however, it was part of a 5-kb *Bgl*II fragment, whereas in the ST1-1 genomic DNA from which the library was prepared it hybridized to a >25-kb *Bgl*II fragment (Fig. 4; data not shown). Other restriction fragments visualized in ST1-1 DNA by this or by flanking CHO probes were also inconsistent with the map of cos8, indicating that this region of the cosmid insert of cos8 was rearranged compared with the ST1-1 genomic sequence. It is possible, therefore, that the common region continues further to the right and still contains parts of the *ERCC-3* gene. This provides a reasonable explanation for the consistent inability of each cosmid alone or in combination to correct the repair defect of 27-1 upon transfection. However, the gene cannot extend more than 9 kb to the right, because the *Bgl*II fragments recognized by probe VI in the DNA of some transformants (e.g., ST1-1; see above) deviated from the corresponding HeLa band (9 kb) (see Fig. 4). This implies that somewhere within this 9-kb fragment the sequences of different transformants start to diverge. From these data we conclude that one end of the *ERCC-3* gene is most likely situated between probes I and II and that the other end is situated somewhere within the 9-kb *Bgl*II fragment.

Efforts to isolate the remaining missing part of *ERCC-3* from ST1-1 DNA or other human cosmid and lambda libraries were discontinued after we screened >60 genome equivalents without success. This included the screening of a lambda library prepared from size-fractionated DNA enriched for the 9-kb human *Bgl*II fragment and the use of *E. coli* hosts recommended for their retention of palindromic sequences. These negative results indicate that this genomic region is rather refractory to cloning. It is possible that the rearranged area encountered in the cosmids containing the pSV3gptH vector (cos19 and cos23, Fig. 4) represents the other side of this refractory segment.

Chromosomal localization of *ERCC-3*. Because of the inability of our cosmids to correct the 27-1 defect, the evidence that the cloned sequences indeed represented *ERCC-3* is based only upon the coinheritance analysis. Although the probability that the retention of the same human DNA

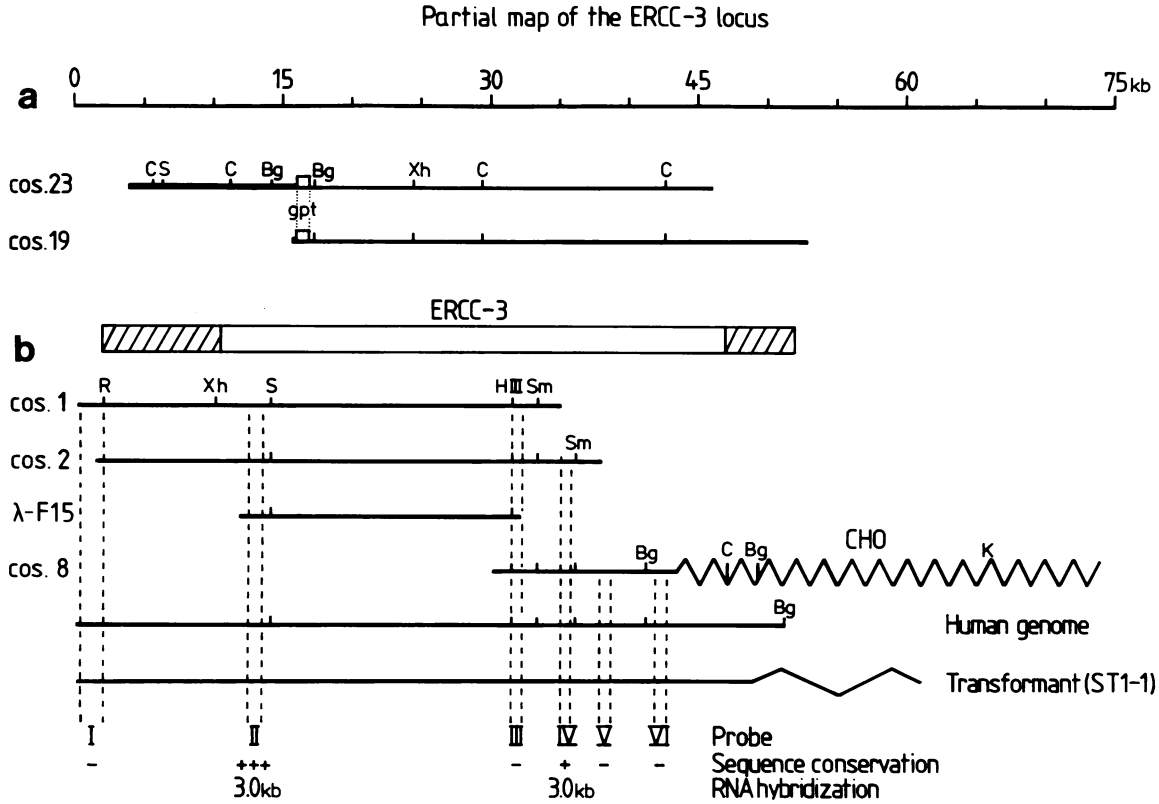

FIG. 4. (a) Physical maps of the two cosmids covering the cloned *Ecogpt* region of ST1-1. The pSV3gptH copy is indicated by the open box. Thin lines to the right of the dominant marker represent human insert sequences that are identical to the ST1-1 genomic DNA. Thick lines to the left of the pSV3gptH copy indicate sequences that, according to their restriction maps, are rearranged in comparison to the ST1-1 genomic DNA and to other cosmids from the same region. (b) Physical maps of three cosmids (designated: cos1, cos2, and cos8) and one genomic lambda clone (F15) covering the *ERCC-3* region. Straight lines represent the human insert of the clones of which the map is identical with the human (HeLa) genome and the human DNA in ST1-1. The wavy line indicates the presence of rearranged CHO DNA. The isolated single-copy probes are indicated by the numbers I to VI. + or − indicates whether the corresponding probe harbors conserved sequences. The boxes denote the minimum (open part) and maximum (shaded plus open part) size of the *ERCC-3* gene as deduced from the coinheritance analysis. Restriction enzymes: *Bgl*II (Bg), *Cla*I (C), *Eco*RI (R), *Hin*dIII (HIII), *Kpn*I (K), *Sal*I (S), *Sma*I (Sm), *Xho*I (Xh). Only the relevant restriction sites are indicated.

segment by nine independent transformants is due to coincidence is extremely low, additional evidence was collected to support the supposition that the cloned region represents the human *ERCC-3* gene. This gene has been assigned previously by Thompson et al. (41) to chromosome 2 on the basis of somatic cell fusion and segregation of noncomplementing chromosomes. Therefore, we determined the chromosomal localization of the common DNA region. With ^{32}P-labeled unique genomic probes, the DNA of 17 human × rodent hybrids was screened for the presence of hybridizing human sequences (Table 4). The highest correlation was found for chromosome 2. Only one case of discordance was found for this chromosome, whereas with the other chromosomes at least five such cases were noted. We conclude that the cloned human segment is derived from chromosome 2, which is in agreement with the previous assignment of *ERCC-3*.

Isolation of functional *ERCC-3* cDNA clones. Some of the isolated *ERCC-3* genomic probes cross-hybridized even under stringent conditions to Chinese hamster fragments (e.g., probes II and IV in Fig. 5). Such a degree of conservation usually implies the presence of coding sequences. This proposition was confirmed by our finding that both probes visualized an mRNA species of 3 kb on Northern blots of HeLa poly(A)$^+$ RNA (Fig. 6, lanes a and b). It suggests that they are derived from the same transcription unit. The probes were subsequently used to screen the Okayama cDNA library prepared in a mammalian expression vector (as well as a human testis cDNA and a K562 cDNA library in lambda gt11 and gt10, respectively). A number of clones with various insert sizes were identified. One clone with an insert of 2.9 kb, designated pcD1, is described in more detail below (Fig. 7). Northern blot analysis of HeLa poly(A)$^+$ RNA revealed that the cDNA hybridized to a ~3-kb transcript, as did the genomic probes (Fig. 6, lane c). Cotransfection of pcD1 with pSV3gptH to 27-1 cells yielded UV-resistant clones with very high efficiency (transfection frequency of UV and MPA resistance was 50 to 100 clones per μg of DNA per 10^6 cells, i.e., >5,000-fold higher than with genomic DNA). The UV survival and UV-induced UDS of the pcD1 transformants were in the CHO wild-type range (Fig. 1, Table 3), indicating that the *ERCC-3* cDNA was functional and corrected the repair deficiency of 27-1 cells.

Specificity of *ERCC-3* complementation. To verify whether a substantial proportion of the transfectants had integrated intact copies of the *ERCC-3* cDNA, Southern blot analysis was performed with *Pst*I-digested DNA of pooled (>500) MPA-resistant clones of each transfected mutant and hybrid-

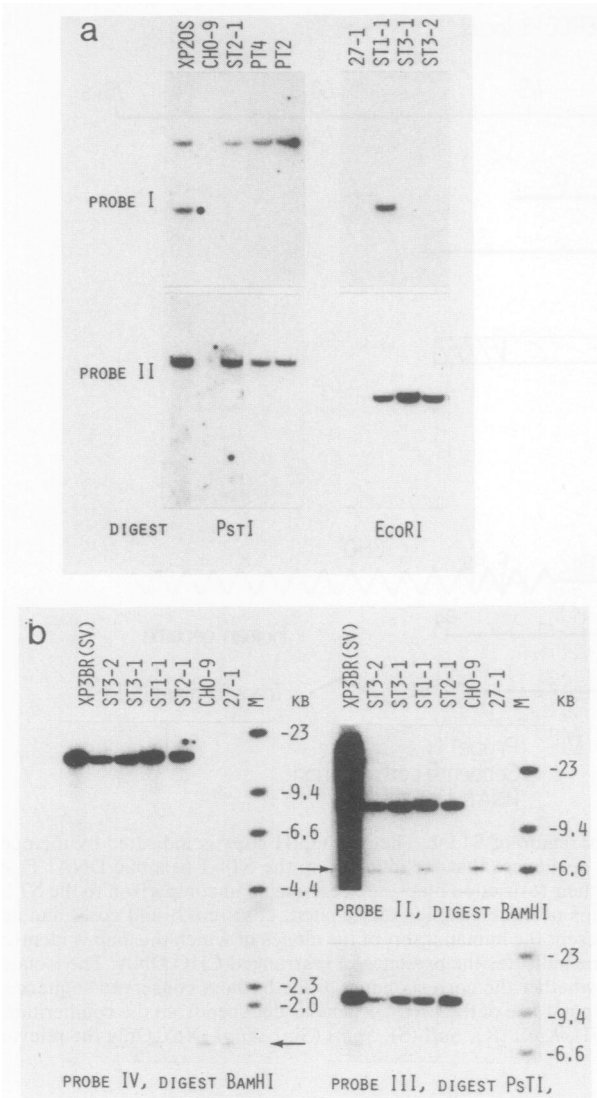

FIG. 5. Southern blot analysis for the presence of coinherited transfected sequences in independent 27-1 transformants. High-molecular-weight DNA (15 μg) of PT4 and PT2 (primary transformants), ST1-1, ST2-1, ST3-1, and ST3-2 (secondary transformants), the parental wild-type CHO-9, 27-1, XP3BR(SV), and XP20S(SV) (as human control cells) was digested with the indicated endonucleases and subjected to electrophoresis in a 0.8% agarose gel. The blots were hybridized with the probes indicated in Fig. 4. Probe I recognizes a polymorphic band in XP20S (SV) DNA (asterisk in panel a). The Chinese hamster fragments cross-hybridizing with probes II and IV are indicated by arrows in panel b.

ization with the entire *ERCC-3* cDNA insert as a probe. Figure 7 shows the presence of the two *Pst*I fragments of 1.5 and 3 kb, expected from the known position of the *Pst*I sites in the *ERCC-3* cDNA and the vector. All transformants contained the fragment, but the number of copies integrated varied considerably between individual mutants (Fig. 7). (The cross-hybridizing 8.5-kb CHO *ERCC-3* genomic fragment [Fig. 7] provided an internal control for the amounts of DNA loaded in each lane.)

To examine whether the *ERCC-3* cDNA is properly expressed in the CHO tranformants, Northern blot analysis was performed. The endogenous *ERCC-3* transcript had the same length as that in HeLa cells, whereas in the transformed cell lines an additional, slightly longer mRNA was detected (Fig. 7b). This cDNA-derived transcript terminates at the SV40 polyadenylation site further downstream and therefore has a slightly increased size. As a control for differences in the amount of RNA loaded onto the gel, the blot was rehybridized with a probe for the rat-specific GAPDH mRNA, which was present in relatively constant amounts (Fig. 7). As with the Southern blot in Fig. 7, the level of cDNA transcripts was quite variable, but in most cases the transfected *ERCC-3* gene was expressed at least to the same extent as the endogenous *ERCC-3* gene. This blot demonstrates furthermore that the mutation in the 27-1 *ERCC-3* gene neither abolished the expression at the RNA level nor affects its size (the wild-type CHO transcript was of the same size and abundance).

TABLE 4. Relationship between the human *ERCC-3* gene and human chromosomes in 17 human-rodent somatic-cell hybrid clones

Chromosome	No. of hybrid clones with the following chromosome/*ERCC-3* gene retension[a]				% Concordance
	+/+	+/−	−/+	−/−	
1	5	6	0	6	65
2	5	1	0	11	94
3	2	5	3	7	53
4[b]	5	7	0	3	53
5	5	7	0	5	59
6[c]	3	7	2	4	44
7	4	7	1	5	53
8	3	7	2	5	47
9	3	7	2	5	47
10	3	6	2	6	53
11[d]	2	5	2	3	36
12	3	7	1	4	64
13	4	5	1	7	65
14	4	7	1	5	53
15	4	4	1	8	71
16	4	8	1	4	47
17	5	12	0	0	29
18	5	5	0	7	71
19	2	8	3	4	35
20	4	8	1	4	47
21	4	8	1	4	47
22[c]	3	9	1	3	38
X	4	8	1	4	47
Y	0	2	15	10	59

[a] In subheadings + and − to the left of the shill (/) indicate the presence and absence, respectively, of the human chromosome; + and − to the right of the shill refer to the presence and absence, respectively, of human *ERCC-3* sequences as detected by Southern blot hybridization.
[b] Two clones containing translocations of this chromosome were excluded.
[c] One clone containing a translocation of this chromosome was excluded.
[d] Six clones containing translocations of this chromosome were excluded.

The UV sensitivity of the various transformants was analyzed to examine whether the transfected *ERCC-3* cDNA corrects the impaired UV survival of one or more CHO complementation groups. UV survival curves of the wild-types AA8 and V79, the mutants, and the cDNA transformants are presented in Fig. 8. The *ERCC-3* cDNA corrected mutant UV24 of complementation group 3 to the same extend as 27-1 (Fig. 1 and data not shown). In contrast, the UV survival of pcD1-transformed populations of the other groups was not corrected. We conclude that *ERCC-3* correction is specific to complementation group 3.

As mentioned above, the 27-1 mutant displayed a high sensitivity to alkylating agents (Table 2), in contrast to other representatives of complementation group 3. It was there-

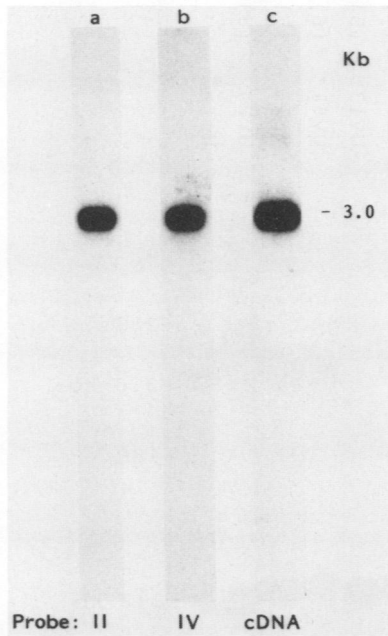

FIG. 6. Northern blot analysis of *ERCC-3* expression in poly(A)$^+$ RNA from HeLa cells. Poly(A)$^+$ RNA (5 μg) was separated in a 1% agarose gel containing formaldehyde and, after transfer to nitrocellulose filters, hybridized to ^{32}P-labeled genomic probe II (lane a), probe IV (lane b), and *ERCC-3* cDNA (lane c).

fore of interest to examine whether *ERCC-3* could correct this defect as well. UV-resistant genomic and cDNA transformants of 27-1 were exposed to MMS. The survival curves (Fig. 1) showed no significant difference in the sensitivity to MMS between the transformants and the 27-1 mutant.

DISCUSSION

DNA-mediated gene transfer to the UV-sensitive CHO-9 mutant 27-1 permitted the cloning of the human excision repair gene *ERCC-3*. The isolation of this gene, however, proved not to be a trivial task, and several lessons of general importance can be learned. Two main problems were encountered.

First, a very low transformation frequency was experienced, particularly with the partially digested DNA. The use of partially cleaved chromosomal DNA ligated in vitro to dominant marker copies did work well for the isolation of the *ERCC-1* gene (15 to 17 kb), which considerably facilitated linked secondary transfection and subsequent cloning (51). In the case of *ERCC-2* (19 kb), sheared human DNA was utilized (49). A prerequisite for the use of chromosomal DNA that is reduced in size, however, is that the gene to be transfected is not too big. From the physical map and the coinheritance analysis of the *ERCC-3* region in independent transformants, we deduce a gene size of 35 to 45 kb (Fig. 4). This provides a reasonable explanation for the very low yield of UV-resistant transformants, with the human DNA digested to an average size of 35 to 50 kb. The subsequent transfections with high-molecular-weight DNA (100 to 300 kb) were approximately 10 times more efficient, although still considerably lower than those with *ERCC-1*. Since the size of the gene to be cloned is generally not known beforehand, it may be wise to attempt both partially digested and high-molecular-weight chromosomal DNA at the same time.

A second pitfall encountered was the presence of a gene segment that proved to be rather refractory to cloning. It is likely that this region prevented the isolation of *ERCC-3* by using the linked dominant marker present in the DNA of ST1-1. Normally, the occurrence of a dominant marker copy in the vicinity of the gene of interest should facilitate its cloning by providing a convenient tag. However, the dominant marker molecule in ST1-1 that was cloned appeared to be close to an area that was rearranged in all cosmids when compared with the parental DNA in the secondary transformant (Fig. 4). Since the human DNA segment of ~40 kb on the other side of this dominant marker was unlinked to the *ERCC-3* gene, we decided to clone directly one of the restriction fragments that are common to all secondary transformants. This was achieved by constructing a lambda library from size-fractionated ST1-1 DNA and screening with human *cot-1* DNA as a probe. This provided us with a unique probe for the *ERCC-3* gene itself. However, the gene could not be cloned in its entirety, because one end (Fig. 4B) containing an essential portion was not present in cosmid and lambda libraries, covering in total approximately 60 times the human haploid genome complement. It is likely that this region is the same as the consistently rearranged area, discussed above, that flanks the dominant marker copy (Fig. 4A). Preliminary evidence suggests that the corresponding part of the murine *ERCC-3* gene is also strongly underrepresented in mouse genomic libraries. However, the isolation of functional *ERCC-3* cDNA clones eliminated the problem caused by the missing genomic segment. The experiences gained in cloning the *ERCC-3* gene may be relevant in strategies for transfection and cloning of other genes (22).

The *ERCC-3* cDNA corrected the UV sensitivity and UV-induced repair synthesis of 27-1 and other mutants of complementation group 3. In contrast to UDS, the level of UV resistance was somewhat lower in all 27-1 transformants than in the repair-proficient CHO-9 parent. The same results were found with mutant 43-3B of complementation group 1 upon introduction of the human *ERCC-1* gene (51, 53). However, in the case of *ERCC-2* most transformants reached wild-type or higher levels of UV survival (49). The incomplete correction by *ERCC-3* may be due to the fact that a human gene has to function in conjunction with a rodent repair system. Alternatively, gene copy number or position effects may account for the observed residual UV sensitivity. Finally, it is possible that this phenomenon is caused by an additional defect of 27-1 cells in a pathway other than the excision repair pathway which is responsible for the unique sensitivity of this mutant to alkylating agents and perhaps also to a minor fraction of UV-induced lesions. It is frequently observed that mutants perturbed in one repair process display mild cross-sensitivity to DNA-damaging agents that induce lesions that are predominantly targets for other repair systems. In this context it is worth noting that the *ERCC-3* gene is unable to compensate for the sensitivity of 27-1 to alkylating agents. This is consistent with the notion that damage induced by small alkylating agents (like MMS) is removed mainly by pathways distinct from long-patch excision. The most logical interpretation of these findings is that *ERCC-3* is not implicated in this process and that 27-1 is disturbed in another gene as well. Such a dual hypersensitivity has been reported for another CHO UV mutant (23). A theoretical possibility is that the same mutation that inactivates *ERCC-3* also affects the second gene, e.g., because of a deletion involving at least two loci. However, Southern and Northern blot analyses of the *ERCC-3* gene in 27-1 did not reveal alterations in gene structure and RNA synthesis

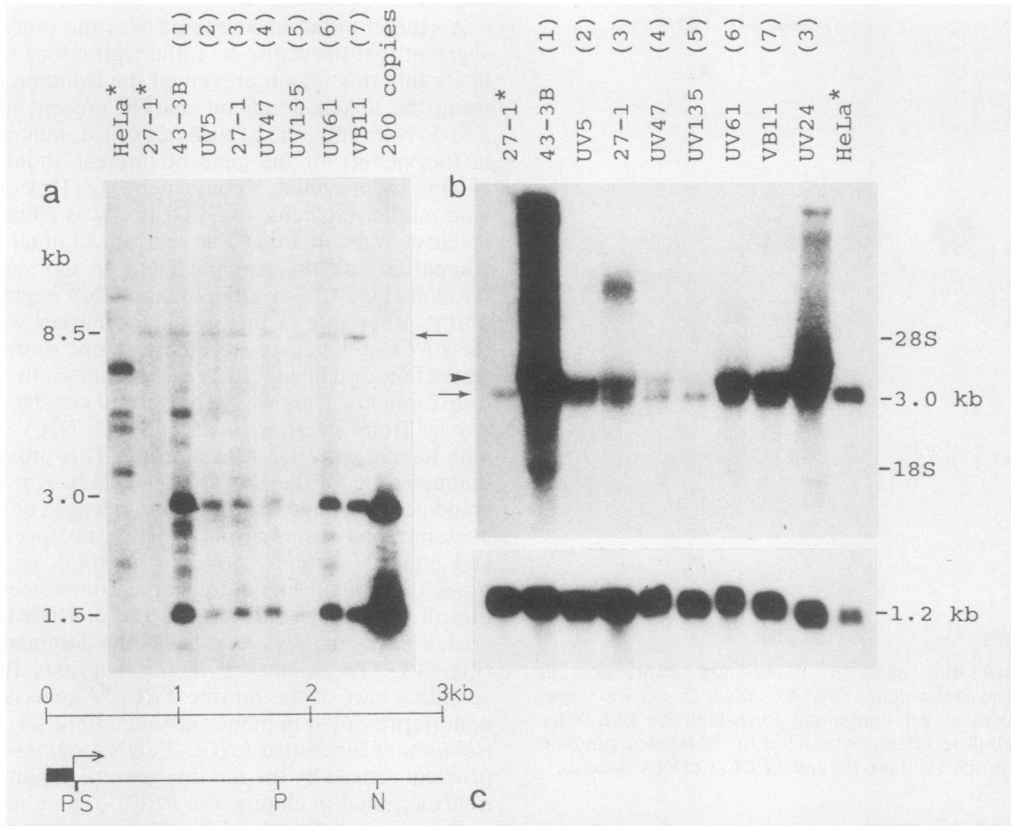

FIG. 7. (a) Southern blot analysis of pcD1-transformed CHO UV-sensitive mutants. High-molecular-weight DNA (15 μg) of the transformed CHO mutants 43-3B, UV5, 27-1, UV24, UV47, UV135, UV61, and VB11, the wild-type CHO-9, and HeLa (human control) was digested with *Pst*I and subjected to electrophoresis in a 0.8% agarose gel. The first lane on the left contains *Pst*I-digested pcD1, equivalent to 200 copies per haploid genome. The blots were hybridized with a ^{32}P-labeled *ERCC-3* cDNA probe as indicated in panel c. A 8.5-kb genomic CHO fragment cross-hybridizing to the human *ERCC-3* probe is indicated (arrow). The asterisk indicates a nontransformed control, and complementation groups are given within parentheses. (b) Northern blot analysis of *ERCC-3* expression in pcD1-transformed mutant cell lines. Total RNA (50 μg) was size fractionated on a 1% agarose gel and, after transfer to nitrocellulose, hybridized to a ^{32}P-labeled *ERCC-3* cDNA probe (upper panel) and a 1.5-kb *Pst*I cDNA probe for the glyceraldehyde-3-phosphate-dehydrogenase gene (GAPDH [10]) (lower panel). Complementation groups are given within parentheses. The endogenous mRNA is indicated with an arrow. The human *ERCC-3* cDNA transcript is indicated with an arrowhead. The asterisk indicates nontransformed control cells. (c) Schematic presentation of the cDNA insert of pcD1. The transcriptional orientation is indicated with an arrow. The small box represent the SV40 early promotor. Abbreviations: *Pst*I (P), *Nar*I (N), *Sst*II (S). The *Sst*II-*Nar*I fragment is used as a human *ERCC-3* cDNA probe.

(Fig. 7 and data not shown). This indicates that the *ERCC-3* gene in 27-1 is probably inactivated by a small-scale mutation.

The correction exerted by *ERCC-3* seems to be complementation group specific: other mutants of group 3 are complemented with the same efficiency as 27-1, whereas representatives of groups 1, 2, and 4 through 7 are not. Control experiments demonstrated that in each case the intact *ERCC-3* cDNA had been integrated into the genome by a substantial fraction of the transformants and that the gene was properly expressed at the RNA level. The correction induced by the other cloned human repair genes *ERCC-1* and *ERCC-2* is also limited to their own complementation groups (46, 49), supporting the validity of the complementation group assignments and consistent with the finding of Thompson and co-workers that most complementation groups are corrected by different human chromosomes (41, 43). In this report we show that the *ERCC-3* gene is located on chromosome 2, the same chromosome found previously to confer UV resistance to other mutants of complementation group 3 (41).

One of the most interesting features emerging from the analysis of the human repair genes cloned thus far is the striking conservation of the encoded proteins. *ERCC-1* was found to be homologous to the *S. cerevisiae* excision repair protein *RAD10*, suggesting that they are descendants of the same ancestral gene (45). The *ERCC-1* gene product possesses, in addition, a C-terminal extension absent in *RAD10*, which shares homology to parts of the *E. coli* repair polypeptides UvrA and UvrC (9, 21). The idea that the strong sequence conservation might hold for other excision repair genes as well is strengthened by the recent finding of Weber et al. (50) that the human *ERCC-2* gene is very similar to the *S. cerevisiae* excision repair gene *RAD3*, which was shown by Sung and co-workers to encode a DNA-dependent helicase (35) with an additional, vital function (19, 29). From the strong cross-hybridization of two of the genomic probes of *ERCC-3* to Chinese hamster sequences under stringent hybridization conditions (Fig. 5), we anticipate that the *ERCC-3* gene also is strongly conserved. Preliminary zoo-blot analysis (Southern blot analysis of DNAs from various species throughout the eucaryotic kingdom) indicates that under identical conditions homologous sequences can be picked up even in *Drosophila* DNA (data not shown).

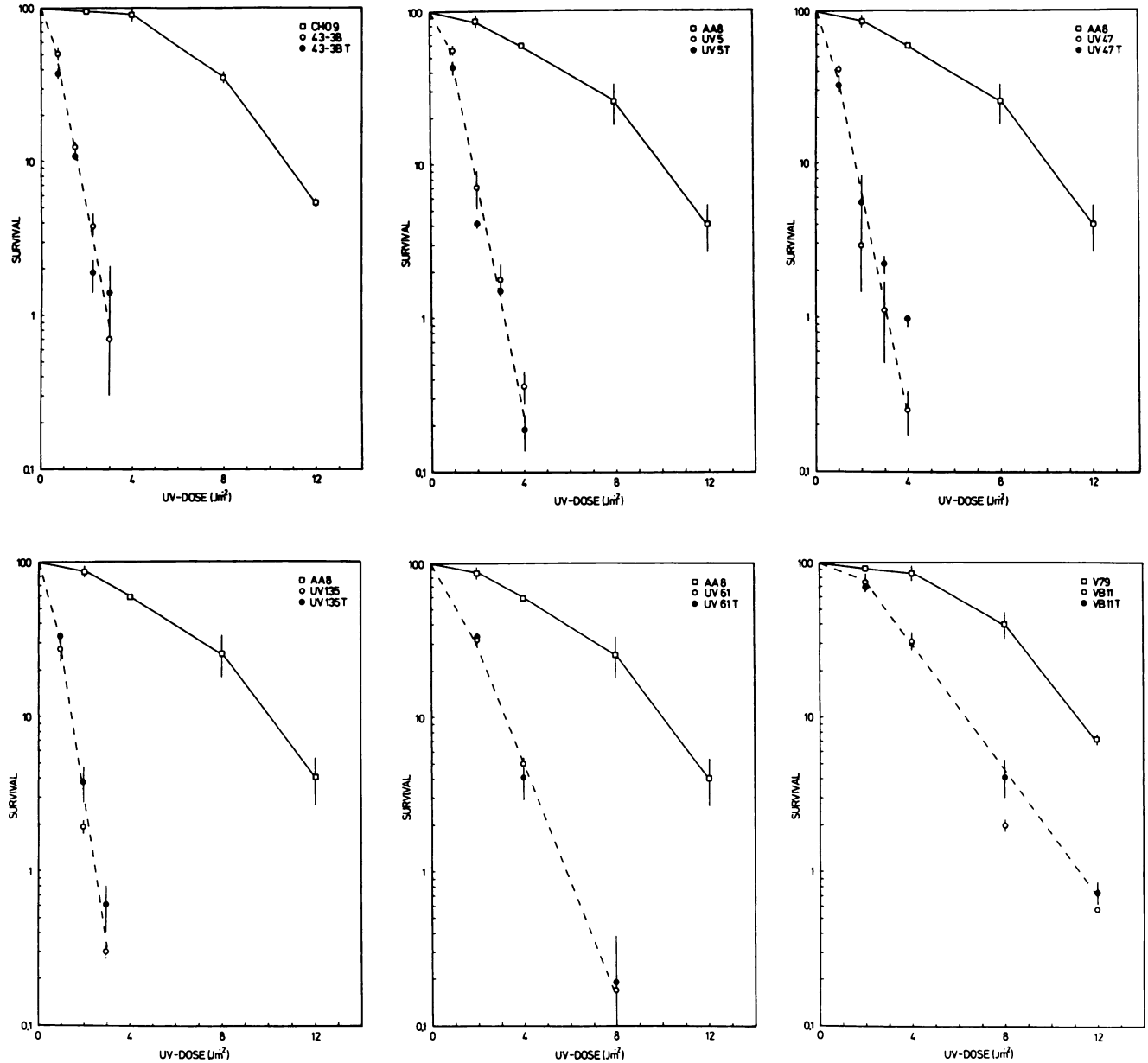

FIG. 8. UV survival curves of wild types (AA8 and V79), mutants (43-3B, UV5, UV47, UV135, UV61, VB11), and transfected mass population (43-3B-T, UV5-T, UV47-T, UV135-T, UV61-T, VB11-T). Error bars represent standard errors of the means.

The phenotype of complementation group 3 mutants is very reminiscent of the human repair syndrome XP in terms of UV sensitivity, patterns of cross-sensitivity toward other DNA-damaging agents, and deficiencies in preincision steps of the excision repair pathway. Until now none of the CHO complementation groups was found to overlap with any of those of XP. However, the cell fusion studies involved have covered only a small fraction of all combinations that would be necessary for a complete analysis. We have shown by Southern and Northern blot analyses and by DNA transfection and microinjection experiments that the *ERCC-1* gene most probably is not involved in any of the XP groups or in Cockayne's syndrome (47). This result can be rationalized in view of the differences in phenotype between CHO group 1 mutants and XP fibroblasts, particularly with respect to the extreme sensitivity of the rodent mutants to cross-linking agents. Therefore, it will be of interest to investigate whether *ERCC-3* is implicated in XP. Such studies are currently under way.

ACKNOWLEDGMENTS

We are grateful to R. D. Wood (Imperial Cancer Research Fund, London, England) for providing the 27-1 mutant cell line, H. Okayama (Osaka, Japan) for giving the mammalian expression library, A. H. M. Geurts van Kessel for the hybrid panel used for the chromosomal localization, L. Thompson (Livermore, Calif.) for providing several UV-sensitive mutant cell lines, G. Grosveld (Rotterdam) for giving the CML-O library. We are indebted to A. Pastink (Leiden) for help in the initial experiments.

This work was financially supported by the Netherlands Organi-

zation for the Advancement of Pure Research through the Foundation of Medical Scientific Research (contract 900-501-091) and by EURATOM (contract BJ6-141-NL).

LITERATURE CITED

1. **Auffray, C., and F. Rougeon.** 1980. Purification of mouse immunoglobin heavy-chain messenger RNAs from total myeloma tumor DNA. Eur. J. Biochem. **107**:303–314.
2. **Bates, P. F., and R. Swift.** 1983. Double cos site vectors: simplified cosmid cloning. Gene **26**:137–146.
3. **Bootsma, D., W. Keyzer, E. G. Jung, and E. Bohnert.** 1989. Xeroderma pigmentosum complementation group XP-I withdrawn. Mutat. Res. **218**:149–151.
4. **Busch, D., C. Greiner, K. Lewis, R. Ford, G. Adair, and L. Thompson.** 1989. Summary of complementation groups of UV-sensitive CHO cell mutants isolated by large-scale screening. Mutagenesis **4**:349–354.
5. **Cleaver, J. E., and K. H. Kraemer.** 1989. Xeroderma pigmentosum, p. 2949–2971. *In* C. R. Scriver, A. L. Beaudet, W. S. Sly, and D. Valle (ed.), Metabolic basis of inherited disease, vol. 2. McGraw-Hill Book Co., New York.
6. **Collins, A., and R. T. Johnson.** 1987. DNA repair mutants in higher eukaryotes. J. Cell. Suppl. **6**:61–82.
7. **De Weerd-Kastelein, E. A., W. Keyzer, and D. Bootsma.** 1972. Genetic heterogeneity of xeroderma pigmentosum demonstrated by somatic cell hybridization. Nature (London) New Biol. **238**:80–83.
8. **De Wit, J., H. L. Hoeksema, D. Bootsma, and A. Westerveld.** 1979. Assignment of structural β-galactosidase loci to human chromosome 3 and 22. Hum. Genet. **51**:259–267.
9. **Doolittle, R. F., M. S. Johnson, I. Husain, B. van Houten, D. C. Thomas, and A. Sancar.** 1986. Domainal evolution of a prokaryotic DNA repair protein and its relationship to active transport proteins. Nature (London) **323**:451–453.
10. **Fort, P., L. Marty, M. Piechaczyk, S. El Sabrouti, C. Dani, P. Jeanteur, and J. M. Blanchard.** 1985. Various rat adult tissues express only one major mRNA species from the glyceraldehyde-3-phosphate-dehydrogenase multigenic family. Nucleic Acids Res. **13**:1431–1442.
11. **Friedberg, E. C.** 1985. DNA repair. W. H. Freeman & Co., San Fransisco.
12. **Friedberg, E. C.** 1988. DNA repair in the yeast *Saccharomyces cerevisiae*. Microbiol. Rev. **52**:70–102.
13. **Frischauf, A. M., H. Lerach, A. Poustka, and N. Murray.** 1983. Lambda replacement vectors carrying polylinker sequences. J. Mol. Biol. **170**:827–842.
14. **Geurts van Kessel, A. H. M., H. ten Brinke, W. A. M. Broere, W. C. den Boer, P. G. de Groot, A. Hagemeyer, P. Meera Khan, and P. L. Pearson.** 1981. Characterisation of the Philadelphia chromosome by gene mapping. Cytogenet. Cell Genet. **30**: 83–91.
15. **Graham, F., and A. J. van der Eb.** 1973. A new technique for the assay of infectivity of human adenovirus 5 DNA. Virology **52**:456–467.
16. **Grossman, L., P. R. Caron, S. J. Mazur, and E. Y. Oh.** 1988. Repair of DNA containing pyrimidine dimers. FASEB J. **2**: 2696–2701.
17. **Haynes, R. H., and B. A. Kunz.** 1981. DNA repair and mutagenesis in yeast, p. 371–414. *In* J. Strathern, E. Jones, and J. Broach (ed.), The molecular biology of the yeast *Saccharomyces*: life cycle and inheritance. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
18. **Hickson, I. D., and A. L. Harris.** 1988. Mammalian repair of mutants, hypersensitive to cytotoxic agents. Trends Genet. **4**:101–106.
19. **Higgens, D. R., S. Prakash, P. Reynolds, R. Polakowska, S. Weber, and L. Prakash.** 1983. Isolation and characterization of the *RAD3* gene of *Saccharomyces cerevisiae* and inviability of *RAD3* deletion mutants. Proc. Natl. Acad. Sci. USA **80**:5680–5684.
20. **Hoeijmakers, J. H. J., H. Odijk, and A. Westerveld.** 1987. Differences between rodent and human cell lines in the amount of integrated DNA after transfection. Exp. Cell. Res. **169**: 111–119.
21. **Hoeijmakers, J. H. J., M. van Duin, A. Yasui, and D. Bootsma.** 1986. Identification of DNA repair genes in the human genome. Cold Spring Harbor Symp. Quant. Biol. **51**:91–101.
22. **Hoeijmakers, J. H. J., A. Westerveld, and D. Bootsma.** 1988. Methods and strategies for molecular cloning of mammalian DNA repair genes by DNA-mediated gene transfer, p. 181–201. *In* E. C. Friedberg and P. C. Hanawalt (ed.), DNA repair, a laboratory manual for research procedures, vol. 3. Marcel Dekker, Inc., New York.
23. **Hoy, C. A., L. H. Thompson, E. P. Salazar, and S. A. Stewart.** 1985. Different genetic alterations underlie dual hypersensitivity of CHO mutant UV-1 to DNA methylating and cross-linking agents. Somatic Cell Mol. Genet. **11**:523–532.
24. **Jeffreys, A. J., and R. A. Flavell.** 1977. A physical map of the DNA region flanking the rabbit β-globin gene. Cell **12**:429–439.
25. **Kraemer, K. H., M. M. Lee, and J. Scotto.** 1984. DNA repair protects against cutaneous and internal neoplasia: evidence from xeroderma pigmentosum. Carcinogenesis **5**:511–514.
26. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
27. **Messing, J., R. Crea, and P. H. Seeburg.** 1981. A system for shotgun DNA sequencing. Nucleic Acids Res. **9**:309–322.
28. **Mulligan, R. C., and P. Berg.** 1981. Selection for animal cells that express the *Escherichia coli* gene coding for xanthine-guanine phosphoribosyltransferase. Proc. Natl. Acad. Sci. USA **78**:2072–2076.
29. **Naumovski, L., and E. C. Friedberg.** 1982. A DNA repair gene required for the incision of damaged DNA is essential for viability in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **80**:4818–4821.
30. **Okayama, H., and P. Berg.** 1983. A cDNA cloning vector that permits expression of cDNA inserts in mammalian cells. Mol. Cell. Biol. **3**:280–289.
31. **Sancar, A., and G. B. Sancar.** 1988. DNA repair enzymes. Annu. Rev. Biochem. **57**:29–67.
32. **Schrier, P. I., R. Bernards, R. T. M. J. Vaessen, A. Houweling, and A. J. van der Eb.** 1983. Expression of class I major histocompatibility antigens switched off by highly oncogenic adenovirus 12 in transformed rat cells. Nature (London) **305**: 771–775.
33. **Sealey, P. G., P. A. Whittaker, and E. M. Southern.** 1985. Removal of repeated sequences from hybridisation probes. Nucleic Acids Res. **13**:1905–1921.
34. **Stefanini, M., W. Keyzer, A. Westerveld, and D. Bootsma.** 1985. Interspecies complementation analysis between xeroderma pigmentosum and UV-sensitive Chinese hamster cells. Exp. Cell Res. **161**:373–380.
35. **Sung, P., L. Prakash, S. W. Matson, and S. Prakash.** 1987. *RAD3* protein of *Saccharomyces cerevisiae* is a DNA helicase. Proc. Natl. Acad. Sci. USA **84**:8951–8955.
36. **Sung, P., L. Prakash, S. Weber, and S. Prakash.** 1987. The *RAD3* gene of *Saccharomyces cerevisiae* encodes a DNA dependent ATPase. Proc. Natl. Acad. Sci. USA **84**:6045–6049.
37. **Tanaka, K., I. Satokata, Z. Ogita, T. Uchida, and Y. Okada.** 1989. Molecular cloning of a mouse DNA repair gene that complements the defect of group-A xeroderma pigmentosum. Proc. Natl. Acad. Sci. USA **86**:5512–5516.
38. **Thompson, L., K. W. Brookman, L. E. Dillehay, C. L. Mooney, and A. V. Carrano.** 1982. Hypersensitivity to mutation and sister-chromatid exchange induction in CHO cell mutants defective in incising DNA containing UV-lesions. Somatic Cell. Genet. **8**:759–773.
39. **Thompson, L., E. P. Salazar, K. W. Brookman, C. C. Collins, S. A. Stewart, D. B. Busch, and C. A. Weber.** 1987. Recent progress with the DNA repair mutants of Chinese hamster ovary cells. J. Cell Sci. Suppl. **6**:97–110.
40. **Thompson, L. H., D. B. Busch, K. W. Brookman, C. L. Mooney, and D. A. Glaser.** 1982. Genetic diversity of UV-sensitive DNA repair mutants of Chinese hamster ovary cells. Proc. Natl. Acad. Sci. USA **78**:3734–3737.
41. **Thompson, L. H., A. V. Carrano, K. Sato, E. P. Salazar, B. F.**

White, S. A. Stewart, J. L. Minkler, and M. J. Siciliano. 1987. Identification of nucleotide-excision repair genes on human chromosomes 2 and 13 by functional complementation in hamster-human hybrids. Somatic Cell Mol. Genet. **13**:539–551.

42. **Thompson, L. H., C. L. Mooney, and K. W. Brookman.** 1985. Genetic complementation between UV-sensitive CHO mutants and xeroderma pigmentosum fibroblasts. Mutat. Res. **150**:423–429.
43. **Thompson, L. H., C. L. Mooney, K. Burkhart-Schultz, A. V. Carrano, and M. J. Siciliano.** 1985. Correction of a nucleotide-excision-repair mutation by human chromosome 19 in hamster-human hybrid cells. Somatic Cell Mol. Genet. **11**:87–92.
44. **Thompson, L. H., T. Shiomi, E. P. Salazar, and S. A. Stewart.** 1988. An eighth complementation group of rodent cells hypersensitive to ultraviolet radiation. Somat. Cell. Mol. Genet. **14**:605–612.
45. **van Duin, M., J. de Wit, H. Odijk, A. Westerveld, A. Yasui, M. H. H. Koken, J. H. J. Hoeijmakers, and D. Bootsma.** 1986. Molecular cloning of the human excision repair gene *ERCC-1*: cDNA cloning and amino acid homology with the yeast DNA repair gene *RAD10*. Cell **44**:913–923.
46. **van Duin, M., J. H. Jansen, J. de Wit, J. H. J. Hoeijmakers, L. H. Thompson, and D. Bootsma.** 1988. Transfection of the cloned human excision repair gene *ERCC-1* to UV-sensitive CHO mutants only corrects the repair defect in complementation group-2 mutants. Mutat. Res. **193**:123–130.
47. **van Duin, M., G. Vredeveldt, L. V. Mayne, H. Odijk, W. Vermeulen, B. Klein, G. Weeda, J. H. J. Hoeijmakers, and D. Bootsma.** 1989. The cloned human excision repair gene *ERCC-1* fails to correct xeroderma pigmentosum complementation group A through I. Mutat. Res. **217**:83–92.
48. **Vermeulen, W., P. Osseweyer, A. J. R. de Jonge, and J. H. J. Hoeijmakers.** 1986. Transient correction of excision repair defects in fribroblasts of 9 xeroderma pigmentosum complementation groups by microinjection of crude human cell extracts. Mutat. Res. **165**:199–206.
49. **Weber, C. A., E. P. Salazar, S. A. Stewart, and L. H. Thompson.** 1988. Molecular cloning and biological characterization of a human gene, *ERCC-2*, which corrects the nucleotide excision repair defect in CHO UV5 cells. Mol. Cell. Biol. **8**:1137–1146.
50. **Weber, C. A., E. P. Salazar, and L. H. Thompson.** 1989. Molecular characterization of *ERCC-2*: a human nucleotide excision repair gene with homology to the yeast *RAD3* repair gene. Environ. Mol. Mutagen. **14**(Suppl. 15):628.
51. **Westerveld, A., J. H. J. Hoeijmakers, M. van Duin, J. de Wit, H. Odijk, A. Pastink, R. D. Wood, and D. Bootsma.** 1984. Molecular cloning of a human DNA repair gene. Nature (London) **310**:425–428.
52. **Wood, R. D., and H. J. Burki.** 1982. Repair capability and the cellular age response for killing and mutation induction after UV. Mutat. Res. **95**:505–514.
53. **Zdzienicka, M. Z., L. Roza, A. Westerveld, D. Bootsma, and J. W. I. M. Simons.** 1987. Biological and biochemical consequences of the human *ERCC-1* repair gene after transfection into a repair deficient CHO cell line. Mutat. Res. **183**:69–74.
54. **Zdzienicka, M. Z., and J. W. I. M. Simons.** 1986. Analysis of repair processes by the determination of the induction of cell killing and mutations in two repair-deficient Chinese hamster ovary cell lines. Mutat. Res. **166**:59–69.
55. **Zdzienicka, M. Z., G. P. van der Schans, and J. W. I. M. Simons.** 1988. Identification of a new seventh complementation group of UV sensitive mutants in Chinese hamster cells. Mutat. Res. **194**:165–170.