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Isolation of the Functional Human Excision Repair Gene *ERCC5* by Intercosmid Recombination

JOHN S. MUDGETT AND MARK A. MACINNES

Genetics Group, Life Sciences Division, Los Alamos National Laboratory, Los Alamos, New Mexico 87545

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The complete human nucleotide excision repair gene *ERCC5* was isolated as a functional gene on overlapping cosmids. *ERCC5* corrects the excision repair deficiency of Chinese hamster ovary cell line UV135, of complementation group 5. Cosmids that contained human sequences were obtained from a UV-resistant cell line derived from UV135 cells transformed with human genomic DNA. Individually, none of the cosmids complemented the UV135 repair defect; cosmid groups were formed to represent putative human genomic regions, and specific pairs of cosmids that effectively transformed UV135 cells to UV resistance were identified. Analysis of transformants derived from the active cosmid pairs showed that the functional 32-kbp *ERCC5* gene was reconstructed by homologous intercosmid recombination. The cloned human sequences exhibited 100% concordance with the locus designated genetically as *ERCC5* located on human chromosome 13q. Cosmid-transformed UV135 host cells repaired cytotoxic damage to levels about 70% of normal and repaired UV-irradiated shuttle vector DNA to levels about 82% of normal. © 1990 Academic Press, Inc.

INTRODUCTION

The genetic defects in most xeroderma pigmentosum patients have been reported to involve early stages of nucleotide excision repair (Friedberg, 1985). Isolation of human DNA repair genes responsible for nucleotide excision repair or other repair pathways would permit the biochemical elucidation of these DNA repair mechanisms. Due, in part, to limitations of using human repair-deficient cell lines as hosts for isolating human DNA repair genes (Lehman, 1985), ultraviolet (UV) radiation-sensitive rodent cell mutants have been isolated. Eight different genetic complementation groups have been identified (Busch *et al.*, 1989; Zdzienicka *et al.*, 1988; Thompson *et al.*, 1988). These Chinese hamster ovary (CHO) cells have been particularly efficient hosts for the isolation of DNA repair mutants due to their partial hemizygosity (Thompson *et al.*, 1989). This hemizygosity has resulted in the isolation of three DNA repair mutants whose affected loci reside on hamster chromosome 9 (Thompson *et al.*, 1989). In addition, rodent repair mutants are efficiently transformed with complementing human DNA, which can be recovered by rescuing segments containing human repetitive sequences.

Research indicated that CHO and human nucleotide excision repair mechanisms were functionally similar. Defects in the first five UV-sensitive CHO complementation groups have been shown to be associated with essential steps of nucleotide excision repair (Thompson *et al.*, 1982), as have the apparent defects in classical xeroderma pigmentosum lines (Friedberg, 1985). It was observed that actively transcribed genes are repaired much more efficiently than bulk DNA in hamster cells (Bohr *et al.*, 1986) and that human cells also exhibit this phenomenon, but to a lesser degree (Mellon *et al.*, 1986). At least one complementing human gene can restore preferential repair to its corresponding CHO repair mutant (Bohr *et al.*, 1988). Specific CHO repair mutants were functionally corrected by creating human/CHO hybrids (Hori *et al.*, 1983; Thompson *et al.*, 1985, 1987), indicating that hamster repair-deficient cell lines could be used to isolate the complementing human DNA repair genes.

Two human nucleotide excision repair genes that complement CHO repair defects have been completely isolated, but others have been more elusive for a variety of reasons. The human gene responsible for complementation of CHO group 1, designated *ERCC1* (*E*xcision *R*epair *C*ross *C*omplementing gene 1), has been cloned and characterized (van Duin *et al.*, 1986; Westerveld *et al.*, 1984). This 15-kbp human DNA repair gene has partial amino acid sequence similarity to the yeast repair gene *RAD10* (van Duin *et al.*, 1986), and the homologous mouse *ERCC1* gene has been cloned (van Duin *et al.*, 1988). The human DNA repair gene *ERCC2* (19 kbp) has also been isolated

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(Weber *et al.*, 1988) and was found to be very similar in amino acid sequence to the *RAD3* repair gene (Weber *et al.*, 1990). These repair protein homologies suggest that some *ERCC* proteins (and their mechanistic functions) are highly conserved through evolution. Other human *ERCC* repair genes have been more difficult to isolate due to various problems encountered in genomic cloning, including apparently unclonable regions (*ERCC3*) (Weeda *et al.*, 1990) and very large gene size (*ERCC6*) (Hoeijmakers *et al.*, 1988). The human repair gene *ERCC4* has not been isolated, due to the probable absence of associated highly repetitive human DNA, which is important for the isolation of human DNA-containing cosmid clones from the CHO genomic background (Dulhanty *et al.*, 1988).

Our previous studies indicated that UV135 cells could be converted to UV-resistant, repair competent transformants using either normal CHO or human genomic DNAs (MacInnes *et al.*, 1984; Strniste *et al.*, 1988). We report in this article the isolation and partial characterization of the functional human DNA repair gene *ERCC5* on overlapping cosmid clones. To overcome some of the problems anticipated in the cloning of repair genes, *ERCC5* was isolated by an effective combination of genetic and molecular approaches. Serial cotransformation with a selectable marker gene, sibling selection enrichment, cosmid alignment, and screening of cosmid groups for functionality were employed in the isolation of *ERCC5*. Southern hybridization analysis conclusively demonstrated that this isolated gene was the locus designated *ERCC5* (Thompson *et al.*, 1987), and the cloned *ERCC5* gene was analyzed to determine some of its molecular characteristics and biological activities.

MATERIALS AND METHODS

Cell Lines and Culture Media

Cells were maintained in minimal Eagle's medium (Gibco) supplemented with 10% fetal bovine serum (Hyclone) and antibiotics (penicillin and streptomycin). The UV-sensitive CHO cell line UV135 was derived from the UV-resistant (UV^r) cell line AA8 and assigned to complementation group 5 (Thompson and Carrano, 1983). The human/CHO hybrids and their UV-sensitive derivatives were obtained as described (Thompson *et al.*, 1987). Primary human skin fibroblast cell line HSF22 (passage 8–11, 46X:Y) was from foreskin tissue obtained locally. Primary, secondary, and tertiary UV^r transformant cell lines of UV135 cells containing the *ecogpt* gene were selected for and cultured in complete medium supplemented with 8 μg/ml mycophenolic acid, 20 μg/ml adenine, and 250 μg/ml xanthine. Cosmid transformants that contained the *neo* gene were selected for and cultured in complete medium containing 350 μg/ml active geneticin (G418).

DNA Isolation, Transfection, and Manipulation

Plasmid and cosmid DNAs were prepared by standard methods (Sambrook *et al.*, 1989). High-molecular-weight genomic DNA was prepared, and genomic and cosmid DNAs were transfected into UV135 host cells by the method of $CaPO_4$ precipitation as previously described (MacInnes *et al.*, 1984). DNA for hybridization probes was radioactively labeled using a random-primed DNA labeling kit (Boehringer Mannheim). Southern blot hybridization analysis was performed using Zetabind membranes (AMF-Cuno) and conditions suggested by the manufacturer.

Sibling Selection Enrichment of UV-Resistant Colonies

A two-step selection enrichment protocol for detection of UV-resistant, antibiotic-resistant cotransformants was previously described (MacInnes *et al.*, 1984). This procedure was used to isolate the primary and secondary cotransformants and was necessary for the detection of functional cosmid groups. In brief, transformation of UV135 cells with either human (HSF22) DNA plus pSV2gpt (*ecogpt*) or with cosmids (*neo*) was followed by transformant selection in medium containing antibiotics (mycophenolic acid or geneticin, respectively). Transformant colonies (20 to 100 per dish) were dispersed after 7 to 9 days of growth and replated as sibling transformant cell populations. The dispersed transformants ($1–5 \times 10^5$ cells per plate) were irradiated with 4.5 J/m^2 UV light (254 nm) at 1, 3, and 5 days after plating. With UV^r colony regrowth, sibling colony populations greatly enhanced our ability to detect very rare UV-resistant cotransformants. Since the number of original transformant colonies was between 20 and 100 per dish, the probability of detecting UV135 revertants was greatly reduced. Negative control experiments performed in parallel, using UV135 genomic DNA and pSV2gpt (MacInnes *et al.*, 1984) or sCos-1 vector DNA, never resulted in UV^r sibling populations.

Cosmid Library Construction and Screening

Secondary transformant 38.4.4 cells ($\sim 7 \times 10^6$ cells per 100-mm plate) were washed twice with PBS and the cell monolayer was frozen without PBS at −80°C *in situ*. Cell lysis buffer (4 ml) (0.05% Sarcosyl, 10 m*M* EDTA, 100 m*M* NaCl, 50 m*M* Tris, pH 8.0, and 25 μg/ml proteinase K) was added to each frozen plate, and the plates were incubated at 37°C for 24 h. An equal volume of 2× restriction buffer (25 m*M*

$MgCl_2$, 100 m*M* NaCl, 2 m*M* DTT, and 200 μg/ml BSA) containing 2 m*M* phenylmethylsulfonyl fluoride (PMSF) and 1 or 2 units of *Mbo*I were added to each plate of lysed cells, and the lysates were digested at 37°C with gentle agitation. Aliquots (1 ml) were removed after 5 to 30 min of incubation and inactivated at 65°C for 20 min. Alkaline phosphatase (5 U, Boehringer Mannheim) was added and the aliquots were incubated overnight at 37°C. The aliquots were extracted twice with phenol:chloroform and twice with chloroform:isoamyl alcohol, ethanol-precipitated, and resuspended in sterile distilled water. Aliquots that contained DNA of a relatively large size were ligated with sCos-1 arms as described (Evans *et al.*, 1989). Ligated DNA was packaged using Gigapack Gold (Stratagene) and used to infect HB101 *Escherichia coli* host cells. This cosmid library procedure yielded between 2×10^6 and 1×10^7 colonies per microgram of genomic DNA. Replicate filters (Colony Screen, DuPont) of the cosmid library were prepared and hybridized with a radioactively labeled total human placental DNA probe, and human DNA-positive cosmids were selected as described (Sambrook *et al.*, 1989).

Grouping of Cosmid DNAs

The cosmid clones were characterized and grouped in the following manner. The individual cosmids were digested with restriction endonucleases (*Hin*dIII and *Eco*RI), electrophoresed on agarose gels, transferred to membranes, and hybridized with a radioactively labeled total human placental DNA probe. The digested cosmids were compared with one another for similarities in the size and pattern of their human-hybridizing and nonhybridizing restriction fragments and were assigned to cosmid groups based on these similarities. This analysis was performed to identify and group overlapping cosmids.

Mapping of Cosmid Clones and Determination of Functional Gene Boundaries

Cosmid clones were mapped for their restriction sites by the method of end-ordered partial digestion (Evans *et al.*, 1989). Single and double digestions of various cosmid regions were performed to augment the end-ordered restriction site data. The functional boundaries of *ERCC5* were assigned indirectly by determining which restriction endonucleases inactivated the repair gene. Cosmids (cH44 or cH75) were digested with specific restriction endonucleases and treated with alkaline phosphatase to help prevent cosmid reassociation. The digested cosmid DNA (2 μg per plate) was transformed into UV135 cells, along with the other cosmid of the pair which had only been linearized with *Pvu*I. Linearizing the cosmids by cutting at the *Pvu*I or *Not*I sites in the sCos-1 vector (Evans *et al.*, 1989) increased the yield of UV-resistant transformants about fourfold. These cosmid transfers were performed using sonicated human placental DNA (10–20 μg per plate) as carrier DNA to block the digested cosmid ends from reassociating. Transformants were selected using geneticin and direct UV selection, without sibling selection enrichment, and the number of UV^r colonies was compared to colony numbers resulting when both cosmids were cut with only *Pvu*I.

UV Irradiation, Transfection, and CAT Assays of Shuttle Vector DNA

The ability of *ERCC5*-transformed UV135 cells to repair UV-irradiated shuttle vector DNA was assayed by the analysis of reporter gene activity. pSVcatSVgpt (a gift from Dr. Miroslava Protić), which contained the active chloramphenicol acetyltransferase (*cat*) reporter gene, was irradiated in TE (0.1 mg/ml) with a calibrated UV light source (254 nm) at room temperature. UV-irradiated shuttle vector DNA (5 μg per plate per dose) was transfected into the CHO cells using $CaPO_4$ precipitation without glycerol treatment. After incubation for 48 h, the cells were extracted and chloramphenicol acetyltransferase (CAT) enzyme assays were performed as described (Sleigh, 1986). Protein determinations of the cell extracts were performed using a Bio-Rad Protein Assay Kit (Bradford method) to ensure that protein yields were comparable.

RESULTS

Production and Validation of Serial Cotransformants of UV135 Cells

Complementation of the UV135 repair defect, via serial cotransformation with human genomic DNA, was described previously (MacInnes *et al.*, 1984; Strniste *et al.*, 1988). Two primary UV- and mycophenolic acid-resistant cotransformants (designated 26.2.1 and 26.5.5) were selected from UV135 cells $CaPO_4$-transformed with simple mixtures of human skin fibroblast (HSF22) genomic DNA and pSV2gpt plasmid DNA. pSV2gpt was included as an independently selectable marker. Another primary transformant was derived using HSF22 genomic DNA that was partially digested with the restriction endonuclease *Sau*3AI to a modal molecular size of about 50 kbp and then enzymatically ligated to pSV2gpt linearized with *Bam*HI. UV135 cells transformed with this ligated material yielded a UV^r and mycophenolic acid-resistant primary cotransformant (designated 33.4.2),

isolated via sibling selection enrichment (described under Materials and Methods).

A secondary cotransformant (designated 38.4.4) was derived from UV135 cells transformed using genomic DNA (>100 kbp size), isolated from the primary transformant 33.4.2, without additional pSV2gpt. This secondary cotransformant was shown to be genetically valid, as its genomic DNA was used to establish tertiary cotransformants. A tertiary cotransformant (designated 40.2.8) was selected by sibling selection enrichment in medium containing mycophenolic acid and three subsequent exposures (1 h each, over 5 days) to *N*-acetoxy acetylaminofluorine (10 μM). Another tertiary cotransformant (designated 46.7D.1) was selected without exposure to UV irradiation, but tested UV resistant after mycophenolic acid-resistant colonies were subcloned.

TABLE 1

Ultraviolet-Resistant Colonies from Cosmid-Pair Cotransfers

Cosmid pair[a]	Overlap (kbp)	UV^r colonies/μg[b]
cH44/cH75	12.2	18 ± 3.7
cH83/cH75	10.8	18 ± 2.3
cH44/cH91	2.5	5 ± 2.4
cH83/cH91	1.1	1 ± 0.5
cH80/cH75	None	0 ± 0
cH80/cH91	None	0 ± 0

[a] Both cosmids were used without restriction endonuclease digestion prior to transformation of 8×10^5 cells per plate.

[b] Average UV-resistant colonies (±SD) from between four and nine determinations performed with two UV irradiations (6 J/m^2) and concurrent geneticin selection without sibling selection enrichment.

Isolation of Cosmid Pairs That Contained the Functional ERCC5 Repair Gene

Secondary transformant 38.4.4 genomic DNA was used for construction of a cosmid library to identify the *ERCC5* repair gene. Approximately 1.6×10^6 cosmid clones, or 20 (haploid) genome equivalents, yielded 101 cosmids that hybridized with a total human DNA probe. Cosmid DNAs were used to transform UV135 cells, but none of the individual cosmids conferred UV resistance to UV135. The cosmid clones were then characterized and assigned to six groups as described under Materials and Methods. These cosmid group assignments were established for the detection of overlaps between cosmid inserts, as *ERCC5* might have been cloned only in overlapping sections.

The cosmid groups were transformed into UV135 cells and transformants were selected with geneticin. The transformants were tested for UV resistance conferred to UV135 cells through reconstruction of a functional *ERCC5* gene by homologous cosmid–cosmid recombination. The cosmid vector sCos-1 was used as a geneticin-resistant, UV-sensitive control. One group of 16 cosmids yielded UV-resistant sibling selected transformant populations (Materials and Methods) from 6 of 12 independent transfer plates. None of the other cosmid groups, including the sCos-1 negative control and a group formed from all the cosmids isolated, resulted in UV-resistant UV135 transformants. The sporadic occurrence of UV-resistant, geneticin-resistant cotransformants observed with the functional group was probably due to the rarity of cosmid cotransfers that could yield a functional *ERCC5* gene. The sibling selection protocol was therefore necessary for the detection of rare UV-resistant cosmid cotransformants.

The cosmids in the active group were tested as groups of a few cosmids, followed by the testing of cosmid pairs. Pairs of cosmids designated cH44 plus cH75 (cH44/cH75) and cH44 plus cH91 (cH44/cH91) gave rise to high frequencies of UV-resistant transformants directly, without requiring sibling selection. About 20% of the cH44/cH75 geneticin-resistant transformants were UV-resistant (data not shown). Cosmid pair cH44/cH75 yielded more than three times as many UV-resistant clones as cosmid pair cH44/cH91 (Table 1). These results suggested that cosmid cH44 contained one end of *ERCC5,* and cosmids cH75 and cH91 overlapped cosmid cH44 and contained additional *ERCC5* sequences required for functionality.

Physical Mapping of ERCC5

The inserts of cosmids cH44, cH75, and cH91 were mapped for restriction endonuclease sites as described under Materials and Methods. The cosmid maps were aligned with each other, and it was confirmed that cosmids cH75 and cH91 overlapped cosmid cH44 (Fig. 1A). The overlap between cH75 and cH44 (12.2 kbp) was greater than that between cH91 and cH44 (2.5 kbp) (Fig. 1A), which supported the previous observation that cosmid pair cH44/cH75 resulted in more UV-resistant colonies than cH44/cH91 (Table 1). Four additional cosmid clones that contained cH44 sequences had also been assigned to the active cosmid group. Two of these additional cosmid clones, designated cH80 and cH83, were mapped to augment the restriction site data obtained from cH44. The smallest cosmid-pair overlap that resulted in UV-resistant colonies was 1.1 kbp between cH83 and cH91 (Table 1); cH80 did not overlap the cosmids containing the other end of *ERCC5* and therefore did not yield UV-resistant colonies (Table 1). The dependence of UV^r transformant frequency on extent of cosmid overlap is completely consistent

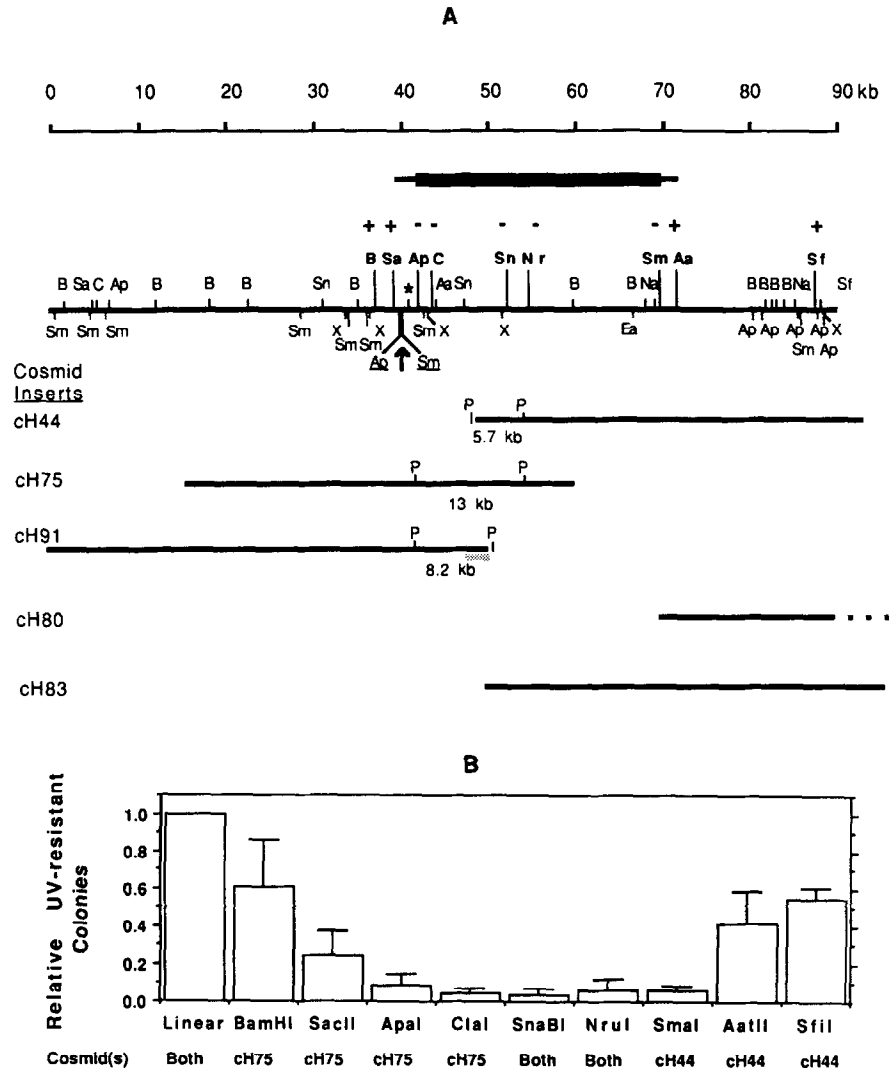

FIG. 1. (A) Restriction and functional map of the human nucleotide excision repair gene *ERCC5*. The letters represent the following restriction sites: Aa, *Aat*II; Ap, *Apa*I; B, *Bam*HI; C, *Cla*I; Ea, *Eag*I; Na, *Nar*I; Nr, *Nru*I; Sa, *Sac*II; Sf, *Sfi*I; Sm, *Sma*I; Sn, *Sna*BI; X, *Xho*I. *Not*I sites flank the cosmid inserts, and there is a unique *Pvu*I site in the sCos-1 vector (6). The asterisk denotes a *Sac*II site that was about 90% refractory to digestion. Restriction sites shown in boldface letters above the general map were used to determine the *ERCC5* boundaries. Restriction sites that inactivated the UV-resistance phenotype were interpreted to lie within an essential region of *ERCC5*. The thick black bar represents the minimum functional extent, and the thin black bar represents the maximum functional extent of the human repair gene *ERCC5*. The letter P represents selected *Pst*I sites in the cosmids cH44, cH75, and cH91. The *Pst*I fragments and their sizes shown are vector–insert junction fragments for cH44 and cH91 and an insert fragment for cH75. These fragments were used to demonstrate that intercosmid recombination actively reconstructed the *ERCC5* gene in the UV135 host cells as described in Fig. 2. The gray box indicates the cH91 fragment from the *Sna*BI site to the sCos-1 *Not*I site which was used as described in Fig. 2. The arrow indicates the cH75 *Apa*I to *Sma*I fragment, shown in underlined letters beneath the map, which was used as described in Fig. 3. (B) Relative numbers of UV-resistant transformants from restriction endonuclease digestions of cosmid pair cH44 and cH75. The cosmid(s) digested is shown for each indicated restriction endonuclease. Eight determinations were performed by UV irradiation and direct selection in geneticin as described in the text. The values given are the mean number (±SD) of UV-resistant transformants from the indicated restriction endonuclease digestions relative to the number determined when both cosmids were linearized at the *Pvu*I site of sCos-1 ("Linear"); the "linear" cosmid transformations averaged 77 ± 19 transformants/μg of each cosmid.

with a recombinatory mechanism of *ERCC5* reconstruction.

The functional boundaries of *ERCC5* were assigned by determining which restriction endonucleases inactivated the repair gene. Cosmid cH44, cosmid cH75, or both cH44 and cH75 were digested prior to transformation of UV135 cells as described under Materials and Methods. The results of cosmid restriction endonuclease digestion on UV-resistant colony formation are shown in Fig. 1B. Unique sites and sites within the repair gene were the relevant sites for this determination (in boldface letters, Fig. 1A). Restric-

tion sites (e.g., *Nru*I) contained in the overlap region did not eliminate *ERCC5* function unless both cosmids were digested. This demonstrated that cosmid overlap was crucial for *ERCC5* functionality and is consistent only with a model in which intercosmid recombination reconstructed a single, functional repair gene. The region from the cH75 *Apa*I sites to the inactivating cH44 *Sma*I site was required for *ERCC5* activity, as shown by the thick black bar in Fig. 1A. The functional ends of *ERCC5* appeared to lie between the cH75 *Sac*II site and the cH44 *Aat*II site. These results gave an approximate minimum functional gene size of 29 kbp and a maximum functional size of 34 kbp (Fig. 1A).

ERCC5 Reconstruction by Intercosmid Recombination

To demonstrate further that UV135 cells reconstructed *ERCC5* by intercosmid recombination, recombinant fragments were determined for cH44/cH91 cotransformants. Genomic DNA from independent cosmid-pair cotransformants was digested with restriction endonuclease *Pst*I and analyzed using a fragment from one end of cH91 (gray box, Fig. 1A) as the hybridization probe. The cosmid pair cH44/cH75 gave rise to two *Pst*I fragments in cotransformant DNA, one from cH44 insertions and the other resulting from either cH75 insertions or intercosmid recombinant insertion (Fig. 2 and shown diagrammatically in Fig. 1A). The cosmid pair cH44/cH91 gave rise to insertion bands from cH44 and cH91, plus a fragment identical to the cH75 cosmid insert (Fig. 2 and Fig. 1A). Cosmids cH44 and cH91 had recombined to lose their junction fragments and form a full-length *ERCC5* fragment. The results shown here support the conclusion that intercosmid recombination reconstructed the functional *ERCC5* gene.

Confirmation of ERCC5 Isolation by Southern Analysis

Southern hybridization analysis was used to confirm that several independent UV-resistant UV135 cotransformants and various UV135/human hybrid cell lines contained sequences from our cloned *ERCC5* gene. These independent, UV-resistant derivatives of UV135 were very unlikely to have these human sequences unless our cosmids contained the complementing human repair gene *ERCC5*. Genomic DNAs were digested with *Eco*RI and hybridized with a human DNA fragment from one end of *ERCC5* (arrow, Fig. 1A). The repair-proficient HeLa and HSF22 cell lines were used as positive controls, and the UV-sensitive CHO cell line UV135 as a negative control. UV-resistant UV135/human hybrid cell lines 135HL2 and 135HL30 (Thompson *et al.*, 1987), the

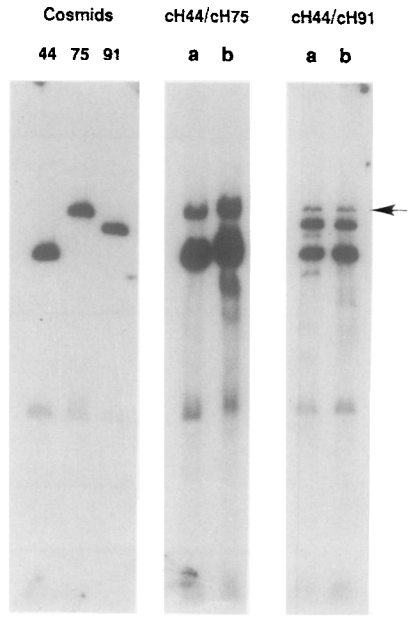

FIG. 2. Southern hybridization analysis of recombinant cosmids in cosmid-transformed cell lines. Cosmid DNAs and genomic DNAs from two independent cosmid-pair transformants for cH44/cH75 and cH44/cH91 were digested with the restriction endonuclease *Pst*I, electrophoresed on an 0.8% agarose gel, transferred to a Zetabind membrane, and hybridized. The radioactively labeled probe used was the cH91 *Sna*BI to *Not*I fragment (Fig. 1A), which hybridized with the vector–insert *Pst*I junction fragments of cH44 and cH91 and a cosmid insert band of cH75 (Fig. 1A); each of these hybridizing *Pst*I fragments was a different size. A hybridizing fragment not found in cH44 or cH91, but identical to the native repair gene (as contained in cH75), was observed in independent cH44/cH91 transformants (indicated by arrow).

three independent primary cotransformants, and the derived secondary and tertiary cotransformants were analyzed for the presence of *ERCC5* sequences. The UV-sensitive segregant 135HL2.1, obtained from the repair-proficient UV135/human hybrid cell line 135HL2 (Thompson *et al.*, 1987), and independent UV-sensitive spontaneous segregants 38.4(S6) and 38.4(S7) of the secondary cotransformant were analyzed to determine whether loss of UV resistance would correspond with loss of human *ERCC5* sequences.

The results of this hybridization analysis are shown in Fig. 3. All of the UV-resistant cell lines examined had a single *Eco*RI fragment (~13 kbp) that hybridized with the *ERCC5* probe, and the UV-sensitive segregants and UV135 did not contain these sequences. The human *ERCC5* fragment exhibited 100% concordance with the UV resistance phenotype in human-derived cotransformants and three UV135/human hybrids. Six additional UV-resistant UV135/human hybrid lines and UV-sensitive segregants (Thompson *et al.*, 1987) have provided further confirmation of these hybridization results (data not

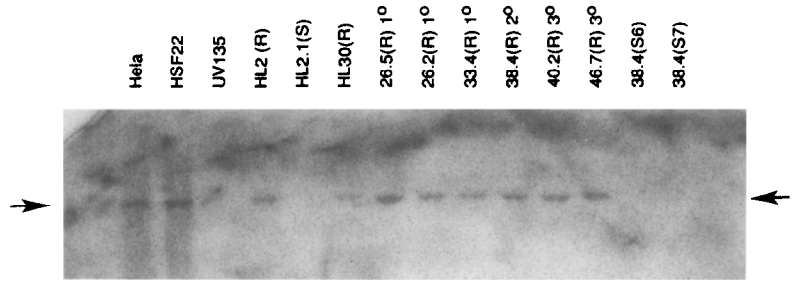

FIG. 3. Southern hybridization analysis of UV-resistant and UV-sensitive cell lines that contained human DNA sequences. Genomic DNA (10 μg) was digested to completion with the restriction endonuclease *Eco*RI, electrophoresed on an 0.8% agarose gel, transferred to a Zetabind membrane, and hybridized as described under Materials and Methods. The radioactively labeled probe used was the indicated *Apa*I to *Sma*I fragment (~400 bp) from cH75 (Fig. 1A, arrow), which was blocked to C_0t 120 as described (23). The cell lines examined and their UV resistances were as follows: (i) HeLa, UV^r human cell line; (ii) HSF22, UV^r human cell line; (iii) UV135, UV^s CHO cell line; (iv) 135HL2, UV^r human/CHO hybrid cell line; (v) 135HL2.1, UV^s segregant from 135HL2; (vi) 135HL30, UV^r human/CHO hybrid cell line; (vii) 26.5.1, UV^r primary transformant cell line; (viii) 26.2.2, UV^r primary transformant cell line; (ix) 33.4.2, UV^r primary transformant cell line; (x) 38.4.4, UV^r secondary transformant cell line; (xi) 40.2.8, UV^r tertiary transformant cell line; (xii) 46.7D.1, UV^r tertiary cell line; (xiii) 38.4(S6), UV^s segregant from 38.4.4; (xiv) 38.4 (S7), UV^s segregant from 38.4.4.

shown). The 100% concordance of hybridization with the UV resistance phenotype confirmed that we have isolated the functional human *ERCC5* locus previously assigned to chromosome 13q.

ERCC5 Complementation of UV135 Sensitivity to UV Light

Transformants of UV135 were assayed for their cellular UV resistance to determine the ability of the human *ERCC5* repair gene to complement the genetic defect. Three independent UV-resistant transformants were isolated from the cosmid-pair transfers cH44/cH75 and cH44/cH91. The 37% survival dose (D_{37}) for the parental AA8 cell line was approximately 9.4 J/m^2, while the D_{37} of the UV-sensitive mutant was 1.5 J/m^2, or about 16% that of AA8 (Fig. 4A). By comparison, the secondary cotransformant, 38.4.4, and cotransformants derived from cosmid pairs had D_{37} values of 7.8 and 6.5 J/m^2, respectively (Fig. 4A). The cosmid-pair cotransformant survival levels were significantly lower than the AA8 levels (about 70% AA8) when the data were compared in a *t*-test analysis (95% confidence level), but were fourfold more resistant to UV radiation than the nucleotide excision repair-deficient UV135 host cells.

ERCC5 Complementation of Shuttle Vector Repair in UV135

The ability of *ERCC5* to reverse the repair defect of UV135 cells was also examined by analyzing repair of shuttle vector DNA. It has been previously demonstrated that DNA damage can inactivate a shuttle vector *cat* gene. The reactivation of *cat* gene expression is an effective indicator of host cell repair proficiency (Klocker *et al.*, 1985; Protić-Sabljić and Kraemer, 1985, 1986) and can be used to assay cloned repair gene function (Henderson *et al.*, 1989). The shuttle vector pSV2catSVgpt was irradiated with UV light and transfected into unirradiated CHO cells. CAT enzyme was subsequently extracted and assayed as described under Materials and Methods. In this repair assay, the parental cell line AA8 had a D_{37} value of 815 J/m^2, while the UV-sensitive mutant UV135 had a D_{37} of 190 J/m^2, four- to fivefold lower (Fig. 4B). The secondary cotransformant, 38.4.4, and the independent *ERCC5* cotransformants (from cosmid pairs cH44/cH75 and cH44/cH91) had D_{37} values of about 670 J/m^2 (Fig. 4B). Although the cotransformant *cat* repair levels were about 82% of parental AA8 levels, these survival levels were not significantly different from AA8 levels when the data were compared in a *t*-test analysis (95% confidence level), suggesting that this *cat* repair assay slightly muted the apparent cotransformant repair deficiencies observed with the cellular resistance studies.

DISCUSSION

The autosomal recessive human syndrome xeroderma pigmentosum results in abnormal sensitivity to ultraviolet radiation and an extremely high incidence of skin cancers, which often lead to premature death (Friedberg, 1985). The biochemical defect in most XP cell lines has been reported to involve nucleotide excision repair, the major pathway for the removal of UV-induced pyrimidine dimers and chemical adducts in DNA (Friedberg, 1985). In an attempt to understand the role of DNA repair in the basic mechanisms of carcinogenesis, human nucleotide excision repair (*ERCC*) genes are being isolated and characterized. In this article, we described the isolation and preliminary characterization of the human DNA repair gene *ERCC5*.

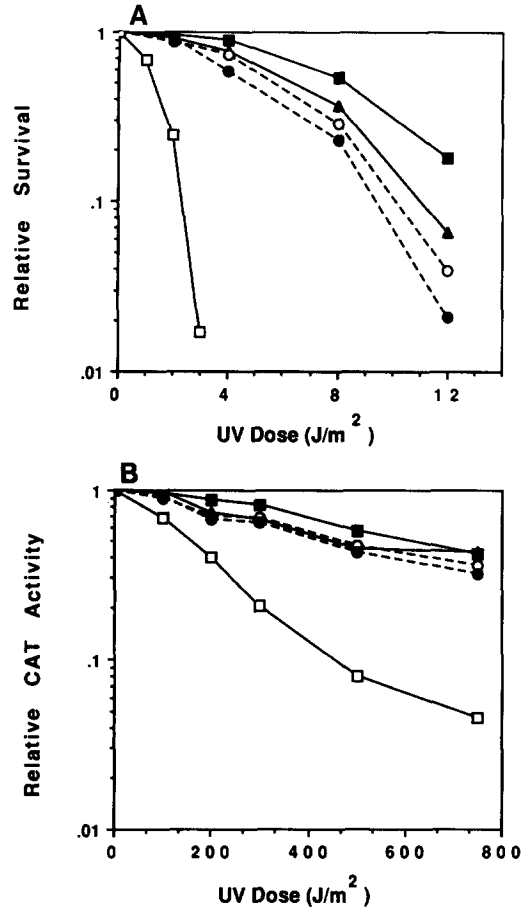

FIG. 4. (A) Relative survival of UV-irradiated parental AA8, mutant UV135, secondary transformant 38.4.4, and cosmid-pair transformant cell lines. Mammalian cells were plated at 400 cells per 100-mm plate 4 h prior to UV irradiation, at which time the medium was removed, the cells were washed once with PBS, and the aspirated plates were irradiated with UV light (254 nm). The plates were stained 1 week later for determination of surviving colony numbers. The solid curves are the average data of four determinations for AA8 (■) and UV135 cells (□) and two determinations for the secondary transformant 38.4.4 (▲). The broken curves are the average data for three independent transformant clones, each assayed twice, for each of the cosmid-pair transformants cH44/cH75 (○) and cH44/cH91 (●). (B) Relative CAT activity of UV-irradiated pSVcatSVgpt in parental AA8, mutant UV135, secondary transformant 38.4.4, and cosmid-pair transformant cell lines. Shuttle vector irradiation, transfection, and extraction and analysis of CAT enzyme activities were performed as described under Materials and Methods. The data plotted are the ratios of the extracted CAT activity (in cpm) at a given dose relative to the CAT activity at zero dose for each cell line analyzed. The solid curves are the average data of four determinations for AA8 (■), UV135 cells (□), and the secondary transformant 38.4.4 (▲). The broken curves are the average data for three independent transformant clones, each assayed twice, for each of the cosmid-pair transformants cH44/cH75 (○) and cH44/cH91 (●).

ERCC5 was cloned on overlapping cosmids and is the largest human nucleotide excision repair gene to be isolated as a functional genomic clone. *ERCC5* is about 32 kbp in size and could have been contained in one cosmid, but the isolated cosmids did not contain the complete *ERCC5* gene on a single insert. Cosmid grouping was therefore crucial for *ERCC5* isolation and identification. Cosmid grouping could also have been beneficial if *ERCC5* did not contain detectable human repetitive sequences, but flanking sequences did. Flanking sequences that contained human repetitive DNA would have been rescued, and the repair gene region reconstructed by cosmid alignment. For this reason, nonhybridizing cosmid fragments were also examined in the assignment of cosmid groups to identify regions of cosmid overlap that did not contain human repetitive sequences. After the transformation of UV135 cells with the cosmid groups, the sibling selection enrichment protocol enabled the detection of (rare) UV-resistant cosmid cotransformants.

The functional cosmid group comprised 16 cosmids, 7 of which contained *ERCC5* sequences. Only 4 additional cosmids, of the 100 tested, were found to contain *ERCC5* sequences. These results indicate that the cosmid-grouping approach efficiently yielded overlapping sets of cosmids. When the cosmid clones were linearized with *Pvu*I or *Not*I, the number of UV-resistant transformants increased about fourfold. Since highly efficient recombination and integration occurred with between 1 and 12 kbp of insert overlap, transformation with grouped and linearized cosmids is a very effective method of isolating and identifying functional genes carried on overlapping clones. Genetic domains of much larger size (>100 kbp), requiring more than 2 cosmid inserts to contain a functional gene, could be reconstructed by this approach as well.

Other investigators have used overlapping cosmids, but not extensive cosmid grouping, to reconstruct functional genes. Cosmid walking was used to locate overlapping clones containing gene sequences in the isolation of a mouse repair gene that complements human xeroderma pigmentosum group-A cells (Tanaka *et al.*, 1989). The thymidylate synthetase gene was completed by the direct cloning of overlapping genomic fragments (Deng *et al.*, 1986). Six *Alu*-positive clones isolated for the human thymidine kinase gene were used in pairwise transfers to reconstitute a functional gene (Lin *et al.*, 1983). In the present study, however, more than 100 cosmids were assigned to groups on the basis of potential overlapping regions without prior information on the location of the repair gene, and a functional group and active cosmid pairs were efficiently identified. By this approach, many cosmid clones can be quickly assayed for a large or rare functional gene.

Hybrid (UV135/human) cell lines had previously been used to determine indirectly that the repair gene *ERCC5* was located on human chromosome 13 between q14 and q34 (Thompson *et al.*, 1987). Probe

DNA derived from the functional region of *ERCC5* hybridized with human cell lines and UV-resistant transformants of UV135. Due to the 100% concordance of the human DNA hybridization probe with the UV resistance phenotype, this DNA was derived from the human locus that complements the UV135 repair deficiency, previously designated *ERCC5*. Preliminary experiments have also demonstrated that a UV-sensitive mouse cell line that lacks *ERCC5* activity, designated Q31 (Hori *et al.*, 1983), is complemented by the cH44 and cH75 cosmid pair (unpublished results).

DNA repair levels, as assayed by shuttle vector *cat* gene reactivation, were returned to near normal (82%) by transforming UV135 with *ERCC5*. Shuttle vector systems assay *cat* transcriptional activity, which is inhibited by both cyclobutane dimers and (6-4)photoproducts in CHO host cells (Mitchell and Nairn, 1989; Protić-Sabljić and Kraemer, 1986). Reactivation of *cat* gene expression (shuttle vector repair) was reported to accurately reflect overall host cell DNA repair capabilities (Klocker *et al.*, 1985; Henderson *et al.*, 1989; Protić *et al.*, 1988; Protić-Sabljić and Kraemer, 1985). In this assay, the UV-sensitive mutant UV135 reactivated the *cat* gene at levels about 23% that of the parental AA8 cells, a difference less than the cell survival differences observed between these two cell lines in a previous study (15%) (Thompson *et al.*, 1982) and in this study (16%). This repair difference is also less than that previously observed by assaying postirradiation strand incision, which showed a UV135 repair level between 4 and 21% of the AA8 level, depending on UV dose (Thompson *et al.*, 1982). The difference between the repair assays is probably due to the much higher density of dimers used in the *cat* assays, as well as to the CAT enzyme assay itself, which reflects *cat* gene repair indirectly. However, the advantages of using shuttle vector reactivation to analyze cellular repair proficiency are that the target substrate for damage and repair is defined and the *in vitro* conditions of irradiation allow defined lesions to be introduced into the substrate without chromosomal damage and possible unrelated metabolic effects on the cells. These advantages offset the fact that *cat* assays are probably not as sensitive as other methods of repair analysis.

The first five CHO complementation groups are deficient in postirradiation strand-incising activity (Thompson *et al.*, 1982), cyclobutane dimer removal (Regan *et al.*, 1990), and the repair of (6-4)photoproducts (Mitchell *et al.*, 1988). The cloned repair gene *ERCC2* reverted the repair-deficient CHO mutant (UV5) to wild-type UV resistance, mutation induction, and strand incision levels (Weber *et al.*, 1988), as well as to normal levels of cyclobutane dimer excision (Regan *et al.*, 1990). *ERCC1* transformants had chemical resistance, mutation induction, cyclobutane dimer removal (Zdzienicka *et al.*, 1987), and preferential repair (Bohr *et al.*, 1988) levels near those of wild-type, but cellular resistance to the cytotoxic effects of UV radiation was slightly deficient (Westerveld *et al.*, 1984; Zdzienicka *et al.*, 1987). *ERCC5*-transformed UV135 cells also appeared to have less than normal (70% AA8) cellular resistance to UV radiation. All six cosmid-pair transformants (three per pair) appeared to be slightly deficient. The UV sensitivities of the first five CHO complementation groups, including *ERCC5*, are reported to correlate better with (6-4)photoproduct repair than with cyclobutane dimer repair (Mitchell and Nairn, 1989; Mitchell *et al.*, 1988). The defect in (6-4)photoproduct repair is completely restored by *ERCC2*, but only partially by *ERCC1* (Mitchell and Nairn, 1989), possibly accounting for the differences observed in complementation of cytotoxic lesion repair. One possible reason that *ERCC5* transformants had slightly deficient resistances to UV irradiation may therefore be an inability of *ERCC5*-transformed UV135 cells to completely repair (6-4)photoproducts. Further investigations into the relative rates and extents that different lesions are repaired by *ERCC5* transformants are required to further elucidate the activities of *ERCC5*.

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REFERENCES

1. Bohr, V. A., Chu, E. H. Y., van Duin, M., Hanawalt, P. C., and Okumoto, D. S. (1988). Human repair gene restores normal pattern of preferential DNA repair in repair defective CHO cells. *Nucleic Acids Res.* **16:** 7397–7403.
2. Bohr, V. A., Okumoto, D. S., and Hanawalt, P. C. (1986). Survival of UV-irradiated mammalian cells correlates with efficient DNA repair in an essential gene. *Proc. Natl. Acad. Sci. USA* **83:** 3830–3833.
3. Busch, D., Greiner, C., Lewis, K., Ford, R., Adair, G., and Thompson, L. (1989). Summary of complementation groups of UV-sensitive CHO cell mutants isolated by large-scale screening. *Mutagenesis* **4:** 349–354.
4. Deng, T. D., Li, D., Jenh, C.-H., and Johnson, L. F. (1986). Structure of the gene for mouse thymidylate synthase. *J. Biol. Chem.* **261:** 16,000–16,005.
5. Dulhanty, A. M., Rubin, J. S., and Whitmore, G. F.

(1988). Complementation of the DNA-repair defect in a CHO mutant by human DNA that lacks highly abundant repetitive sequences. *Mutat. Res.* **194:** 207–217.

6. EVANS, G. A., LEWIS, K., AND ROTHENBERG, B. E. (1989). High efficiency vectors for cosmid microcloning and genomic analysis. *Gene* **79:** 9–20.
7. FRIEDBERG, E. C. (1985). "DNA Repair," Freeman, San Francisco.
8. HENDERSON, E. E., VALERIE, K., GREEN, A. P., AND DE RIEL, J. K. (1989). Host cell reactivation of CAT-expression vectors as a method to assay for cloned DNA-repair genes. *Mutat. Res.* **220:** 151–160.
9. HOEIJMAKERS, J. H. J., VAN DUIN, M., WEEDA, G., VAN DER EB, A. J., TROELSTRA, C., EKER, A. P. M., JASPERS, N. G. J., WESTERVELD, A., AND BOOTSMA, D. (1988). Analysis of mammalian excision repair: From mutants to genes and gene products. *In* "Mechanisms and Consequences of DNA Damage Processing" (E. C. Friedberg and P. C. Hanawalt, Eds.), UCLA Symposium on Molecular and Cellular Biology New Series, Vol. 83, pp. 281–287, A. R. Liss, New York.
10. HORI, T., SHIOMI, T., AND SATO, K. (1983). Human chromosome 13 compensates a DNA repair defect in UV-sensitive mouse cells by mouse–human cell hybridization. *Proc. Natl. Acad. Sci. USA* **80:** 5655–5659.
11. KLOCKER, H., SCHNEIDER, R., BURTSCHER, H. J., AUER, B., HIRSCH-KAUFFMANN, M., AND SCHWEIGER, M. (1985). Transient expression of a plasmid gene, a tool to study DNA repair in human cells: Defect of DNA repair in Cockayne syndrome; one thymine dimer is sufficient to block transcription. *Eur. J. Cell Biol.* **39:** 346–351.
12. LEHAMN, A. R. (1985). Use of recombinant techniques in cloning DNA repair genes and in the study of mutagenesis in mammalian cells. *Mutat. Res.* **150:** 61–67.
13. LIN, P.-F., ZHAO, S.-Y., AND RUDDLE, F. H. (1983). Genomic cloning and preliminary characterization of the human thymidine kinase gene. *Proc. Natl. Acad. Sci. USA* **80:** 6528–6532.
14. MACINNES, M. A., BINGHAM, J. M., THOMPSON, L. H., AND STRNISTE, G. F. (1984). DNA-mediated cotransfer of excision repair capacity and drug resistance into Chinese hamster ovary mutant cell line UV-135. *Mol. Cell. Biol.* **4:** 1152–1158.
15. MELLON, I., BOHR, V. A., SMITH, C. A., AND HANAWALT, P. C. (1986). Preferential repair of an active gene in human cells. *Proc. Natl. Acad. Sci. USA* **83:** 8878–8882.
16. MITCHELL, D. L., HUMPHREY, R. M., ADAIR, G. M., THOMPSON, L. H., AND CLARKSON, J. M. (1988). Repair of (6-4)photoproducts correlates with split-dose recovery in UV-irradiated normal and hypersensitive rodent cells. *Mutat. Res.* **193:** 53–63.
17. MITCHELL, D. L., AND NAIRN, R. S. (1989). The biology of the (6-4)photoproduct. *Photochem. Photobiol.* **49:** 805–819.
18. PROTIĆ, M., ROILIDES, E., LEVINE, A. S., AND DIXON, K. (1988). Enhancement of DNA repair capacity of mammalian cells by carcinogen treatment. *Somat. Cell Mol. Genet.* **14:** 351–357.
19. PROTIĆ-SABLJIĆ, M., AND KRAEMER, K. H. (1985). One pyrimidine dimer inactivates expression of a transfected gene in xeroderma pigmentosum cells. *Proc. Natl. Acad. Sci. USA* **82:** 6622–6626.
20. PROTIĆ-SABLJIĆ, M., AND KRAEMER, K. H. (1986). Reduced repair of non-dimer photoproducts in a gene transfected into xeroderma pigmentosum cells. *Photochem. Photobiol.* **43:** 509–513.
21. REGAN, J. D., THOMPSON, L. H., CARRIER, W. L., WEBER, C. A., FRANCIS, A. A., AND ZDZIENICKA, M. Z. (1990). Cyclobutane-pyrimidine dimer excision in UV-sensitive CHO mutants and the effect of the human *ERCC2* repair gene. *Mutat. Res.* **235:** 157–163.
22. SAMBROOK, J., FRITSCH, E. F., AND MANIATIS, T. (1989). "Molecular Cloning: A Laboratory Manual," Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
23. SEALEY, P. G., WHITTAKER, P. A., AND SOUTHERN, E. M. (1985). Removal of repeated sequences from hybridisation probes. *Nucleic Acids Res.* **13:** 1905–1922.
24. SLEIGH, M. L. (1986). A nonchromatic assay for expression of the chloramphenicol acetyltransferase gene in eukaryotic cells. *Anal. Biochem.* **156:** 251–256.
25. STRNISTE, G. F., CHEN, D. J.-C., DEBRUIN, D., LUKE, J. A., MCCOY, L. S., MUDGETT, J. S., NICKOLS, J. W., OKINAKA, R. T., TESMER, J. G., AND MACINNES, M. A. (1988). Restoration of Chinese hamster cell radiation resistance by the human repair gene *ERCC5* and progress in molecular cloning of this gene. *In* "Mechanisms and Consequences of DNA Damage Processing" (E. C. Friedberg and P. C. Hanawalt, Eds.), UCLA Symposium on Molecular and Cellular Biology New Series, Vol. 83, pp. 301–306, A. R. Liss, New York.
26. TANAKA, K., SATOKATA, I., OGITA, Z., UCHIDA, T., AND YOSHIO, O. (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.
27. THOMPSON, L. H., BACHINSKI, L. L., STALLINGS, R. L., DOLF, G., WEBER, C. A., WESTERVELD, A., AND SICILIANO, M. J. (1989). Complementation of repair gene mutations on the hemizygous chromosome 9 in CHO: A third repair gene on human chromosome 19. *Genomics* **5:** 670–679.
28. THOMPSON, L. H., BROOKMAN, K. W., DILLEHAY, L. E., MOONEY, C. L., AND CARRANO, A. V. (1982). Hypersensitivity to mutation and sister-chromatid-exchange induction in CHO cell mutants defective in incising DNA containing UV lesions. *Somat. Cell Genet.* **8:** 759–773.
29. THOMPSON, L. H., AND CARRANO, A. V. (1983). Analysis of mammalian cell mutagenesis and DNA repair using in vitro selected CHO cell mutants. *In* "Cellular Responses to DNA Damage" (E. C. Friedberg and B. R. Bridges, Eds.), UCLA Symposium on Molecular and Cellular Biology New Series, Vol. 11, pp. 125–143, A. R. Liss, New York.
30. THOMPSON, L. H., CARRANO, A. V., SATO, K., SALAZAR, E. P., WHITE, B. F., STEWART, S. A., MINKLER, J. L., AND SICILIANO, M. J. (1987). Identification of nucleotide-excision-repair genes on human chromosomes 2 and 13 by functional complementation in hamster–human hybrids. *Somat. Cell Mol. Genet.* **13:** 539–551.
31. THOMPSON, L. H., MOONEY, C. L., BUKHART-SCHULTZ, K., CARRANO, A. V., AND SICILIANO, M. J. (1985). Correction of a nucleotide-excision-repair mutation by human chromosome 19 in hamster–human hybrid cells. *Somat. Cell Mol. Genet.* **11:** 87–92.
32. THOMPSON, L. H., SHIOMI, T., SALAZAR, E. P., AND STEWART, S. A. (1988). An eighth complementation group of rodent cells hypersensitive to ultraviolet radiation. *Somat. Cell Mol. Genet.* **14:** 605–612.
33. VAN DUIN, M., DE WIT, J., ODIJK, H., WESTERVELD, A., YASUI, A., KOKEN, M. H. M., HOEIJMAKERS, J. H. J., AND BOOTSMA, D. (1986). Molecular characterization 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.
34. VAN DUIN, M., VAN DEN TOL, J., WARMERDAM, P., ODIJK, H., MEIJER, C., WESTERVELD, A., BOOTSMA, D., AND HOEIJ-

MAKERS, J. H. J. (1988). Evolution and mutagenesis of the mammalian excision repair gene *ERCC-1*. *Nucleic Acids Res.* **16:** 5305–5322.

35. WEBER, C. A., SALAZAR, E. P., STEWART, S. A., AND THOMPSON, L. H. (1988). Molecular cloning and biological characterization of a human gene, *ERCC2*, that corrects the nucleotide excision repair defect in CHO UV5 cells. *Mol. Cell. Biol.* **8:** 1137–1146.
36. WEBER, C. A., SALAZAR, E. P., STEWART, S. A., AND THOMPSON, L. H. (1990). *ERCC2*: cDNA cloning and molecular characterization of a human nucleotide excision repair gene with high homology to yeast RAD3. *EMBO J.* **9:** 1437–1447.
37. WEEDA, G., VAN HAM, R. C. A., MASUREL, R., WESTERVELD, A., ODIJK, H., DE WIT, J., BOOTSMA, D., VAN DER EB, A. J., AND HOEIJMAKERS, J. H. J. (1990). Molecular cloning and biological characterization of the human excision repair gene *ERCC-3*. *Mol. Cell. Biol.* **10:** 2570–2581.
38. WESTERVELD, A., HOEIJMAKERS, J. H. J., VAN DUIN, M., DE WIT, J., ODIJK, H., PASTINK, A., WOOD, R. D., AND BOOTSMA, D. (1984). Molecular cloning of a human DNA repair gene. *Nature (London)* **310:** 425–429.
39. ZDZIENICKA, M. Z., ROZA, L., WESTERVELD, A., BOOTSMA, D., AND SIMONS, J. W. I. M. (1987). Biological and biochemical consequences of the human *ERCC-1* repair gene after transfection into a repair-defective CHO cell line. *Mutat. Res.* **183:** 69–74.
40. ZDZIENICKA, M. Z., VAN DER SCHANS, G. P., AND SIMONS, J. W. I. M. (1988). Identification of a new seventh complementation group of UV-sensitive mutants in Chinese hamster cells. *Mutat. Res.* **194:** 165–170.