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Localization of the Nucleotide Excision Repair Gene *ERCC6* to Human Chromosome 10q11–q21

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We have cloned the human DNA excision repair gene *ERCC6* by virtue of its ability to correct the uv sensitivity of Chinese hamster ovary cell mutant UV61. This mutant is a member of complementation group 6 of the nucleotide excision repair-deficient rodent mutants. By means of *in situ* hybridization and Southern blot analysis of mouse × human somatic cell hybrids, the gene was localized to human chromosome 10q11–q21. An RFLP detected within the *ERCC6* locus can be helpful in linkage analysis.

INTRODUCTION

To protect DNA from deleterious accumulation of damage and permanent mutations, an intricate network of DNA repair systems has evolved (reviewed by Friedberg, 1985). One of the major DNA repair processes is the nucleotide excision repair pathway. This system removes a broad range of DNA lesions, such as uv-induced cyclobutane pyrimidine dimers and (6–4) photoproducts, bulky chemical adducts, and DNA crosslinks.

Two human genetic diseases in which the excision repair mechanism is defective are known: xeroderma pigmentosum (XP) and Cockayne syndrome (CS). The autosomal, recessive disorder XP is clinically characterized by extreme sensitivity of the skin to sunlight (uv), pigmentation abnormalities, predisposition to skin cancer, and frequently neurological complications (Cleaver and Kraemer, 1989). Cell fusion experiments have identified at least seven excision-deficient XP complementation groups (De Weerd-Kastelein *et al.*, 1972; Vermeulen *et al.*, 1991, and references therein). CS patients exhibit sun sensitivity, dwarfism, microcephaly, wizened appearance, deafness, and severe mental retardation. CS is, unlike XP, not associated with an elevated risk for skin tumor formation (reviewed by Lehmann, 1987). Recently, CS cells were found to be disturbed in a subpathway of the nucleotide excision repair: the preferential repair of actively transcribed genes (Venema *et al.*, 1990). Also, CS is genetically heterogeneous; at least three complementation groups have been identified (Lehmann, 1982).

A second class of mammalian excision repair-deficient cell lines consists of laboratory-induced, uv-sensitive, rodent cell lines. Eight complementation groups have been identified (reviewed by Collins and Johnson, 1987; Busch *et al.*, 1989). So far, only one overlap between these two classes has been detected: the gene correcting XP complementation group B is identical to the gene correcting complementation group 3 of the rodent mutant cell lines (Weeda *et al.*, 1990).

Recently, we have cloned the human *ERCC6* gene (*e*xcision *r*epair *c*ross *c*omplementing rodent repair deficiency). This gene is capable of correcting a uv-sensitive CHO mutant belonging to group 6: UV61 (Troelstra *et al.*, 1990). Mutant UV61 is remarkable in harboring a specific deficiency in the repair of cyclobutane pyrimidine dimers and bulky chemical adducts, but permitting apparently normal repair of (6–4) photoproducts (Thompson *et al.*, 1988). This suggests that the gene product affected in UV61 is, directly or indirectly, involved in damage recognition. Whether *ERCC6* is implicated in one of the XP or CS complementation groups remains to be elucidated.

Here we report the chromosomal localization of the *ERCC6* gene by *in situ* hybridization with biotinylated cDNA probes and Southern blot hybridization of *ERCC6* probes to DNA of somatic cell hybrids. Furthermore, an RFLP that can be used for linkage analysis has been identified within the *ERCC6* locus.

MATERIALS AND METHODS

In situ hybridization. Treatment of human lymphocyte metaphase spreads prior to hybridization was as described by Weeda *et al.* (1991).

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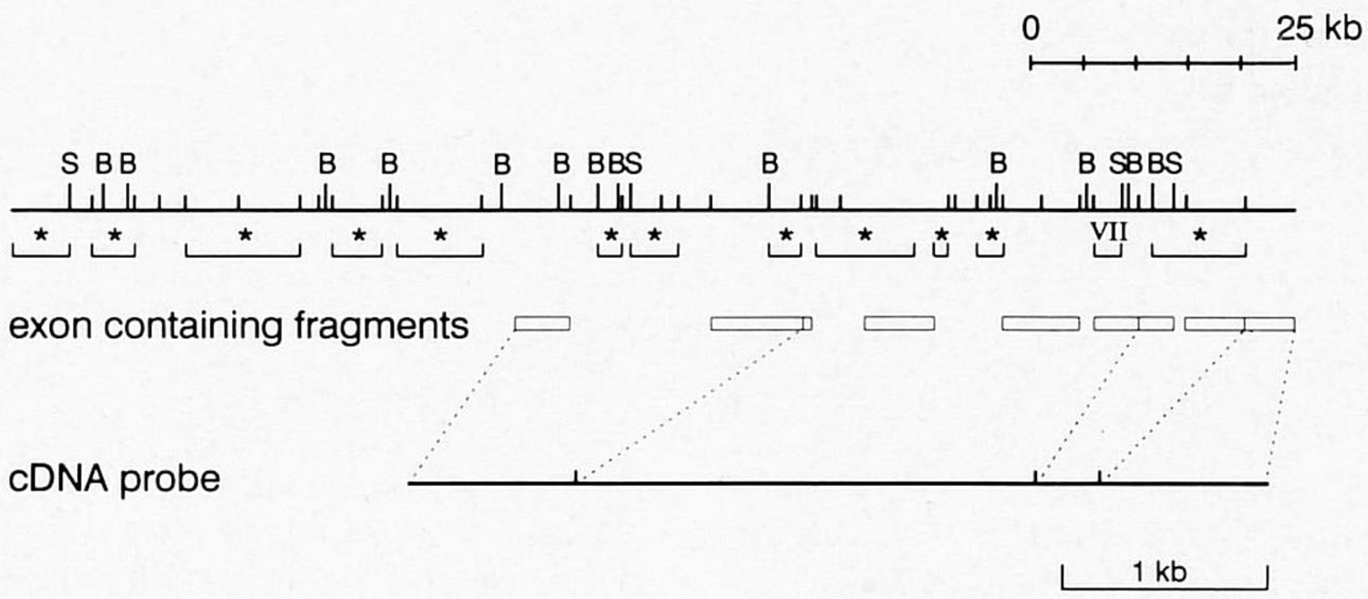

FIG. 1. Restriction map of the human *ERCC6* locus. The cDNA probe used for *in situ* hybridizations and the genomic probe VII (see Troelstra *et al.*, 1990) used on Southern blots are shown. Exon containing fragments recognized by the cDNA probe are indicated. Short bars represent *Eco*RI restriction sites. Symbols: B, *Bam*HI; S, *Sal*I; *, repeat containing fragment; VII, genomic probe VII.

In situ hybridization experiments using the *ERCC6* cDNA fragment shown in Fig. 1 or cDNAs extending more 5′, a chromosome 8-specific marker (thyroglobulin gene), and the centromere-specific marker D10Z1 as biotin-labeled probes were performed as described elsewhere (Pinkel *et al.*, 1986). Both probe and target DNA were denatured at 80°C for 5 min. Hybridization was 16 h at 37°C in 50% formamide, 2× SSC, 40 m*M* sodium phosphate (pH 7.0), 10% dextran sulfate, 100 ng sonicated salmon sperm DNA, 100 ng yeast tRNA, and 20 ng labeled probe. The slides were washed with 50% formamide, 2× SSC, pH 7, at 45°C followed by 4× SSC plus 0.05% Tween 20 at room temperature. Slides were incubated with 5 μg/ml avidin D–FITC (Vector, U.S.A.), and the fluorescent signal was amplified with biotinylated goat anti-avidin D, washed, dehydrated with ethanol, and air-dried. The slides were either counterstained with propidium iodide in antifade medium or banded with 4′,6′-diamidino-2-phenylindole (DAPI) and actinomycin D.

Cell lines. Somatic cell hybrids CY18 and CY5 are mouse–human hybrid cell lines containing a single human chromosome 16 and a der 10t(10;16)(q26;q22) translocation chromosome, respectively, as their only human material (Callen, 1986). The original mouse cell line used for fusion was GM346A, a HPRT- and APRT-deficient mouse L-cell.

Southern blot analysis. Digestion of DNA with the indicated restriction enzymes, gel electrophoresis, labeling of DNA probes, and hybridizations were performed using routine procedures as described (Maniatis *et al.*, 1982). Southern blotting to Zeta probe blotting membranes was performed by alkaline transfer, as described by the manufacturer (Bio-Rad, Richmond, CA).

RESULTS

In Situ Hybridization

To map the *ERCC6* locus, *in situ* hybridization was carried out using biotinylated *ERCC6* cDNA probes. The 3.6-kb probe (Fig. 1) represents the 3′ half of the smallest of the two *ERCC6* transcripts of about 5 and 7.5 kb detectable on Northern blots (unpublished results). This 3.6-kb cDNA fragment covers 75 kb of the *ERCC6* locus. Also, longer cDNA probes, extending more 5′, have been used in *in situ* hybridizations. Two representative *in situ* hybridizations are shown in Fig. 2. Although the chromosome banding is not as detailed as that in a routine G-banding procedure, the hybridizing chromosome can be identified as human chromosome 8 or 10, in the area close to the centromere. *In situ* hybridization, simultaneously using the *ERCC6* cDNA probe and a probe specific for either chromosome 8 (a single-copy probe from the thyroglobulin gene) or chromosome 10 (an alpha satellite DNA probe, D10Z1), clearly demonstrated the *ERCC6* locus to be on chromosome 10q11–q21 (results not shown).

Hybridization to DNA from Somatic Cell Hybrids

To confirm the assignment of the *ERCC6* gene to chromosome 10, two human × mouse somatic cell hybrids (CY5 and CY18) were used. Hybrid CY5 contains almost the complete chromosome 10 and part of chromosome 16 as the only human chromosomes; in hybrid CY18 only human chromosome 16 is present (Callen, 1986). The probe used in these experiments was a unique 2.2-kb human genomic DNA fragment (probe VII, see Fig. 1) that recognizes a *Taq*I fragment of 3.2 kb in HeLa DNA (Fig. 3). In DNAs of both hybrids and mouse cells, a cross-hybridizing fragment (4.3 kb) from the mouse *ERCC6* homologue is detected, indicating that the probe contains a conserved sequence. In CY5 the human *ERCC6 Taq*I fragment (3.2 kb) is present (Fig. 3). In hybrid CY18 no human fragment is detected, meaning that the *ERCC6* locus segregates with human chromosome 10, completely in accordance with the results from the *in situ* hybridization.

Restriction Fragment Length Polymorphism in the ERCC6 Gene

The genetic defect in multiple endocrine neoplasia type 2 (MEN2) has been mapped to the pericentromeric region of chromosome 10 (Mathew *et al.*, 1987; Simpson *et al.*, 1987; Norum *et al.*, 1990; Wu *et al.*, 1990; Lairmore *et al.*, 1991). The *ERCC6* gene might therefore reside in the vicinity of the MEN2 locus. This prompted us to look for RFLPs within the *ERCC6* region. DNAs of 30

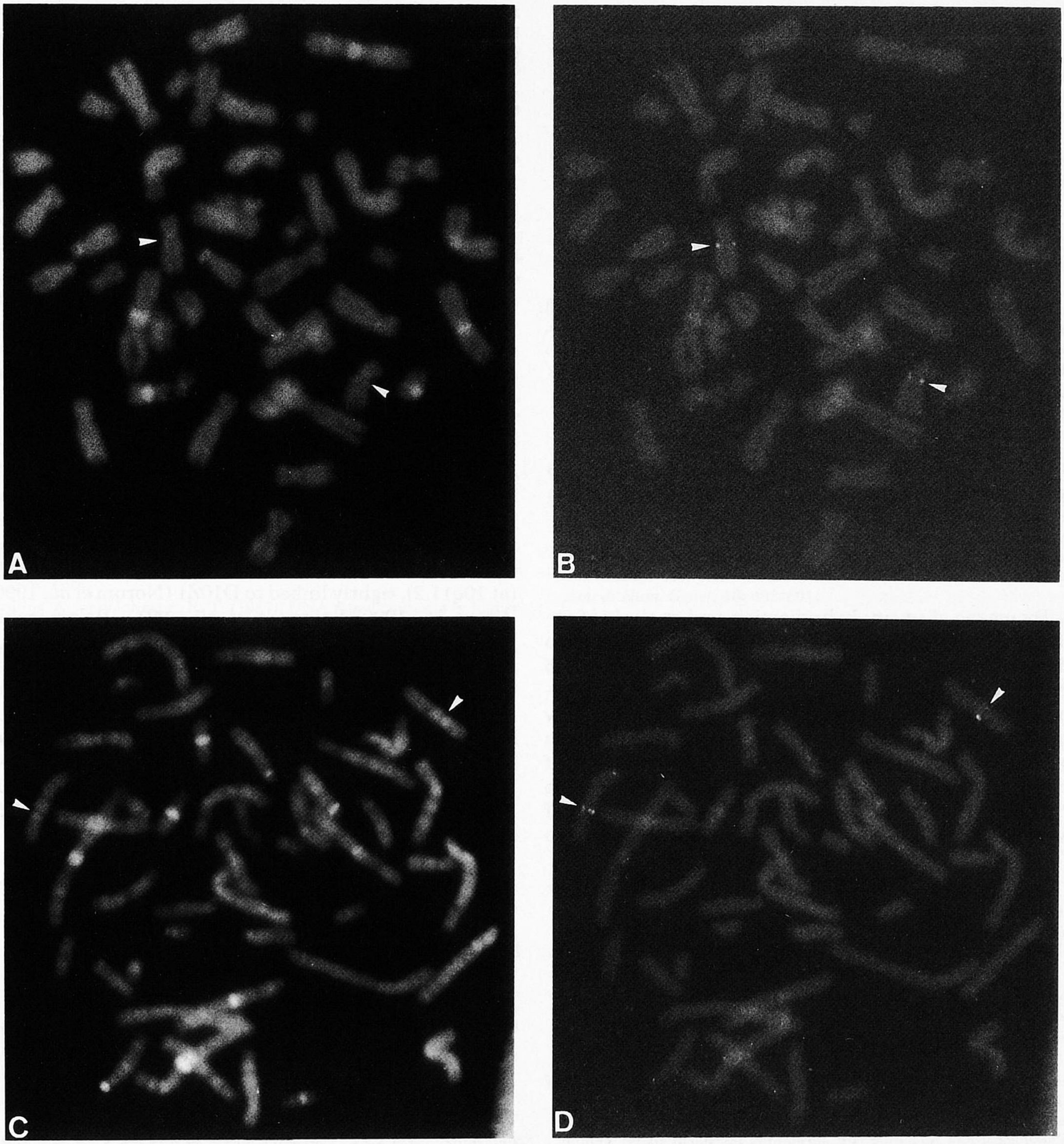

FIG. 2. Localization of *ERCC6* by *in situ* hybridization. (**A, C**) Karyotypes of human lymphocyte metaphase spreads, stained with DAPI; arrowheads indicate chromosome 10. (**B, D**) Photographs showing the corresponding fluorescent *in situ* hybridization with the human *ERCC6* cDNA fragment as a probe. Arrowheads indicate the fluorescent label on chromosome 10.

unrelated European Caucasians were digested with *Ban*I, *Bgl*II, *Eco*RI, *Hin*dIII, *Msp*I, *Pst*I, *Taq*I, and *Xba*I. Southern blots were hybridized to various *ERCC6* probes and checked for the presence of polymorphisms. Using probe 4J.ES3 (probe VII in Fig. 1, results shown from analysis of a family) *Taq*I identified a two-allele polymorphism (A1 = 10.3 kb, A2 = 3.2 kb) without a constant band (Fig. 4, individuals homozygous for A1 not shown). Based on the analysis of DNA from these 30 unrelated European Caucasians, an allelic frequency of

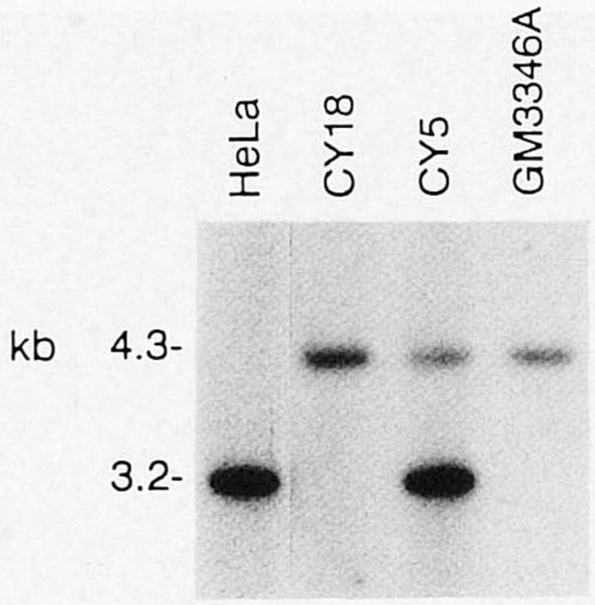

FIG. 3. Localization of *ERCC6* by hybridization to somatic cell hybrids. Southern blot analysis of genomic DNA (20 μg) from HeLa, somatic cell hybrids CY18 and CY5, and mouse cell line GM346A, digested with *Taq*I and hybridized with genomic probe VII (Fig. 1).

0.42 for A1 and 0.58 for A2 can be calculated. No RFLPs were detected with this probe in the other digests tested.

DISCUSSION

By means of *in situ* hybridization and analysis of somatic cell hybrids, we have assigned the *ERCC6* locus to human chromosome 10, region q11–q21. The *ERCC6* gene product participates in the nucleotide excision repair pathway, which removes a wide range of potentially mutagenic and carcinogenic lesions in the DNA. As such, the *ERCC6* protein is involved in the prevention of carcinogenesis. Whether mutations or deletions in the *ERCC6* locus are of any importance for development of specific tumors is unknown. It is, however, interesting to note that during progression from astrocytoma to glioblastoma (part of) chromosome 10 is frequently found to be lost (James *et al.*, 1988).

Several other human DNA repair genes have been assigned to various chromosomes (Mohrenweiser *et al.*, 1989; Smeets *et al.*, 1990; Weeda *et al.*, 1991; Siciliano *et al.*, 1986; Thompson *et al.*, 1987; Kaur and Athwal, 1989; Ishizaki *et al.*, 1990). A remarkable clustering of repair genes occurs on chromosome 19, in the q13.2–q13.3 area. This chromosome contains *ERCC1, ERCC2, XRCC1* (*X*-ray-*r*epair *c*ross *c*omplementing rodent repair deficiency gene) and ligase I. *ERCC1* and *ERCC2* have been shown to be separated by less than 250 kb (Mohrenweiser *et al.*, 1989; Smeets *et al.*, 1990). *ERCC6* is the second DNA repair gene assigned to chromosome 10, the other being a gene encoding a human methyltransferase (Rydberg *et al.*, 1990).

Previous reports have mapped the genetic defect in the MEN2 syndrome to the pericentromeric region of chromosome 10, possibly in the vicinity of the *ERCC6* locus. Two clinical types of MEN2 exist: MEN2A and MEN2B. By linkage analysis, both have been located more precisely: to a small region (~11 cM) around the centromere, bordered by FNRB (at 10p11.2) and RBP3 (at 10q11.2), tightly linked to D10Z1 (Norum *et al.*, 1990; Wu *et al.*, 1990, Lairmore *et al.*, 1991). Polymorphic DNA markers that are closely linked to the gene for a genetic disease have proved to be of great value for determining the genotypes at the disease locus of members of afflicted families. In addition, such markers are a valuable tool in the molecular cloning of the disease gene. Although it remains to be determined how closely linked the *Taq*I RFLP reported here and the MEN2 locus are, the RFLP might be of importance in future MEN2 studies.

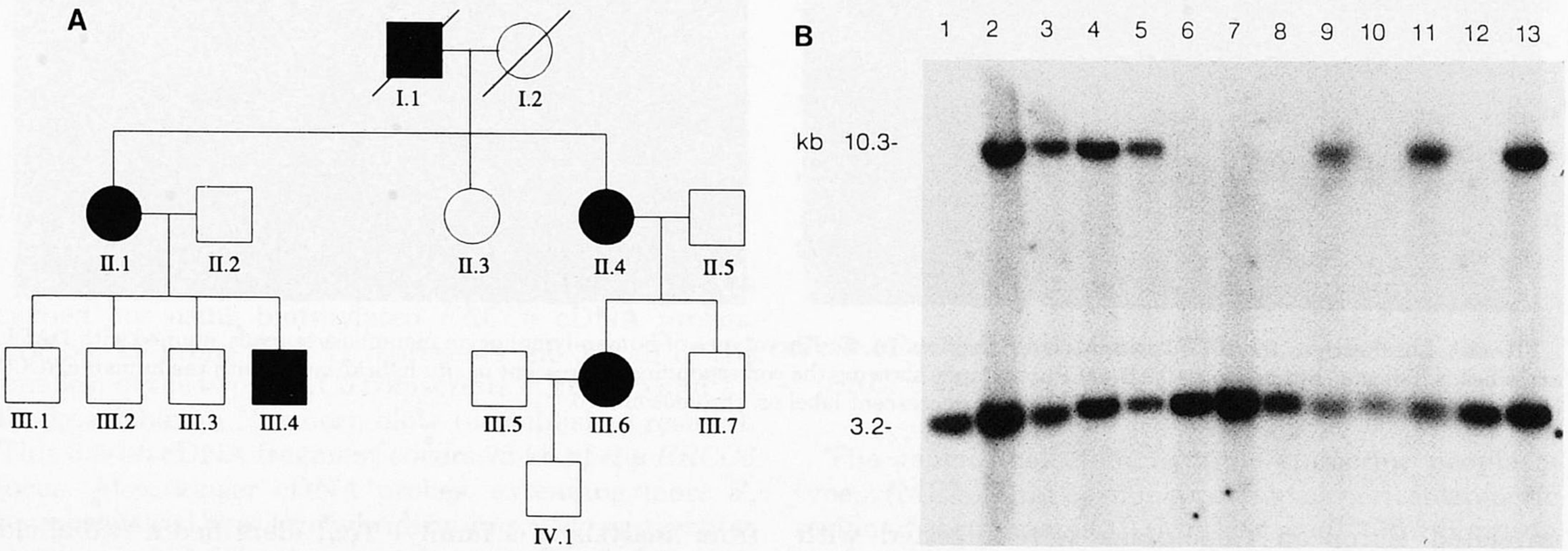

FIG. 4. Detection of an RFLP within the *ERCC6* gene. (**A**) Pedigree of the MEN2A family used in **B.** (**B**) Southern blot analysis of genomic DNA (20 μg) of 13 family members digested with *Taq*I and hybridized with the genomic probe VII (Fig. 1) detecting the A1 and A2 alleles indicated. Lane **1,** II.1; lane **2,** II.2; lane **3,** III.1; lane **4,** III.2; lane **5,** III.3; lane **6,** III.4; lane **7,** II.3; lane **8,** II.4; lane **9,** II.5; lane **10,** III.6; lane **11,** III.5; lane **12,** IV.1; lane **13,** III.7.

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Note added in proof. After the acceptance of this manuscript a clinical report appeared (Fryns *et al.* (1991) *Am. J. Med. Genet.* **40:** 343–344) presenting a Cockayne syndrome patient with an interstitial deletion of the long arm of chromosome 10 (del(10)(q11.23q21.2)). By Southern blot analysis of DNA from this patient, we have obtained indications that one copy of the *ERCC6* gene is missing in DNA of this patient. This makes *ERCC6* a candidate gene for the excision repair disorder Cockayne syndrome.

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