Chromosomal Localization of Three Repair Genes: The Xeroderma Pigmentosum Group C Gene and Two Human Homologs of Yeast RAD23

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Received March 30, 1994; revised June 18, 1994

The nucleotide excision repair (NER) disorder xeroderma pigmentosum (XP) is characterized by sun (UV) sensitivity, predisposition to skin cancer, and extensive genetic heterogeneity. Recently, we reported the cloning and analysis of three human NER genes, XPC, HHR23A, and HHR23B. The previously cloned XPC gene is involved in the common XP complementation group C, which is defective in excision repair of non-transcribed sequences in the genome. The XPC protein was found to be complexed with the product of HHR23B, one of the two human homologs of the Saccharomyces cerevisiae NER gene RAD23. Here we present the chromosomal localization by in situ hybridization using haptenized probes of all three genes. The HHR23A gene was assigned to chromosome 19p13.2. Interestingly, the HHR23B and XPC genes, the product of which forms a tight complex, were found to colocalize on band 3p25.1. Pulsed-field gel electrophoresis revealed that the HHR23B and XPC genes possibly share a Mlu1 restriction fragment of about 625 kb. Potential involvement of the HHR23 genes in human genetic disorders is discussed. © 1994 Academic Press, Inc.

INTRODUCTION

The integrity of the DNA is under constant assault by genotoxic agents, such as ultraviolet light, X rays, and numerous chemical compounds that can damage the genetic material. A network of repair systems has evolved to minimize the deleterious effects of DNA injury. One of these pathways, the nucleotide excision repair (NER) process, removes a broad range of DNA lesions, such as UV-induced cyclobutane pyrimidine dimers and (6-4) photoproducts, bulky chemical adducts, and certain DNA crosslinks in a multienzyme reaction (Hoeijmakers, 1993a,b). Two NER subpathways can be discerned: a rapid and efficient repair of the transcribed strand of active genes (transcription-coupled repair) and a more slow and less efficient repair of the bulk DNA, designated herein genome overall repair (Bohr, 1991; Hanawalt and Mellon, 1993). The enzymes involved in NER play a role in inherited diseases such as xeroderma pigmentosum (XP), Cockayne syndrome (CS), and PIBIDS, in which the excision repair mechanism is defective.

The autosomal recessive disorder XP is clinically characterized by extreme sensitivity of the skin to sunlight (UV), sunlight-induced pigmentation abnormalities, and predisposition to skin cancer. Frequently, neurological complications are seen due to progressive neurodegeneration (for a review see Cleaver and Kraemer, 1994). Cell fusion experiments have identified at least seven excision-deficient XP complementation groups (designated XP-A to XP-G) (Vermeulen et al., 1991) in addition to a form of XP, called XP-variant, that is defective in postreplication repair (Lehmann et al., 1975). This indicates involvement of a minimum of seven distinct NER genes in XP. Most of the XP complementation groups are defective in both NER subpathways, the overall genome repair, and the transcription-coupled repair. However, patients belonging to XP group C, one of the most common complementation groups, are only defective in genome overall repair and are proficient in the removal of lesions from the transcribed strand of active genes (Venema et al., 1991), indicating that specific factors are implicated in this subpathway of excision repair.

CS patients exhibit sun sensitivity, dwarfism, microcephaly, wizened appearance, deafness, and severe mental retardation. The neurological symptoms in this disorder are related to neurodysmyelination. CS is, unlike XP, not associated with an elevated risk for skin tumor formation (Lehmann, 1987). Two complementation groups have been identified within the classical form of the disease: CS-A and CS-B (Tanaka et al.,
A third recently discovered NER disorder is PIBIDS, an acronym for photosensitivity, ichthyosis, brittle hair and nails, impaired intelligence, decreased fertility, and short stature (Stefanini et al., 1993). Brittle hair and nails are hallmarks of trichothiodystrophy (TTD), a much broader genetic disease that includes PIBIDS. In many clinical aspects CS and PIBIDS resemble each other (Bootsma and Hoeijmakers, 1993; Hoeijmakers, 1993b). At least two complementation groups have been described, one of which overlaps with XP-D (Stefanini et al., 1993). Finally, rare cases have been identified displaying simultaneously the clinical hallmarks of XP and CS. These patients are assigned to XP groups B, D, and G (Hoeijmakers, 1993b). Recently, many of the clinical features of CS and TTD have been ascribed to subtle defects in the vital process of basal transcription, as the proteins affected appear to be involved in NER as well as in transcription initiation (Bootsma and Hoeijmakers, 1993; Hoeijmakers, 1993b; Vermeulen et al., submitted for publication).

A second class of mammalian excision repair-deficient mutants is represented by laboratory-induced, UV-sensitive, rodent cell lines. Eleven complementation groups have been identified (Busch et al., 1994; Riboni et al., 1992). Human genes correcting these rodent mutants are designated excision repair cross complementing (ERCC) genes. Biochemical and genetic analyses of mammalian and yeast NER genes and proteins have revealed that the entire NER pathway is strongly conserved in euakaryotic evolution (reviewed in Hoeijmakers, 1993a,b).

Recently, we described the purification of a NER protein complex consisting of the 125-kDa XPC gene product and a 58-kDa protein with overall homology to the product of the Saccharomyces cerevisiae RAD23 NER gene (Masutani et al., 1994). Simultaneously, we cloned a closely related second homolog of the yeast gene designated HHR23A (for Human Homolog of RAD23), whereas the former was called HHR23B. This represents the first example of a NER gene duplication during eukaryotic evolution. The RAD6 gene encoding an ubiquitin-activating enzyme involved in postreplication repair is also duplicated in mammals (Koken et al., 1992). RAD23 mutants show a partial defect in excision repair, and the encoded protein begins with a strongly conserved ubiquitin-like domain that is essential for its repair function (Watkins et al., 1993). The XPC/HHR23B complex displays a strong affinity for ssDNA and appears to be selectively involved in the genome-overall NER subpathway. Here we report the chromosomal localization of these genes.

**MATERIALS AND METHODS**

In situ hybridization. Normal human lymphocytes were used for the preparation of metaphase spreads prior to in situ hybridization. In situ hybridization experiments using the XPC cDNA in a pBluescript vector, the HHR23A genomic phage IV, the HHR23B genomic phage II, the HHR23B cDNA in a pBluescript vector, and the chromosome 19 centromere-specific marker, pG-A16 (Chérif et al., 1990), as biotin-labeled or digoxigenin-labeled probes were performed as described elsewhere (Pinkel et al., 1986).

After incubation with avidin D–FITC (Vector, U.S.A.), the biotin-labeled probes were visualized. The fluorescent signal was amplified with biotinylated goat anti-avidin D. The digoxigenin-labeled probes were visualized by incubation with sheep anti-digoxigenin TRITC followed by amplification with donkey anti-sheep Texas red conjugates (Fab fragments, Boehringer Mannheim). After immunohistochemical staining, the slides were dehydrated with ethanol and air-dried. The slides were counterstained with propidium iodide and 4,6-diamidino-2-phenylindole (DAPI) in antifade medium.

In the case of hybridization with HHR23A, slides were banded with bisbenzimide H33258 (Hoechst), UV-irradiated, and heat-denatured before amplification (Chérif et al., 1990).

**General procedures.** Isolation, digestion, and gel electrophoresis of the genomic λ colonies hybridizing to the HHR23A and HHR23B cDNAs were performed according to established procedures (Sambrook et al., 1989). Labeling of DNA probes and hybridizations of Southern blots were carried out using routine protocols. Southern blotting to Zeta probe membranes was performed by alkaline transfer, as described by the manufacturer (Bio-Rad, Richmond, CA). Membranes were exposed at –80°C to Fuji RX film with intensifying screens. After exposure, blots were stripped in 10 mM Tris, 1 mM EDTA, 1% SDS at 90°C for 5 min and rehybridized.

The HHR23 genomic phages were derived from a λ EMBL-3 library prepared from genomic DNA of the CML-0 cell line (generously provided by Dr. G. Grosveld). Phages were used to infect Escherichia coli LE392 cells. Analysis and identification of HHR23 genomic fragments were carried out by restriction enzyme site mapping and hybridization using HHR23 cDNA probes.

DNA for restriction fragment length polymorphism (RFLP) analysis was isolated from peripheral blood leukocytes. For pulsed-field gel electrophoresis (PFGE), agarose-embedded leukocytes of a normal individual were lysed and digested with the appropriate restriction endonucleases according to the manufacturers’ instructions. PFGE was carried out as detailed elsewhere (van Ommen and Verkerk, 1986).

**RESULTS**

**In Situ Hybridization**

HHR23A. Hybridization with the HHR23A genomic probe yielded a clear hybridizing signal with a chromosome that on the basis of Hoechst banding can be identified as human chromosome 19, in the area close to the p13.3–p13.2 border (Fig. 1A). To verify the chromosome identification, the chromosome 19-specific centromere probe pG-A16 was used in combination with the HHR23A genomic probe. Figure 2 shows that the HHR23A signals reside on the same chromosome as that of the centromere probe. As an independent confirmation of the assignment of HHR23A to the p arm of chromosome 19, we also performed simultaneous hybridization with a genomic probe of another known chromosome 19 gene, ERCC1, located on 19q13.2 (Mohrenweiser et al., 1989). The results obtained (not shown) were in complete agreement with the localization of the HHR23A gene to 19p13.2. in every metaphase analyzed.

HHR23B. For mapping of the HHR23B locus, in situ hybridization experiments were performed on
metaphase spreads using the biotinylated **HHR23B** cDNA in a pBluescript vector as well as the genomic λ phage. The **HHR23B** genomic probe II contains a 7-kb EcoRI fragment present in the 3′ end of the **HHR23B** gene. In agreement with the cDNA, the genomic probe gave specific hybridization on 3p25.1 (see Fig. 1B). Since unequivocal identification of chromosome 3 is possible on the basis of morphology and banding pattern, no double hybridizations with a control probe were performed. A representative example of chromosome 3 showing hybridization and the clear morphology in combination with the banding pattern is depicted in Fig. 2.

**XPC.** For localization of the xeroderma pigmento-

sum group C correcting gene (**XPC**), the 3.6-kb cDNA was biotinylated and used for *in situ* hybridization. A representative *in situ* hybridization for **XPC**, of the more than 50 metaphases analyzed, is depicted in Fig. 1C. Interestingly, like **HHR23B**, the **XPC** gene was also assigned to chromosome 3p25.1. Since both genes are located on 3p25.1, a double hybridization with both **XPC** and **HHR23B** was performed to see whether they hybridize to discernable locations. To this aim, the **XPC** cDNA probe was haptenized with biotin and visualized by FITC-labeled antibodies, whereas the **HHR23B** probe was provided with a digoxigenin label and visualized by TRITC/Texas red-labeled antibodies. The results (Fig. 3) indicate a similar cytogenetic position on
FIG. 2. In situ hybridization of metaphase chromosomes with biotinylated genomic HHR23 probes. (Left) In situ hybridization signal on chromosome 19 with the biotinylated genomic HHR23A probe and a cocktail of a centromeric probe specific for chromosome 19 in combination with HHR23A. (Right) The signal of the biotinylated HHR23B probe on chromosome 3.

the chromosome. The cytogenetic colocalization of both probes is apparent from the fact that only one spot is present with a mixed color composed of the green of XPC and red of the HHR23B probe. Detectable hybridization of both probes is also demonstrated by the finding of distinct spots in interphase nuclei (data not shown). Occasionally, in more elongated chromosomes, distinct green (XPC) and orange/red (HHR23B) spots were observed, with the XPC hybridization in each case located more to the telomere. These data suggest the following order of these genes on the chromosome: centromere/HHR23B–XPC/telomere.

Characterization of the Genomic Context of the XPC and HHR23 Genes by Pulsed-Field Gel Electrophoresis

Since the cytogenetic data do not permit conclusions about the physical distance by which XPC and HHR23B are separated and in fact do not exclude the possibility that they are very close together, pulsed-field gel electrophoresis experiments were conducted. The following restriction enzymes were utilized: EagI, MluI, SfiI, BssHII, NolI, NruI, and SalI. The results summarized in Table 1a indicate that all but one enzyme generate different fragments for each gene. However, interestingly, XPC and part of the HHR23B gene hybridize to a MluI fragment of about 625 kb, raising the possibility that both genes reside on this fragment. The fact that the HHR23B cDNA probe visualizes two MluI bands implies that this site is situated within the HHR23B gene. In view of the large size of the MluI fragments (~625 and >1000 kb), it was not feasible to perform partial digestions to rule out the possibility that the hybridization of both genes to a similar-size fragment is due to a coincidental correspondence in size. From the results obtained with EagI and BssHII, one can conclude that XPC and HHR23B must be at least 250 kb apart, assuming that both genes are not larger than 50 kb. The MluI digest sets an upper limit to the distance between both loci of 625 kb if they indeed are located on the same fragment. Finally, the findings of more than one HHR23B hybridizing fragment for four rare-cutting enzymes (EagI, MluI, BssHII, and SalI) strongly suggests the presence of a CpG island within this gene. The XPC gene appears to contain sites for EagI and SalI.

We have also characterized the genomic context of the HHR23A gene by PFGE. The results are presented in Table 1b. From these data we can conclude that there are at least two NruI sites and one ClaI site present in the HHR23A gene. Furthermore, the NruI digests indicate that the gene has a minimal size of 16 kb.

RFLPs in the Areas of HHR23A and HHR23B

To facilitate linkage analysis we have searched for RFLPs in the genomic areas of both HHR23 genes. The

FIG. 3. In situ hybridization of metaphase chromosomes with biotinylated HHR23B and XPC probes. The arrowheads indicate the hybridization signals on the short arm of chromosome 3. Digoxigenin-labeled HHR23B (visualized with TRITC/Texas red) was hybridized in combination with biotin-labeled XPC (visualized with FITC). (A) In situ hybridization results. (B) The DAPI banding of the same metaphase spread.
TABLE 1
Pulsed-Field Gel Electrophoresis Analysis of XPC, HHR23A, and HHR23B

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>HHR23B fragment (kb)</th>
<th>XPC fragment (kb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EcoRI</td>
<td>≈700</td>
<td>≈310</td>
</tr>
<tr>
<td></td>
<td>≈290</td>
<td>≈40</td>
</tr>
<tr>
<td>MluI</td>
<td>&gt;1000?</td>
<td>≈625</td>
</tr>
<tr>
<td></td>
<td>≈625</td>
<td></td>
</tr>
<tr>
<td>BspHI</td>
<td>≈725</td>
<td>&lt;100</td>
</tr>
<tr>
<td></td>
<td>≈310</td>
<td></td>
</tr>
<tr>
<td>NotI</td>
<td>≈800</td>
<td>≈750</td>
</tr>
<tr>
<td>SfiI</td>
<td>≈300</td>
<td>≈240</td>
</tr>
<tr>
<td>SalI</td>
<td>&gt;1000?</td>
<td>≈450</td>
</tr>
<tr>
<td></td>
<td>≈100</td>
<td>&lt;50</td>
</tr>
</tbody>
</table>

(b) Enzyme | HHR23A fragment (kb) |
-------------|----------------------|
ClinI        | ≈300                 |
|            | 60–70                |
NruI         | ≈240                 |
|            | ≈18                  |
|            | ≈16                  |
NotI         | ≈190                 |
MluI         | <80                  |
BspHI        | <80                  |

HHR23A gene, on the short arm of chromosome 19, does not appear to be highly polymorphic for EcoRI, PstI, RsaI, MspI, and TaqI. Similarly, the HHR23B 3p25.1 locus does not appear to be highly polymorphic for PstI, RsaI, MspI, PvuII, and TaqI. However, the HHR23B locus seems to contain a BglII-polymorphic site. Figure 4 shows part of the blot in which the RFLP is presented. The allelic frequency of the less common b allele is estimated from the small sample to be on the order of 25% in the Caucasian population.

DISCUSSION

The consequences of inefficient or deficient repair are illustrated by genetic repair diseases that in general predispose individuals to cancer due to the fact that mutations arising from unrepaired lesions accumulate at a high rate. The genetics of NER in mammalian (including human) cells is very complex since many genes are involved in this pathway. Rodent mutant cell lines defective in NER fall into at least 11 distinct genetic complementation groups. The human NER-defective hereditary disease XP is characterized by at least 7 genetic complementation groups. CS and PIBIDS account for at least 3 other complementation groups. Furthermore, one of the PIBIDS complementation groups shows overlap with XP (XP group D). The parallelism between yeast repair genes and these NER syndromes indicates the importance of this evolutionarily conserved pathway (Hoeijmakers, 1993a,b). Here we report the chromosomal localization of 3 recently isolated human repair genes. The XPC gene is localized on the short arm of chromosome 3, like the HHR23B gene. The HHR23A gene maps on the short arm of chromosome 19.

In the process of reviewing our manuscript, we became aware of earlier work by Legerski et al. (1994) providing unequivocal evidence in favor of the localization of XPC on 3p25 based on Southern blotting of hybrids and in situ hybridization studies and contrasting with preliminary results by Kauer and Atwali (1993) suggesting chromosome 5, based on microcell-mediated correction studies. Our independent observation strongly corroborates Legerski’s finding and finally settles the question of the XPC assignment in favor of chromosome 3p25.

As shown in Table 2, the human NER genes mapped to date are distributed over the genome. This resembles the situation in S. cerevisiae. Since yeast represents the other end of the eukaryotic spectrum, it is likely that random distribution of NER genes occurs in all eukaryotes. Many repair genes are found on chromosome 19: ERCC1, 2, the gene for DNA ligase I, and the X-ray repair gene XRCC1 all reside on 19q13.2. The HHR23A gene can be added to this list, but in contrast to the others this gene resides on the short arm. The location of a considerable fraction of repair genes on chromosome 19 is in line with the known high density of genes on this chromosome (Human Gene Mapping 11, 1991). In humans, 2 pairs of NER genes are localized close together. Previously we reported that the ERCC1 and ERCC2 genes are situated 250–300 kb apart on 19q13.2 (Smeets et al., 1990). Here we find close proximity of the XPC and HHR23B genes, on band 3p25.1 at a distance of 250–625 kb. This colocalization is a remarkable finding, since the gene products form a tight complex, which is not the case for ERCC1.

**FIG. 4.** A BglII polymorphism in the HHR23B gene. Autoradiogram of BglII-digested DNA of two unrelated Caucasian individuals hybridized with the full-length HHR23B cDNA probe. The polymorphic band was detected in DNA of four of eight unrelated individuals. Lane 1, DNA with the a/b haplotype; lane 2, DNA with the a/a haplotype.
TABLE 2
Chromosomal Localization of Human NER Genes

<table>
<thead>
<tr>
<th>Human gene</th>
<th>Yeast homolog</th>
<th>Corrected human NER syndrome</th>
<th>Chromosomal localization</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>XPA</td>
<td>RAD14</td>
<td>XPA</td>
<td>9q34</td>
<td>Kauer and Athwal, 1989</td>
</tr>
<tr>
<td>XBP (ERCC3)</td>
<td>RAD25</td>
<td>XBP</td>
<td>2q21</td>
<td>Ishizaki, 1990</td>
</tr>
<tr>
<td>XPC</td>
<td>RAD4</td>
<td>XPC</td>
<td>3p25.1</td>
<td>Weeda, 1989</td>
</tr>
<tr>
<td>XPD (ERCC2)</td>
<td>RAD3</td>
<td>XPD</td>
<td>19q13.2</td>
<td>This report</td>
</tr>
<tr>
<td>XPF (ERCC5)</td>
<td>RAD2</td>
<td>XPF</td>
<td>13q32–q33</td>
<td>Siciliano, 1986</td>
</tr>
<tr>
<td>ERCC1</td>
<td>RAD10</td>
<td>Unknown*</td>
<td>19q13.2</td>
<td>Mudgett and MacInnes, 1990</td>
</tr>
<tr>
<td>ERCC4</td>
<td>Unknown</td>
<td>Unknown</td>
<td>16p13.13–p13.2</td>
<td>Mohrenweiser et al., 1989</td>
</tr>
<tr>
<td>HHR23A</td>
<td>RAD23</td>
<td>Unknown</td>
<td>19p13.2</td>
<td>Liu et al., 1993</td>
</tr>
<tr>
<td>HHR23B</td>
<td>RAD23</td>
<td>Unknown</td>
<td>3p25.1</td>
<td>This report</td>
</tr>
<tr>
<td>CSB (ERCC6)</td>
<td>Unknown</td>
<td>CSB</td>
<td>10q11–q21</td>
<td>Troelstra et al., 1992</td>
</tr>
<tr>
<td>DNA ligase</td>
<td>Ligase</td>
<td>46BR</td>
<td>19q13.2</td>
<td>Barnes et al., 1992</td>
</tr>
</tbody>
</table>

* Not any of the known NER-deficient complementation groups.

and ERCC2. The question remains whether this is pure coincidence or whether the colocalization has a functional significance, for instance, coregulation at the transcription level. At present it is not known whether other genes are located in between XPC and HHR23B as with ERCC1 and ERCC2.

The Chromosomal Context of HHR23A

Loss of heterozygosity studies indicate an underrepresentation of the involvement of chromosome 19 in human cancers, which is in contrast to chromosome 3p (Seizinger et al., 1991). Many expressed genes have been mapped to the 19p13 region (Mckusick, 1990), as have breakpoints for several translocations (Kamps et al., 1990). The t(1;19)(q23;p13) chromosomal translocation is observed in 25% of children with pre-B-cell acute lymphoblastic leukemia (ALL) (Hunger et al., 1991). However, the gene(s) disrupted by the translocation have not yet been cloned, and the possibility that the HHR23A gene is involved cannot be excluded at present. Furthermore, it should be noted that many other breakpoints involved in ALL have been described (Ahuja and Cline, 1988).

A rare heritable folate-sensitive fragile site at 19p13 was detected in four brothers (Chodirker et al., 1987). The clinical significance of this fragile site and possible involvement of HHR23A are unknown. Other well-characterized loci on 19p are the low density lipoprotein receptor (Francke et al., 1984), insulin receptor (Yang-Feng et al., 1985), and the human Ro ribonucleoprotein (52 kDa) autoantigen (McCauliffe et al., 1990). The MHC class II regulatory factors RFX1 and RFX2, defective in hereditary HLA II deficiency (Bare lymphocyte syndrome), respectively mapped to 19p13.1 and 19p13.2–p13.3 (Pugliatti et al., 1992). These data in combination with the HHR23A locus should help guide molecular studies to characterize further 19p13 breakpoints and mapping of genes to this chromosomal region.

The Chromosomal Context of HHR23B and XPC

Like HHR23B, the XPC gene maps to 3p25.1. A number of studies suggested the presence of important genetic loci on the short arm of chromosome 3. Chromosomal abnormalities of 3p have been observed in breast cancer, lung cancer, renal cell carcinoma, ovarian carcinoma, various hematological malignancies, and cervical cancer (Naylor and Carratt, 1991). Loss of 3p in some of these malignancies suggests the presence of one or multiple tumor suppressor genes on the short arm of chromosome 3. One of these is the gene responsible for the genetic disorder von Hippel–Lindau, which was recently cloned (Latif et al., 1993). Furthermore, the developmental disorder Greig craniosynostaly syndrome has been associated with a t(3;17)(p21;p13) balanced translocation. The 3p25 region has been characterized quite well by several groups working on the von Hippel–Lindau disease gene. Chromosome 3p allele loss has been described for four tumor types: renal cell carcinoma, hemangioblastoma, pheochromocytoma, and pancreatic tumor, suggesting a common mechanism of tumorigenesis in all types of tumor in von Hippel–Lindau disease. Our data obtained by pulsed-field analysis were not easy to implement in the map generated for the von Hippel–Lindau region (Szymanski et al., 1993).

The close vicinity of the XPC and HHR23B genes opens the possibility of a common deletion inactivating both genes. Since impairment of the two functions may yield a phenotype more severe than regular XPC, we searched for XPC patients with additional features. An XPC patient (XP1MI) was described having a unique combination of symptoms that correspond to two sun-sensitive conditions: xeroderma pigmentosum (XPC) conferring sensitivity to UV-B, and systemic lupus erythematosus, with an exaggerated response to UV-A (Hananian and Cleaver, 1980). This XPC patient shows no detectable mRNA on Northern blot analysis (Leger and Peterson, 1992) and a point mutation in one of
the alleles of XP1MI was reported using RT-PCR (Li et al., 1993). No functional studies demonstrating inactivation of the gene by this mutation have been performed, and it is not known whether this sequence alteration is present on one or both alleles nor whether the patient has lost the second allele. However, in situ hybridization on lymphocytes and fibroblasts of patient XP1MI (our own unpublished data) show that both alleles of XPC and HHR23B are present. This was also found in another XPC patient (Halley et al., 1979) showing no detectable mRNA in Northern blot analysis is XP4PA (Legerski and Peterson, 1992). Recently, a mutation was reported in the DNA of this patient involving a deletion of two nucleotides at the mRNA level and a frameshift in the central part of the protein (Li et al., 1993). No information on the other allele was provided.

**Relationship of the HHR23A and B Genes with Human NER Syndromes**

Virtually all NER-deficient XP, CS, TTD, and rodent complementation groups for which no repair gene is isolated have been tested for the ability of the HHR23A gene to correct their defect. However, no correction was found (P. J. van der Spek and W. Vermeulen, unpublished results). Similar studies using the HHR23B gene are in progress. Given the high amino acid sequence homology between both gene products (57% identity and 76% similarity) it is possible the HHR23A and HHR23B proteins have largely overlapping functions. When functional redundancy exists, it would require the unlikely event of simultaneous inactivation of both HHR23 genes for clinical symptoms to become manifest, which may explain the absence of a known repair disorder for HHR23A and possibly also for HHR23B. Targeted gene replacement in mouse embryonal stem cells opens the possibility of generating HHR23-defective cell lines and mice in the laboratory. Via cloning of the mouse homologs, valuable insight can be gained in translation of a molecular defect in the HHR23 function into clinical features, particularly the predisposition to cancer and other clinical hallmarks of human NER disorders.

**ACKNOWLEDGMENTS**

We are grateful to Arjenne Hesseling-Janssen, D. J. J. Halley, Peter Heutink, Guido Breedveld, and Cécile Visser for their help with the PFGE and RFLP experiments. Furthermore, we thank Christine Troelstra and Professor D. Bootsma for the helpful suggestions and discussions. We also thank Dr. M. Siciliano for sharing his independent results on the XPC assignment. Mirko Kuit, Ruud Koppenol, and Tom de Vries Lentsch are acknowledged for photography. This work was financially supported by a subsidy of the Dutch government to the Research School Medical Genetics Centre (MGC).

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