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

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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 nontranscribed 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 *Mlu*I restriction fragment of about 625 kb. Potential involvement of the *HHR23* genes in human genetic disorders is discussed.

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.,*

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1981; Lehmann, 1982). CS cells were found to be selectively disturbed in the transcription-coupled repair subpathway of NER (Venema *et al.,* 1990).

A third recently discovered NER disorder is PIBIDS, an acronym for *p*hotosensitivity, *i*chthyosis, *b*rittle hair and nails, *i*mpaired intelligence, *d*ecreased fertility, and *s*hort 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 *e*xcision *r*epair *c*ross *c*omplementing (*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 eukaryotic 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 *H*uman *H*omolog 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 immunochemical 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 clones 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 m*M* Tris, 1 m*M* 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

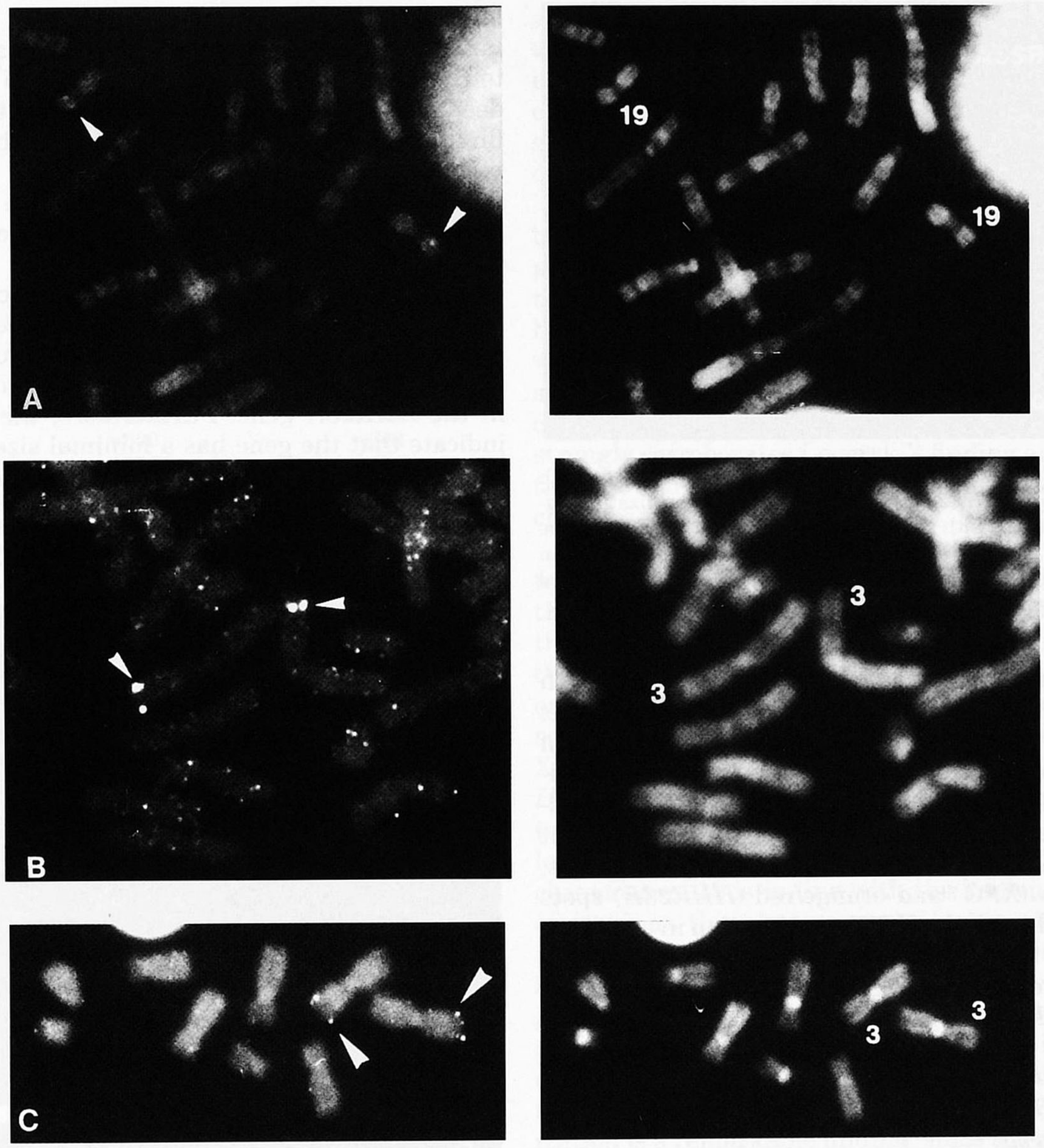

FIG. 1. (**A**) *In situ* hybridization of metaphase chromosomes with biotinylated genomic *HHR23A* probe. Hybridization on a metaphase spread (partly shown) with the genomic *HHR23A* probe. The arrowheads point to the region with a specific signal on chromosome 19p13.2. (**Left**) The *in situ* hybridization results. (**Right**) The Hoechst banding of the same metaphases. (**B**) *In situ* hybridization of metaphase chromosomes with biotinylated genomic *HHR23B* probe. The arrowheads indicate the hybridization signal on chromosome 3p25.1. (**Left**) *In situ* hybridization results. (**Right**) The DAPI banding of the same metaphase. (**C**) *In situ* hybridization of metaphase chromosomes with biotinylated *XPC* cDNA probe. The arrowheads indicate the hybridization signal on chromosome 3p25.1. (**Left**) *In situ* hybridization results. (**Right**) The DAPI banding of the same metaphase.

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 *Eco*RI 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 pigmentosum 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

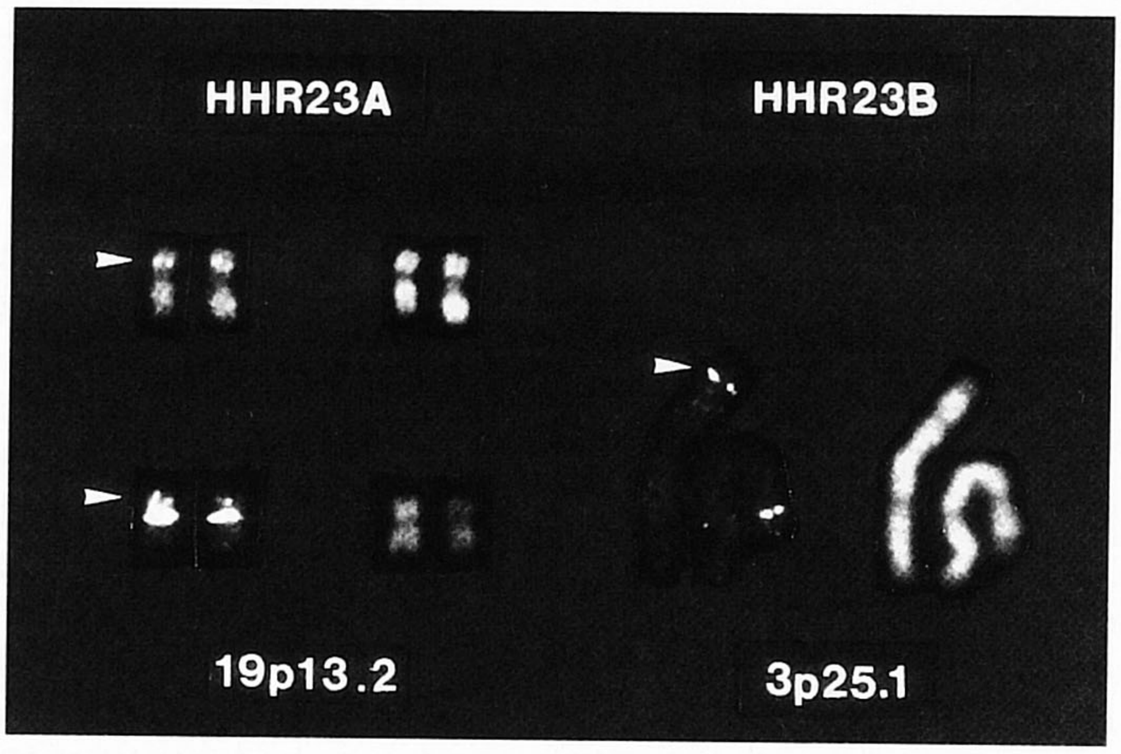

FIG. 2. *In situ* hybridization of metaphase chromosomes with biotinylated genomic *HHR23* probes. (**Left**) *In situ* hybridization signal on chromosome 19 with the biotinylated genomic *HHR23A* probe and a cocktail of a centromeric probe specific for chromosome 19 in combination with *HHR23A*. (**Right**) 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: *Eag*I, *Mlu*I, *Sfi*I, *Bss*HII, *Not*I, *Nru*I, and *Sal*I. 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 *Mlu*I fragment of about 625 kb, raising the possibility that both genes reside on this fragment. The fact that the *HHR23B* cDNA probe visualizes two *Mlu*I bands implies that this site is situated within the *HHR23B* gene. In view of the large size of the *Mlu*I 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 *Eag*I and *Bss*HII, 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 *Mlu*I 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 (*Eag*I, *Mlu*I, *Bss*HII, and *Sal*I) strongly suggests the presence of a CpG island within this gene. The *XPC* gene appears to contain sites for *Eag*I and *Sal*I.

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 *Nru*I sites and one *Cla*I site present in the *HHR23A* gene. Furthermore, the *Nru*I 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

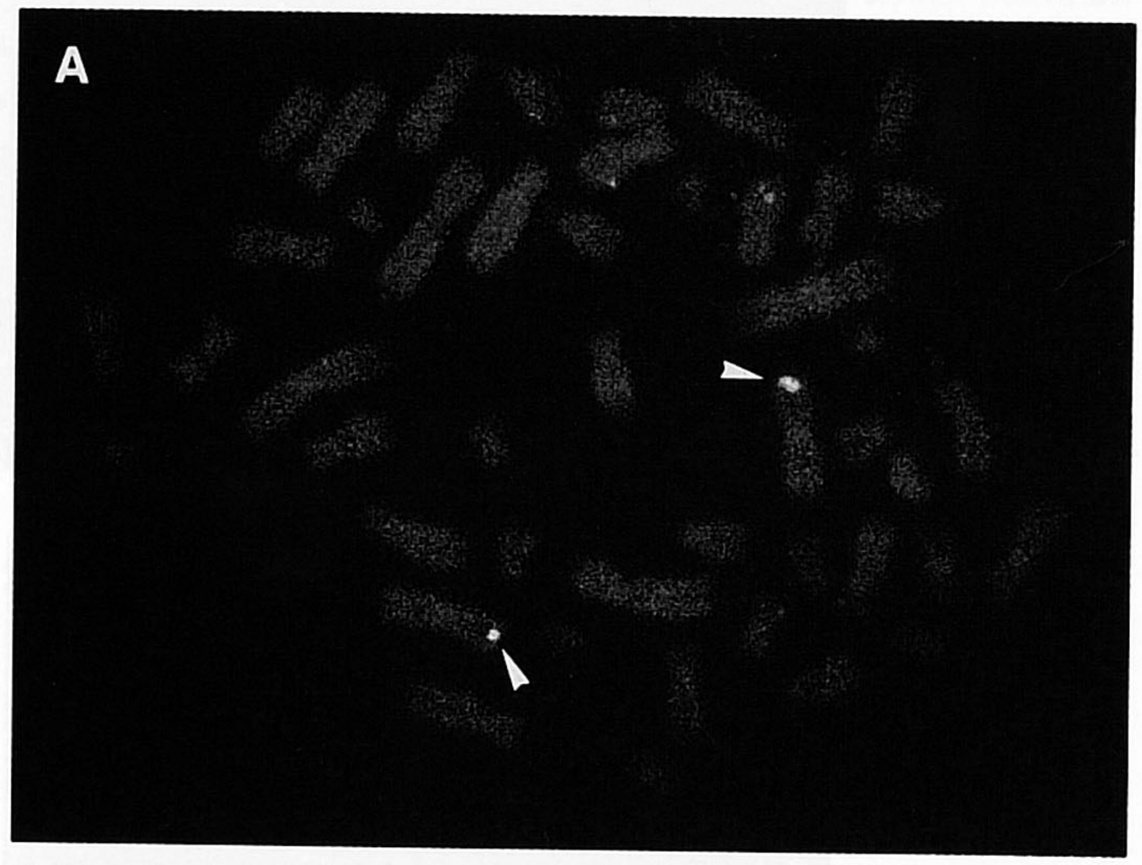

FIG. 3. *In situ* hybridization of metaphase chromosomes with biotinylated *HHR23B* and *XPC* probes. The arrowheads indicate the hybridization signals on the short arm of chromosome 3. Digoxigenin-labeled *HHR23B* (visualized with TRITC/Texas red) was hybridized in combination with biotin-labeled *XPC* (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*

(**a**)

Enzyme	*HHR23B* fragment (kb)	*XPC* fragment (kb)
*Eag*I	≈700	≈310
	≈290	<40
*Mlu*I	>1000?	≈625
	≈625	
*Bss*HII	≈725	<100
	≈310	
*Not*I	≈800	≈750
*Sfi*I	≈300	≈240
*Sal*I	>1000	≈450
	≈100	<50

(**b**)

Enzyme	*HHR23A* fragment (kb)
*Cla*I	≈300
	60–70
*Nru*I	≈240
	≈18
	≈16
*Not*I	≈190
*Mlu*I	<80
*Bss*HII	<80

HHR23A gene, on the short arm of chromosome 19, does not appear to be highly polymorphic for *Eco*RI, *Pst*I, *Rsa*I, *Msp*I, and *Taq*I. Similarly, the *HHR23B* 3p25.1 locus does not appear to be highly polymorphic for *Pst*I, *Rsa*I, *Msp*I, *Pvu*II, and *Taq*I. However, the *HHR23B* locus seems to contain a *Bgl*II-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 of 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 Atwahl (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*

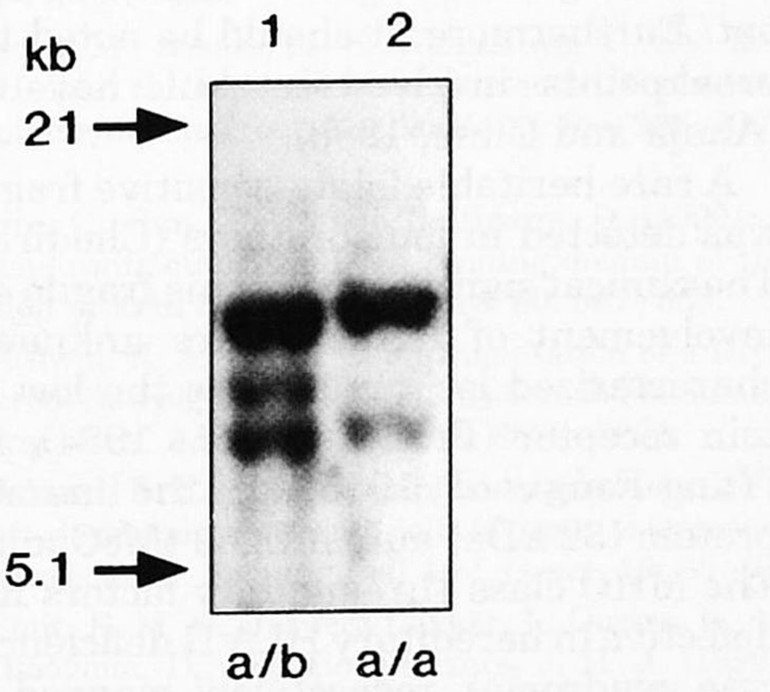

FIG. 4. A *Bgl* II polymorphism in the *HHR23B* gene. Autoradiogram of *Bgl* II-digested DNA of two unrelated Caucasian individuals hybridized with the full-length *HHR23B* cDNA probe. The polymorphic band was detected in DNA of four of eight unrelated individuals. Lane **1,** DNA with the a/b haplotype;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

TABLE 2

Chromosomal Localization of Human NER Genes

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

[a] 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 Carritt, 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 craniopolysyndactyly 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 (Legerski 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.

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REFERENCES

Ahuja, H. G., and Cline, M. J. (1988). Genetic and cytogenetic changes in acute lymphoblastic leukemia. *Med. Oncol. Tumor. Pharmacother.* **5:** 211–222.

Barnes, D. E., Tomkinson, A. E., Lehmann, A. R., Webster, A. D., and Lindahl, T. (1992). Mutations in the DNA ligase I gene of an individual with immunodeficiencies and cellular hypersensitivity to DNA-damaging agents. *Cell* **69:** 495–503.

Bohr, V. A. (1991). Gene specific DNA repair. *Carcinogenesis* **12:** 1983–1992.

Bootsma, D., and Hoeijmakers, J. H. J. (1993). DNA repair: Engagement with transcription. *Nature* **363:** 114–115.

Busch, D., Greiner, C., Rosenfeld, K. L., Ford, R., de Wit, J., Hoeijmakers, J. H. J., and Thompson, L. H. (1994). Complementation group assignments of moderately UV-sensitive CHO mutants isolated by large scale screening (FAECB). *Mutagenesis,* in press.

Chérif, D., Julier, C., Delattre, O., Derré, J., Lathrop, G. M., and Berger, R. (1990). Simultaneous localization of cosmids and chromosome R-banding by fluorescence microscopy: Application to regional mapping of human chromosome 11. *Proc. Natl. Acad. Sci. USA* **87:** 6639–6643.

Chodirker, B. N., Chudley, A. E., Ray, M., Wickstrom, D. E., and Riordan, D. L. (1987). Fragile 19p13 in a family with mental illness. *Clin. Genet.* **31:** 1–6.

Cleaver, J. E., and Kraemer, K. H. (1994). Xeroderma pigmentosum. *In* "The Metabolic Basis of Inherited Disease," 7th ed. (C. R. Scriver, A. L., Beaudet, W. S., Sly, and D. Valle, Eds.), Vol. 2, pp. 2949–2971, McGraw–Hill, New York.

Francke, U., Brown, M. S., and Goldstein, J. L. (1984). Assignment of the human gene for the low density lipoprotein receptor to chromosome 19: Synteny of a receptor, a ligand, and a genetic disease. *Proc. Natl. Acad. Sci. USA* **81:** 2826–2830.

Halley, D. J. J., Keijzer, W., Jaspers, N. G. J., Niermeijer, M. F., Kleijer, W. J., Boué, J., Boué, A., and Bootsma, D. (1979). *Clin. Genet.* **16:** 137–146.

Hananian, J., and Cleaver, J. E. (1980). Xeroderma pigmentosum exhibiting neurological disorders and systemic lupus erythematosus. *Clin. Genet.* **17:** 39–45.

Hanawalt, P., and Mellon, I. (1993). Stranded in an active gene. *Curr. Biol.* **3:** 67–69.

Hoeijmakers, J. H. J. (1993a). Nucleotide excision repair I: From *E. coli* to yeast. *Trends Genet.* **9:** 173–177.

Hoeijmakers, J. H. J. (1993b). Nucleotide excision repair II: From yeast to mammals. *Trends Genet.* **9:** 211–217.

Human Gene Mapping 11 (1991). *Cytogenet. Cell Genet.* **58:** 1–2200.

Hunger, S. P., Galili, N., Carroll, A. J., Crist, W. M., Link, M. P., and Cleary, M. L. (1991). The t(1;19)(q23;p13) results in consistent fusion of E2E and PBX1 coding sequences in acute lymphoblastic leukemias. *Blood* **77:** 687–693.

Ishizaki, K., Oshimura, M., Sasaki, M. S., Nakamura, Y., and Ikenaga, M. (1990). Human chromosome 9 complements UV sensitivity of xeroderma pigmentosum group A cells. *Mutat. Res.* **235:** 209–215.

Kamps, M. P., Murre, C., Sun, X. H., and Baltimore, D. (1990). A new homeobox gene contributes the DNA binding domain of the t(1;19) translocation protein in pre-B ALL. *Cell* **60:** 547–555.

Kauer, G. P., and Athwal, R. S. (1989). Complementation of a DNA repair defect in xeroderma pigmentosum cells by transfer of human chromosome 9. *Proc. Natl. Acad. Sci. USA* **86:** 8872–8876.

Kauer, G. P., and Athwal, R. S. (1993). Complementation of a DNA repair defect in xeroderma pigmentosum cells of group C by transfer of human chromosome 5. *Somatic Cell Mol. Genet.* **19:** 83–93.

Koken, M. H. M., Smit, E. M. E., Jaspers-Dekker, I., Oostra, B. A., Hagemeijer, A., Bootsma, D., and Hoeijmakers, J. H. J. (1992). Localization of two human homologs, HHR6A and HHR6B, of the yeast DNA repair gene RAD6 to chromosomes Xq24–q25 and 5q23–q31. *Genomics* **12:** 447–453.

Latif, F., Tory, K., Gnarra, J., Yao, M., Duh, F. M., Orcutt, M. L., Stackhouse, T., Kuzmin, I., Modi, W., Geil, L., Schmidt, L., Zhou, F., Li, H., Wei, M. H., Chen, F., Glenn, G., Choyke, P., Walther, M. M., Weng, Y., Duan, D. S. R., Dean, M., Glavac, D., Richards,