Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 6423–6427, July 1992
Genetics

Localization of a DNA repair gene (*XRCC5*) involved in double-strand-break rejoining to human chromosome 2

(microcell fusion/γ-irradiation/monochromosomal hybrids/complementation)

P. A. Jeggo*†, M. Hafezparast*, A. F. Thompson*, B. C. Broughton*, G. P. Kaur‡, M. Z. Zdzienicka§¶, and R. S. Athwal‡

*Medical Research Council Cell Mutation Unit, Sussex University, Falmer, Brighton BN1 9RR, United Kingdom; ‡Department of Microbiology and Molecular Genetics, New Jersey Medical School, Newark, NJ 07103-2757; §Department of Radiation Genetics and Chemical Mutagenesis, State University of Leiden, Sylvius Laboratories, Wassenaarseweg 72, 2333 AL Leiden, The Netherlands; and ¶J. A. Cohen Institute, Interuniversity Research Institute for Radiopathology and Radiation Protection, Leiden, The Netherlands

Communicated by Richard B. Setlow, March 12, 1992 (received for review December 10, 1991)

ABSTRACT **Complementation of the repair defect in hamster *xrs* mutants has been achieved by transfer of human chromosome 2 using the method of microcell-mediated chromosome transfer. The *xrs* mutants belong to ionizing radiation complementation group 5, are highly sensitive to ionizing radiation, and have an impaired ability to rejoin radiation-induced DNA double-strand breaks. Both phenotypes were corrected by chromosome 2, although the correction of radiation sensitivity was only partial. Complementation was achieved in two members of this complementation group, *xrs6* and *XR-V15B*, derived independently from the CHO and V79 cell lines, respectively. The presence of human chromosome 2 in complemented clones was examined cytogenetically and by PCR analysis with primers directed at a human-specific long interspersed repetitive sequence or chromosome 2-specific genes. Complementation was observed in 25/27 hybrids, one of which contained only the q arm of chromosome 2. The two noncomplementing hybrids were missing segments of chromosome 2. The use of a back-selection system enabled the isolation of clones that had lost the human chromosome and these regained radiation sensitivity. Transfer of several other human chromosomes did not result in complementation of the repair defect in *XR-V15B*. These data show that the gene defective in *xrs* cells, *XRCC5*, which is involved in double-strand break rejoining, is located on human chromosome 2q.**

A DNA double-strand break (dsb) is a major lesion that destroys the integrity of the DNA molecule. Such damage is introduced by ionizing radiation (IR) and may also arise during the repair of other DNA lesions or as an intermediate in the recombination step involved in metabolic processes such as the rearrangement of immunoglobulin genes and mating-type switching (1, 2). All organisms so far examined possess mechanisms to repair this important class of DNA damage (3–6). Several genes involved in dsb repair have been isolated from *Escherichia coli* and *Saccharomyces cerevisiae* but not, to date, from mammalian cells (7–9). However, a number of mutants defective in the repair of DNA dsb have been identified in rodent cells and classified into at least three distinct complementation groups (10–15). One complementation group includes six mutants isolated from the CHO cell line *xrs1–6* and one mutant (*XR-V15B*) derived from V79 cells (16, 17). All these mutants exhibit high sensitivity to IR, an impaired ability to rejoin DNA dsb but little or no sensitivity to UV irradiation (10, 18). The repair gene defective in these mutants has been designated *XRCC5* and the complementation group has been designated IR complementation group 5.

Attempts to clone the *XRCC5* gene by DNA transfection have so far been unsuccessful because of the high reversion frequency of the *xrs* mutants (19). We describe here an alternative approach involving initially identification of the complementing human chromosome. Studies aimed at mapping and ultimately at cloning the repair gene remain to be done.

For this study, we have used a panel of mouse–human monochromosomal hybrid cell lines each containing a single, different human chromosome "tagged" with a dominant selectable marker, the guanine phosphoribosyltransferase (*gpt*) gene (20, 21). Individual chromosomes were transferred into repair-deficient recipient cells by microcell-mediated chromosome transfer (20–22). Recipient cells carrying the human chromosomes were isolated by selection for the *gpt* marker and analyzed for phenotypic complementation of the repair defect to identify the complementing chromosome. This approach has been used successfully to identify the chromosomal location of other genes involved in DNA repair (21, 23, 24). We show here that the repair gene, *XRCC5*, which complements the defect in *xrs* cells, is located on the long arm of human chromosome 2.

MATERIALS AND METHODS

Cell Strains and Growth Conditions. Two members of the IR complementation group 5, *xrs6* and *XR-V15B*, isolated from the hamster CHO and V79 cell lines, respectively, were used in this study (16, 17). Spontaneous thioguanine-resistant (TG^R) derivatives were obtained from both strains for use as recipients for chromosome transfer. A panel of mouse–human hybrid cell lines, each containing a single, different human chromosome tagged with the *gpt* gene, a dominant selectable marker, were used as microcell donors (20, 21). All strains were routinely cultured in minimal essential medium (MEM; GIBCO) supplemented with nonessential amino acids and 10% fetal calf serum. All other conditions were as described (16). Medium was supplemented with mycophenolic acid (10 μg/ml) and xanthine (70 μg/ml) (MX medium) for selection and maintenance of gpt^+ clones. 6-Thioguanine (TG) (2 μg/ml) was used to select TG^R clones.

Microcell-Mediated Chromosome Transfer. Microcells were prepared from mouse–human monochromosomal hybrid cell lines as described (21). *xrs6* or *XR-V15B* recipient cells were seeded at 5×10^5 or 1×10^6 cells per 10-cm dish 36 h before fusion. For fusion, microcells suspended in 0.4 ml of serum-free medium containing phytohemagglutinin (100 μg/ml) were overlaid on a monolayer of recipient cells and

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: dsb, double-strand break(s); IR, ionizing radiation; TG, 6-thioguanine; TG^R, TG resistance.

†To whom reprint requests should be addressed.

incubated at 37°C for 15 min. The medium was then removed and 1.5 ml of PEG 1500 [50% (wt/vol) in 0.05 M Hepes] was added for 2 min. After careful washing with serum-free medium, cells were incubated overnight and then treated with trypsin and plated in MX selection medium. MX-resistant clones, isolated individually, were propagated in MX medium for further analysis.

Analysis of Hamster–Human Hybrid Clones. *(i) Survival following IR.* Cells suspended in medium were exposed to a single (routinely 2 Gy) or various doses of γ-rays and plated at densities varying from 10^2 to 10^3 cells per 6-cm dish. Survival was estimated from the number of clones in irradiated relative to unirradiated dishes after 7–10 days incubation.

(ii) Cytogenetic analysis. Standard metaphase preparations were made from all hybrid clones and parental strains as described (25). Metaphase spreads were stained for G11 and G-banding to determine the presence and structural integrity of the human chromosome (21).

(iii) PCR analysis. Genomic DNAs, prepared from each hamster–human hybrid cell line by standard procedures (26), were amplified by PCR using the L1-H primer and the procedure of Ledbetter *et al.* (27) with minor modifications. Samples (100 μl) containing 100 ng of DNA and 0.35 μM L1-H primer in 10 mM Tris·HCl/50 mM KCl/1.5 mM $MgCl_2$/0.01% gelatin/250 μM each dNTP/1 unit of *Taq* polymerase were amplified for 15 cycles by denaturation at 94°C (1 min), annealing at 55°C (2 min), and extension at 70°C (3 min). Another 1 unit of *Taq* polymerase was then added and amplification continued for a further 15 cycles. Twenty microliters of the PCR product was analyzed by gel electrophoresis. PCR amplification with chromosome 2-specific primers was carried out as described above except that each sample contained 1 μg of DNA and 1.25 μg of each primer (1.6 μM). Annealing was at 60°C rather than at 55°C. Chromosome 2-specific primers were as described (28).

(iv) Southern blot analysis. Genomic DNA (15 μg) was restriction digested, transferred to nylon filters, and hybridized by standard procedures (26). Plasmid pCD1-1 was digested with *Eco*RI to release a 2.7-kilobase (kb) insert containing part of the ERCC-3 coding region.

Measurement of dsb Rejoining. dsb rejoining after irradiation with 60 Gy using a ^{137}Cs source was measured by the pulsed-field gel electrophoresis (PFGE) procedure described by Whitaker and McMillan (29) with modification for use with the Waltzer apparatus (Tribotics, West Witney, UK; ref. 30). PFGE was carried out in 0.8% agarose gels in 0.5× TBE buffer (1× TBE = 45 mM Tris borate/1 mM EDTA) for 22 h at 16°C at 200 V (7.7 V/cm) and a pulse time of 70 sec (30). The fraction retained in the plug (*FR*) = (dpm per plug)/(dpm per plug + dpm per lane). Results are expressed as percentage rejoining index, which represents

$$\frac{FR_{\mathrm{I,t}} - FR_{\mathrm{I,O}}}{FR_{\mathrm{u}} - FR_{\mathrm{I,O}}} \times 100,$$

where I,t represents cells irradiated and repaired, I,O represents cells irradiated and not repaired, and u represents unirradiated cells. Representative values of FR_{u} and $FR_{\mathrm{I,O}}$ were 0.96 and 0.54, respectively.

RESULTS

Chromosome Transfer and Identification of the Complementing Chromosome. For all initial experiments, a TG^R ($HPRT^-$) derivative of *XR-V15B*, a member of IR complementation group 5 derived from the V79 cell line, was used as the recipient (17). Single human chromosomes present in the mouse–human monochromosomal hybrid cell lines were transferred to *XR-V15B* by microcell fusion. Hybrid clones of *XR-V15B* containing the transferred human chromosome were isolated by selection in MX medium. Complementation of the repair defect was analyzed by measuring survival after IR.

The human chromosomes transferred to the *XR-V15B* strain are listed in Table 1. With the exception of hybrids receiving human chromosome 2, all other hybrids constructed were as sensitive to IR as the parent strain *XR-V15B*. In initial experiments, human chromosome 2 appeared capable of correcting the defect in *XR-V15B*. Subsequently, human chromosome 2 originating from two different sources was transferred into two different recipient cell lines belonging to complementation group 5. The monochromosomal hybrid donor lines were RA3-5 and RA-2, which contained human chromosome 2 derived from a transformed and a primary human line, respectively. Two recipient cell lines, *XR-V15B* TG^R and *xrs6* TG^R, were used to verify that chromosome 2 could complement two independently derived members of complementation group 5. In these experiments, a total of 27 hamster–human hybrid clones were isolated (Table 1). Survival following a range of γ-ray doses was measured for eight representative hybrid clones (Fig. 1) and the remainder were examined for survival after exposure to a single dose of radiation (2 Gy). Twenty-five of the 27 hybrid clones showed elevated resistance to radiation when compared to the sensitive parent, while two clones (*XR*–RA3-5 E1 and *XR*–RA3-5 H27) maintained their sensitivity (Fig. 1*a* and Table 1; survival data only shown for H27).

These results show that the radiation sensitivity of both the V79- and CHO-derived members of IR complementation group 5 is corrected specifically by human chromosome 2. In all cases, however, only partial correction was observed, which appeared to be intermediate between mutant and parental levels.

Table 1. Human chromosomes transferred to *XR-V15B* and *xrs6*

Donor hybrid	Human chromosome present	Recipient	γ-Ray sensitivity	No. of clones examined	Nomenclature of hybrids
RA5-5	5	*XR-V15B*	S	2	
RA6	6	*XR-V15B*	S	1	
RA9	9	*XR-V15B*	S	4	
RA1	12q	*XR-V15B*	S	4	
CHH13	13	*XR-V15B*	S	2	
RA12-2	15	*XR-V15B*	S	4	
RA17	17	*XR-V15B*	S	1	
RA21	21	*XR-V15B*	S	3	
RA3-5	2	*XR-V15B*	19R, 2S	21	*XR*–RA3-5
RA3-5	2	*xrs6*	R	5	*xrs*–RA3-5
RA2	2	*XR-V15B*	R	1	*XR*–RA2

S, sensitive to IR; R, resistant to IR. See Fig. 1 for survival levels.

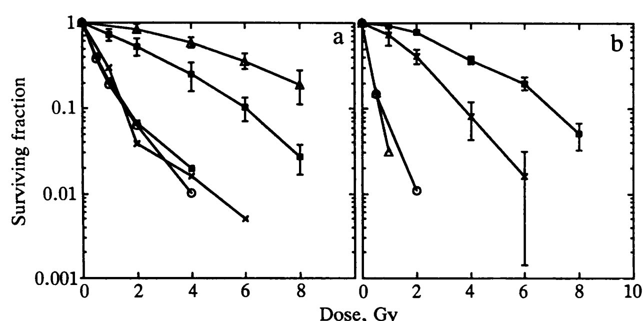

FIG. 1. (*a*) Survival of *XR*–RA3-5 hybrid clones after exposure to IR. Results for *XR*–RA3-5 hybrids represent the mean of one or two survival curves carried out on six different hybrids. All other survival curves represent the mean of at least four experiments on the same line. Error bars represent 1 SD. For clarity, error bars are not shown on the highly sensitive lines. △, V79; ⊡, *XR*–RA3-5 hybrids; ○, *XR-V15B* TG^R; ×, *XR*–RA3-5 H27; ■, TG^R segregant derived from *XR*–RA3-5 H1. (*b*) Survival of *xrs*–RA3-5 hybrids after exposure to IR. For *xrs*–RA3-5 hybrid clones, results represent the mean of three experiments carried out on two different hybrids. Other survival curves were the mean of at least three experiments. ⊡, CHO-K1; ×, *xrs*–RA3-5 hybrids; ○, *xrs6* TG^R; △, TG^R segregant derived from *xrs*–RA3-5 D5.

Complementation of the Defect in dsb Rejoining. The radiation sensitivity of the IR complementation group 5 mutants is associated with a defect in dsb repair (10, 17, 18). To investigate complementation of this phenotype, one hybrid (*XR*–RA3-5 H1) and the parental mutant and normal cell lines (*XR-V15B* and V79) were compared for their ability to rejoin radiation-induced DNA dsb by pulsed-field gel electrophoresis. The results show that DNA dsb rejoining in the hybrid cells was as proficient as the parent V79 strain, while *XR-V15B* had a reduced ability for dsb rejoining (Fig. 2).

Cosegregation of Chromosome 2 and Radiation Resistance. Since *XR-V15B* and *xrs6* are hprt$^-$, it is possible to isolate cells that have lost the gpt tagged human chromosome by back-selection in TG. Cells from five complemented hybrid clones—*XR*–RA3-5 H1, H21, H24, *xrs6*–RA3-5 D5, and *XR*–RA2 P1—were cultured in nonselective medium and then plated in medium containing TG. Three independent clones were isolated from each hybrid. Survival following a range of γ-ray doses was examined for one clone from each hybrid, and the remaining clones were analyzed for survival following a single dose of γ-rays (2 Gy). All back-selected clones exhibited the radiosensitive phenotype characteristic of the *XR-V15B* or *xrs6* parent strain (shown in Fig. 1*a* for one hybrid). These data show that the radiation resistance of hybrid clones cosegregates with human chromosome 2. This provides strong evidence that γ-ray resistance is due to the presence of human chromosome 2 and is not the result of a reversion event or due to the presence of a mouse chromosome that may have been transferred along with the human chromosome. Loss of human chromosome 2 in the TG^R segregants was confirmed by molecular analysis (see below).

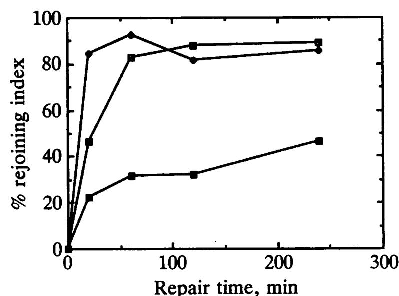

FIG. 2. dsb rejoining in *XR*–RA3-5 H1. Cells were irradiated with 60 Gy. Results represent the mean of duplicate samples from two independent experiments. ⊡, V79; ■, *XR-V15B* TG^R; ◆, hybrid *XR*–RA3-5 H1.

Identification of Human DNA in the Hybrid Clones. *(i) PCR analysis using long interspersed repetitive sequences.* Specific amplification of human DNA in a somatic cell hybrid can be achieved by using a human specific primer (L1-H) directed at the middle long interspersed repetitive sequence (27). This sequence occurs as an inverted repeat less frequently than *Alu* sequences and normally yields a pattern of 5–10 amplification products per chromosome. This technique can therefore act as a form of chromosome fingerprinting and was used to verify the presence of human DNA in the hamster–human hybrids. Using the L1-H primer and DNA from the human–mouse donor hybrid, RA3-5, a pattern of amplification products specific for chromosome 2 was observed consisting of five bands designated A–E (Fig. 3). No amplification products were produced by using either hamster or mouse DNA. All of the hybrid clones bearing chromosome 2 were analyzed by PCR amplification using the L1-H primer. Twenty-one of the complemented hybrids gave PCR products identical to the RA3-5 donor strain (shown in Fig. 3 for two hybrids—*XR*–RA3-5 H1 and H24). Four of the complemented hybrids—*XR*–RA3-5 H22, H26, H33, and H53 (shown in Fig. 3 for *XR*–RA3-5 H33 and *XR*–RA3-5 H53)—and the two noncomplemented hybrids *XR*–RA3-5 E1 and H27, however, produced an aberrant pattern with some bands missing (Fig. 3).

No detectable human DNA was present in three TG^R segregants examined by this method (Fig. 3 and Table 2), thus confirming our conclusion that the human DNA present in the hybrid clones is lost on back-selection.

(ii) PCR analysis using chromosome 2-specific primers. To verify the identity of the human chromosome, PCR analysis of some hybrids was also carried out with primers specific for this chromosome (28, 31). These data are summarized in Table 2. All the hybrid clones, including the two noncomplemented, were positive for the marker placental alkaline phosphatase. This suggests that the *gpt* selective marker, which must be retained in all hybrids, is tightly linked to the ALPP marker, which maps in the region 2q37. Retention of ALPP in the two radiosensitive hybrids suggests that the *XRCC5* gene is less closely linked to this marker. For all other

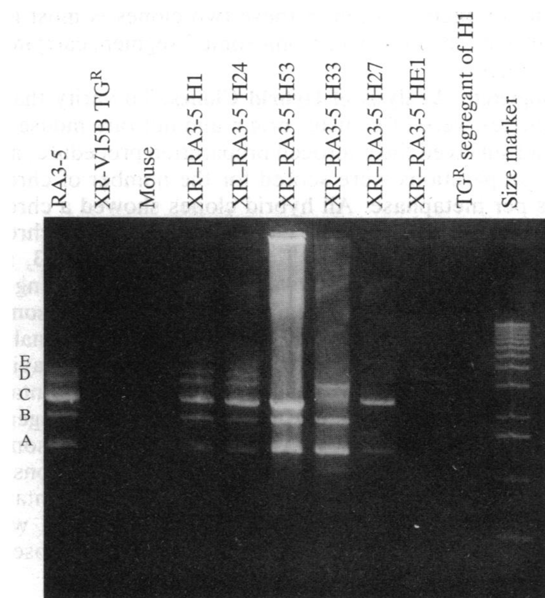

FIG. 3. Analysis of PCR products with L1-H primers. DNA from parental and hybrid clones was amplified with the L1-H primer and products were separated by electrophoresis in 0.8% agarose.

Table 2. PCR analysis of hybrids

Hybrid	γ-Ray sensitivity	PCR bands using L1-H primers: A	B	C	D	E	Others	Chromosome 2-specific primers: ALPP (2q37)	IL1A (2q12–q21)	POMC (2p23)
XR-V15B	S	–	–	–	–	–		–	–	–
RA3-5	R	+	+	+	+	+		+	+	+
XR-RA3-5 E1	S	–	–	–	–	–	1 weak band	+	–	–
XR-RA3-5 H27	S	+	–	+	–	–		+	–	–
XR-RA3-5 H22	R	+	+	–	+	–	1 extra band	+	+	–
XR-RA3-5 H33	R	+	+	–	+	–		+	–	–
XR-RA3-5 H26	R	+	+	+	–	–		+	+	+
XR-RA3-5 H53	R	+	+	+	–	–		+	+	+
XR-RA3-5 H1	R	+	+	+	+	+		+	+	–
XR-RA3-5 H24	R	+	+	+	+	+		+	+	+
XR-RA3-5 B2	R	+	+	+	+	+		+	+	+
XR-RA3-5 B4	R	+	+	+	+	+		+	+	+
xrs-RA3-5 D5	R	+	+	+	+	+		+	–	+
xrs-RA3-5 D12	R	+	+	+	+	+		+	+	+
XR-RA2 P1	R	+	+	+	+	+		+	+	–
XR-RA3-5 H1.TG^R	S	–	–	–	–	–		–	–	–

S, sensitive to IR; R, resistant to IR.

markers examined, both positive and negative clones were obtained, and there was no obvious correlation between radiation resistance and the presence of a specific marker.

(iii) Southern blot analysis using a chromosome 2-specific probe. Finally, to verify further the transfer of chromosome 2 to the hamster lines, DNAs from human placenta, RA3-5, hamster (*XR-V15B*), and two hybrids (*XR*-RA3-5 B4 and *XR*-RA2 P1) were digested with *Eco*RI and hybridized with a fragment derived from plasmid pCD1-1, which contains *ERCC-3* cDNA, a gene previously shown to map to 2q21. With human DNA this probe gave three bands (5.6, 4.2, and 3.0 kb) as shown previously (32) while a single band (7.1 kb) was obtained with hamster DNA. The two hybrids examined yielded four bands showing the presence of both the human and hamster genes (data not shown).

These data verify that human chromosome 2 has been transferred to the complemented hybrids but indicate that loss of segments of the chromosome has occurred in some hybrids. The two noncomplemented clones E1 and H27 were positive for only one of the three markers examined. Thus, lack of complementation in these two clones is most likely explained by the loss of a chromosome 2 segment carrying the *XRCC5* gene.

Cytogenetic Analysis of Hybrid Clones. To verify that the gpt$^+$ clones were of hamster origin and not rare mouse cells that had survived the microcell preparation procedure, metaphase preparations were scored for the number of chromosomes per metaphase. All hybrid clones showed a chromosome complement typical of hamster cells (21 or 22 chromosomes). Selected hybrids (*XR*–RA3-5 H1, H13, H33, H53, H26, H27, and E1) were examined by G11 staining and G-banding to assess the integrity of human chromosome 2. Hybrid H1 (a clone also used for segregation analysis) contained either an intact chromosome 2 or one having a small deletion. The remaining hybrids examined contained one or more fragments of chromosome 2. These cytogenetic data support the conclusion that human chromosome 2 material is present in these hybrids, but that deletions and rearrangements are frequent. Because of the fragmentation, it was not possible by this analysis to determine which specific segments of chromosome 2 were present or absent in the hybrids.

Localization of *XRCC5* to the Long Arm of Chromosome 2. Cytogenetic analysis of hybrid *XL*–RA2 P1 revealed a mixed population with some cells bearing a single arm of chromosome 2. This hybrid was therefore subcloned to obtain two clones (P1-7 and P1-8), which contained only one arm of chromosome 2 (Fig. 4). PCR analysis using chromosome 2-specific primers showed that two q arm sequences were present and one p arm sequence was absent (Table 2). Clones P1-7 and P1-8 were resistant to IR and TG^R segregants from both clones regained γ-ray sensitivity (data not shown). These data show that *XRCC5* is located on the q arm of chromosome 2.

DISCUSSION

We have used the technique of microcell-mediated chromosome transfer to show that human chromosome 2 complements the repair defect in two radiation-sensitive mutants, *xrs6* and *XR-V15B*, and have mapped the complementing gene (*XRCC5*) to the q arm of this chromosome. Hamster mutants in this complementation group are sensitive to IR and have a defect in dsb rejoining, and both phenotypes are corrected by chromosome 2. Two different mouse–human monochromosomal hybrids, RA-2 and RA3-5, carrying chromosome 2 derived from primary human or transformed cells, respectively, were used as microcell donor strains. Two members of the IR complementation group 5, derived from two different hamster lines, were corrected by human chromosome 2. PCR analysis of the chromosome transfer hybrid clones showed that two noncomplemented hybrids were missing substantial portions of chromosome 2 but retained the ALPP marker, which maps to 2q37, suggesting that *XRCC5* is not closely linked to this marker. However, one

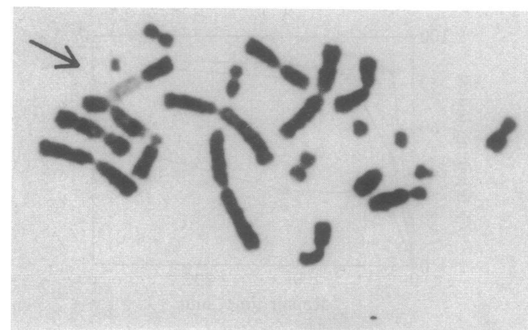

FIG. 4. Metaphase spread of hybrid *XR*–RA2 P1-7 stained by the G11 method. The q arm of human chromosome 2 (arrow) was also identified by G-banding (data not shown).

complemented hybrid, obtained by subcloning, contained only the q arm of chromosome 2, indicating that *XRCC5* does reside on this arm. These data were confirmed by the cosegregation of 2q and radiation resistance in clones obtained by back-selection. It is interesting that another gene involved in DNA repair *(ERCC3)* is located at 2q21 (32). We do not know at present whether or not *XRCC5* is closely linked to *ERCC3*. Evidence for limited clustering of DNA repair genes has been observed previously on chromosome 19 (33–35). None of the other chromosomes when transferred to *XR-V15B* showed complementation of the repair defect.

Although the use of the *xrs* mutants for gene cloning and mapping studies has been hampered by their propensity to revert, the microcell-mediated chromosome transfer technique described here has enabled us to overcome this problem in two ways. First, selection for rare hybrids bearing chromosome 2 does not require selection for radiation resistance and thus minimizes the likelihood of selecting revertant clones. Second, the use of a two-way selection system has enabled us to show that loss of the human chromosome 2 restores radiation sensitivity. Taken together, these data provide strong evidence that a gene involved in dsb rejoining is located on human chromosome 2 and also support the prior conclusion that the two mutants (*xrs6* and *XR-V15B*) are members of the same complementation group. Furthermore, our results show that this technique is applicable for mapping genes defective in mutant strains for which a strong selection does not exist and/or that have a tendency to revert.

It was interesting that extensive fragmentation and loss of human chromosome 2 segments occurred in many of the hybrid clones examined. This property may relate to the defect in dsb repair of the recipient strain. If so, this cell line could be a useful recipient when chromosome fragmentation is specifically desired, as, for example, in subchromosomal mapping.

Only partial restoration of γ-ray resistance was observed in all complementing hybrids. Since the same results were achieved by using chromosome 2 originating from two independent sources, it is unlikely that partial correction results from a partial defect in the human gene. One explanation is that the human gene cannot fully complement the defect in the hamster mutant and might indicate that the gene product acts as part of a repair complex. An alternative possibility is that more than one gene is required for complete restoration of the repair defect. The explanation awaits cloning of the *XRCC5* gene.

In conclusion, in this study we have isolated a panel of chromosome transfer hybrids bearing fragments of human chromosome 2 both complementing and noncomplementing the *xrs* repair defect. We have thus shown that the *XRCC5* gene, whose product is involved in dsb rejoining, is located on the long arm of human chromosome 2. These hybrids will be important intermediates in further studies aimed at cloning the *XRCC5* gene.

We wish to acknowledge the help and support of Dr. A. Lehmann in this work, Dr. S. Povey for a gift of some PCR primers, and Dr. S. Whittacker for help in setting up the pulsed-field gel electrophoresis technique. We thank Dr. G. Weeda for the plasmid pCD1-1. Work contributing to this study was funded by Grant DE-FG02-89ER60866 to R.S.A. from the U.S. Department of Energy. G.P.K. was supported by a fellowship from the New Jersey Commission on Cancer Research and the Association of Brain Tumor Research.

1. Hendrickson, E. A., Qin, X., Bump, E. A., Schatz, D. G., Oettinger, M. & Weaver, D. T. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 4061–4065.
2. Malone, R. E. & Esposito, R. E. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 503–507.
3. Kitayama, S. A. & Matsuyama, A. (1968) *Biochem. Biophys. Res. Commun.* **33,** 418–422.
4. Hariharan, P. V. & Hutchinson, F. (1973) *J. Mol. Biol.* **75,** 479–494.
5. Ho, K. Y. (1975) *Mutat. Res.* **30,** 327–334.
6. Resnick, M. A. & Martin, P. (1976) *Mol. Gen. Genet.* **143,** 119–129.
7. Schild, D., Konforti, B., Perez, C., Gish, W. & Mortimer, R. K. (1983) *Curr. Genet.* **7,** 85–92.
8. Calderon, I. J., Contopoulou, C. R. & Mortimer, R. K. (1983) *Curr. Genet.* **7,** 93–100.
9. Picksley, S. M., Attfield, P. V. & Lloyd, R. G. (1984) *Mol. Gen. Genet.* **195,** 267–274.
10. Kemp, L. M., Sedgwick, S. G. & Jeggo, P. A. (1984) *Mutat. Res.* **132,** 189–196.
11. Giaccia, A., Weinstein, R., Hu, J. & Stamato, T. D. (1985) *Somat. Cell Mol. Genet.* **11,** 485–491.
12. Evans, H. L., Ricanati, M. & Horng, M.-F. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 7562–7566.
13. Whitmore, G. F., Varghese, A. J. & Gulyas, S. (1989) *Int. J. Radiat. Biol.* **56,** 657–665.
14. Jeggo, P. A., Tesmer, J. & Chen, D. J. (1991) *Mutat. Res.* **254,** 125–133.
15. Thacker, J. & Wilkinson, R. E. (1991) *Mutat. Res.* **254,** 135–142.
16. Jeggo, P. A. & Kemp, L. M. (1983) *Mutat. Res.* **112,** 313–327.
17. Zdzienicka, M. Z., Tran, Q., Van Der Schans, C. P. & Simons, J. W. I. M. (1988) *Mutat. Res.* **194,** 239–243.
18. Weibezahn, K. F., Lohrer, H. & Herrlich, P. (1985) *Mutat. Res.* **145,** 177–183.
19. Jeggo, P. A. & Holliday, R. (1986) *Mol. Cell. Biol.* **6,** 2944–2949.
20. Athwal, R. S., Smarsh, M., Searle, B. M. & Deo, S. S. (1985) *Somat. Cell Mol. Genet.* **11,** 177–187.
21. Kaur, G. P. & Athwal, R. S. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 8872–8876.
22. Athwal, R. S. & Dhar, V. (1984) in *Gene Transfer and Cancer*, eds. Pearson, M. L. & Sternberg, N. L. (Raven, New York), pp. 21–29.
23. Saxon, P. J., Schultz, R. A., Stanbridge, E. J. & Friedberg, E. C. (1989) *Am. J. Hum. Genet.* **44,** 474–485.
24. Ishizaki, K., Oshimura, M., Sasaki, M. S., Nakamura, Y. & Ikenaca, M. (1990) *Mutat. Res.* **235,** 209–215.
25. Jeggo, P. A. (1985) *Mutat. Res.* **146,** 265–270.
26. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
27. Ledbetter, S. A., Garcia-Heras, J. & Ledbetter, D. H. (1990) *Genomics* **8,** 614–622.
28. Theune, S., Fung, J., Todd, S., Sakaguchi, A. Y. & Naylor, L. (1991) *Genomics* **9,** 511–516.
29. Whitaker, S. J. & McMillan, T. J. (1992) *Int. J. Radiat. Biol.* **61,** 29–41.
30. Southern, E. M., Anand, R., Brown, W. R. A. & Fletcher, D. S. (1987) *Nucleic Acids Res.* **15,** 5925–5943.
31. Abbott, C. & Povey, S. (1991) *Genomics* **9,** 73–77.
32. Weeda, G., Wiegant, J., Van Der Ploeg, M., Van Kessel, A. H. M. G., Van Der Eb, A. J. & Hoeijmakers, J. H. J. (1991) *Genomics* **10,** 1035–1040.
33. Siciliano, M. J., Carrano, A. V. & Thompson, L. H. (1986) *Mutat. Res.* **174,** 303–308.
34. Mohrenweiser, H. W., Carrano, A. V., Fertitta, A., Perry, B., Thompson, L. H., Tucker, J. D. & Weber, C. A. (1989) *Cytogenet. Cell Genet.* **52,** 11–14.
35. Smeets, H., Bachinski, L., Corrwinkel, M., Schepens, J., Hoeijmakers, J. H. J., Van Duin, M., Grzeschik, L. H., Weber, C. A., De Jong, P., Siciliano, M. J. & Wirringa, B. (1990) *Am. J. Hum. Genet.* **46,** 492–501.