SHORT COMMUNICATION

Assignment of the *XRCC2* Human DNA Repair Gene to Chromosome 7q36 by Complementation Analysis

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The V79 hamster cell line irs1 is a repair-deficient mutant hypersensitive to radiation and DNA-reactive chemical agents. Somatic cell hybrids were formed by fusing irs1 cells with human lymphocytes and selecting for complementation in medium containing concentrations of mitomycin C (MMC) that are toxic to irs1. Thirty-eight MMC-resistant hybrids showed extensive segregation of human chromosomes, with 35 of them retaining human chromosome 7, as indicated by molecular marker and cytogenetic analyses. Inter-*Alu*-PCR products from the DNA of hybrids, when used as fluorescence *in situ* hybridization probe onto normal human metaphases, indicated that one resistant hybrid was monochromosomal for chromosome 7 and that the three resistant hybrids shown to be negative for chromosome 7 markers have retained portions of chromosome 7, with region 7q36 being the smallest common region. MMC-sensitive subclones of a resistant hybrid lost human chromosome 7. Therefore, the gene complementing the repair defect, *XRCC2* (X-ray repair cross complementing), is assigned to human chromosome 7q36.

The isolation and analysis of repair-deficient mutants of Chinese hamster cells have provided a powerful system for identifying and cloning human DNA repair genes by functional complementation. In the nucleotide excision repair pathway, CHO UV-sensitive mutants were used to clone the *ERCC1* through *ERCC6* human repair genes, and several of these genes have been shown to be involved in the human repair disorder xeroderma pigmentosum (9, 26). Human genes involved in ionizing radiation sensitivity (XRCC, to denote X-ray repair cross complementing) are also being mapped, cloned, and characterized. The CHO mutant line EM9, identified on the basis of hypersensitivity to ethyl methanesulfonate, was used to clone the human *XRCC1* gene (25). *XRCC1* was mapped to chromosome 19q13.2–q13.3 (18, 20) and shown to correct the defective repair of single-strand breaks and the high level of sister-chromatid exchange in EM9 cells (25). *XRCC1* protein appears to act at the final step of ligation in base excision repair, as recent evidence shows an association of *XRCC1* with DNA ligase III (2). The *XRCC4* and *XRCC5* genes, which are required for repairing double-strand breaks, were mapped to chromosomes 5 and 2q35, respectively (3, 7, 8). *XRCC5* proved to be equivalent to the Ku80 autoantigen, a DNA end-binding protein (21).

The V79 Chinese hamster mutant irs1 was isolated as an ionizing-radiation-sensitive (irs) mutant (~3-fold hypersensitive), which was found to have a very broad spectrum of hypersensitivity to other agents, including UV radiation (~3-fold), ethyl methanesulfonate (10-fold), and mitomycin C (60-fold) (12). Subsequent studies showed that the extreme sensitivity seen with mitomycin C extended to other DNA-cross-linking agents, namely cisplatin, nitrogen mustard, melphalan, chlorambucil, and 1,3-butadiene diepoxide (1, 14). To date, complementation analysis has demonstrated that irs1 is the unique representative of its assigned group for both ionizing radiation sensitivity (10, 13, 24) and mitomycin C sensitivity (11, 15, 19).

A specific DNA repair defect in irs1 has not been identified, as the repair of single-strand and double-strand breaks, measured by both filter elution and sucrose gradient sedimentation techniques, appeared normal in assays performed on total genomic DNA (15, 22). However, the greatly elevated levels of chromosomal aberrations induced in irs1 by both ionizing radiation and mitomycin C, including numerous chromatid deletions and exchanges, suggest that some lesions result in double-strand breaks that remain unrepaired or become misrepaired (23, 27). Using a vector-mediated assay, Debenham and co-workers (5) presented evidence that irs1 has a reduced efficiency of faithfully rejoining a restriction-enzyme-mediated scission in the *gpt* gene of a transfected plasmid molecule when later assayed for *gpt* function. A better understanding of the biochemical defect in irs1 cells in terms of the repair pathway that is affected may result from characteriz-

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TABLE 1

Percentage of the 38 Resistant Hybrids Retaining at Least One of the Markers Tested for Each Human Chromosome

	Human chromosome																					
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X
Percentage of hybrids with marker	53	58	45	45	25	53	92	47	18	53	53	63	39	55	47	34	18	47	55	53	55	47	13

ing the defective gene. In this study we used somatic cell hybrids to identify a human gene, which we define as *XRCC2,* that complements the mutagen sensitivities of irs1, and we report a regional chromosomal localization for this gene.

Hybrids were formed by fusing irs1 mutant cells with human lymphocytes from fresh blood of healthy donors as described previously (15) and grown in α-MEM medium supplemented with 10% fetal bovine serum and 100 μg/ml streptomycin sulfate + 100 units/ml penicillin (Gibco). A total of 38 primary hybrids were isolated by selecting for cells that formed colonies in the presence of 30–100 n*M* mitomycin C (MMC), a concentration range that is fully toxic to irs1 mutant cells. After expansion to mass culture in the presence of MMC, hybrids were analyzed for their content of human chromosomes. The presence or absence of human chromosomes was determined by a combination of isozyme and Southern blot analyses of 42 chromosomally assigned biochemical and molecular markers representing all autosomes and the X chromosome as previously described (4).

The expectation was that every hybrid should contain the human chromosome that carries the gene responsible for the complementation. The results of that analysis are summarized in Table 1, which shows that 92% of the hybrids retained the isozyme marker for human chromosome 7 (*GUSB*), while markers for other human chromosomes were present in from 13 to 63% of the hybrids. In one hybrid (1HL11), *GUSB* was the only human marker detected and the only human chromosome detected by cytogenetic analysis (data not shown), suggesting that the hybrid was monochromosomal for human chromosome 7. This inference was verified by performing inter-*Alu*-PCR on the DNA from 1HL11 and using it as competitive fluorescence *in situ* hybridization (FISH) probe to normal human metaphase spreads (16). This procedure, called "paint-back," identifies the human genomic regions present in hybrid cells. As shown in Fig. 1A, the human DNA derived from 1HL11 resulted in the painting of only chromosomes 7 in such spreads. This result indicated that 1HL11 was monochromosomal for human chromosome 7 and, taken together with the marker analysis data for all of the hybrid cells, suggested that the complementing gene, which we have defined as *XRCC2* (X-ray repair cross complementing gene 2), is present on human chromosome 7.

To confirm the human chromosome 7 localization of *XRCC2,* hybrid 1HL8 was allowed to grow in the absence of MMC selection for several weeks and subclones were isolated. Two resistant (1HL8-1 and 1HL8-2) and two sensitive (1HL8-3 and 1HL8-4) subclones were analyzed. The two resistant subclones retained human *GUSB,* and the two subclones that had segregated the human sequences responsible for MMC resistance did not have human *GUSB.* Paint-backs of the human DNA from the resistant and sensitive hybrid subclones were performed using inter-*Alu*-PCR DNA derived from each of them. Paint-backs to human chromosomes 7 were seen for the probes derived from resistant subclones, but the probes from the sensitive subclones did not result in painting of chromosomes 7 (data not shown). These results confirmed the marker analysis and verified the human chromosome 7 location of *XRCC2.*

Three resistant hybrids not scoring positive for *GUSB*—1HL2, 1HL12, and 1HL13—resulted in only 92% of the hybrids scoring positive for chromosome 7 rather than the hypothesized 100% as discussed above. To obtain a subchromosomal localization of *XRCC2,* these discordant hybrids were analyzed in greater detail. Although they could conceivably have been revertant irs1 cells that had regained resistance to MMC, they were deemed true hybrids because 1HL12 retained human markers for chromosomes 2 and 8, 1HL13 retained the human marker for chromosome 13, and 1HL2 was shown to contain human repeat sequences (data not shown). We concluded that they were hybrids that had retained portions of human chromosome 7 containing *XRCC2* and had lost the portions containing *GUSB.* Therefore, determination of the portions of chromosome 7 retained in these hybrids would effectively identify the regional location of *XRCC2.*

A series of molecular markers (*PDGFA, IL6, TCRG, EGFR, D7S325, GUSB,* and *MET*) for the identification of different regions of human chromosome 7 (p22, p21–p15, p15–p14, p12, p11–q11, q22, and q31, respectively) was used in Southern hybridization studies to determine what portions, if any, of the chromosome were retained in hybrids 1HL2, 1HL12, and 1HL13. All of these regional markers were absent in the discordant hybrids but present in a representative set of positive controls (resistant hybrids that had been shown to retain *GUSB*). These data suggest that if there is a complementing portion of chromosome 7 present in hybrids

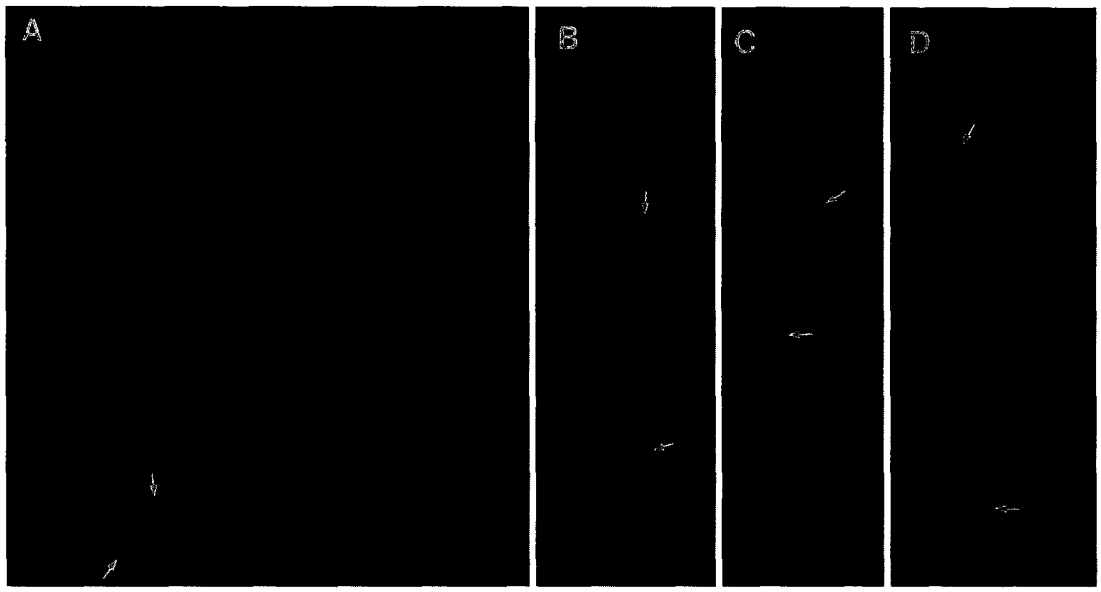

FIG. 1. Fluorescence *in situ* hybridization (FISH) or paint-back experiments. For the molecular cytogenetic identification of human chromosomal regions present in hybrids, human DNA was amplified by inter-*Alu*-PCR, labeled with biotin and/or digoxigenin, and competitively hybridized onto normal human metaphase preparation with human low-Cot DNA to block nonspecific repetitive DNA from participating in the reaction. Regions of hybridization were detected by avidin–fluorescein and/or rhodamine as described in Liu *et al.* (16), for the biotin–avidin–fluorescein system, and in Marlton *et al.* (17) for the multiple color system. Arrows indicate centromeres of human chromosomes 7. (**A**) Chromosomes 7 only were visualized and R-banded by probe from hybrid 1HL11. (**B**) Partial metaphase in which probe from hybrid 1HL11 was used to identify the chromosomes 7 (red), and probe from 1HL13 was used to identify any regions of the chromosome (green overlaying red producing yellow) present in that hybrid. (**C**) Same as **B** but replacing probe from 1HL13 with probe from 1HL12. (**D**) Same as **B** but replacing probe from 1HL13 with probe from 1HL2.

1HL2, 1HL12, and 1HL13, it is most probably the end of either the p or the q arm (the regions not represented in the markers used). To test this presumption, FISH experiments were performed by inter-*Alu*-PCR extraction of the human DNA from the three hybrids of concern and painting those DNAs back onto normal human chromosomes. These data are illustrated in Figs. 1B–1D and indicate that hybrid 1HL2 retains only the q36 portion of human chromosome 7, while hybrids 1HL12 and 1HL13 retain regions q34–q36 and q32–q36, respectively. The smallest region of overlap being 7q36, the very end of the q arm, enables us to assign *XRCC2* to that region.

The *XRCC2* gene that we have localized to 7p36 appears to complement the broad-spectrum mutagen sensitivity of mutant irs1. Previous data on several of the hybrids used in the present study showed that the resistance of irs1 to MMC and UV radiation was restored to normal levels, whereas the resistance to gamma rays and ethyl methanesulfonate was fully restored in some hybrids but partially restored in others (15). Thus, a single gene (the putative hamster homolog of *XRCC2*) seems to be mutated and responsible for the complex mutagen sensitivity phenotype of irs1 cells. Our regional assignment should be of assistance in cloning this human gene, an activity that should result in an understanding of the type of repair defect present in irs1 cells. It should be of great interest to determine how that activity compares with that of *XRCC3* (the cDNA of which we have recently cloned—R. S. Tebbs, L. H. Thompson, and M. J. Siciliano, submitted), which corrects the CHO mutant irs1SF (6), since that mutant shares with irs1 the same broad-spectrum mutagen sensitivity. Such observations suggest that *XRCC2* and *XRCC3* may be involved in the same repair pathway.

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Note added in proof. While this article was in press, the following independent work placing *XRCC2* in essentially the same position as that reported here was published: Thacker, J., Tambini, C. E., Simpson, P. J., Tsui, L.-C., and Scherer, W. (1995). Localization to chromosome 7q36.1 of the human *XRCC2* gene, determining sensitivity to DNA-damaging agents. *Hum. Mol. Genet.* **4:** 113–120.

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