Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 6354–6358, July 1995
Genetics

Correction of chromosomal instability and sensitivity to diverse mutagens by a cloned cDNA of the *XRCC3* DNA repair gene

(irs1SF/crosslinking agents/genetic complementation/chromosomal aberrations)

ROBERT S. TEBBS*†, YING ZHAO‡, JAMES D. TUCKER*, JULIA B. SCHEERER‡, MICHAEL J. SICILIANO‡, MONA HWANG*, NAN LIU*, RANDY J. LEGERSKI‡, AND LARRY H. THOMPSON*§

*Biology and Biotechnology Research Program, Lawrence Livermore National Laboratory, Livermore, CA 94551-0808; and ‡Department of Molecular Genetics, University of Texas Cancer Center, Houston, TX 77030

Communicated by Evelyn Witkin, Princeton, NJ, March 24, 1995

ABSTRACT **The mutagen-sensitive CHO line irs1SF was previously isolated on the basis of hypersensitivity to ionizing radiation and was found to be chromosomally unstable as well as cross-sensitive to diverse kinds of DNA-damaging agents. The analysis of somatic cell hybrids formed between irs1SF and human lymphocytes implicated a human gene (defined as *XRCC3*; x-ray repair cross-complementing), which partially restored mitomycin C resistance to the mutant. A functional cDNA that confers mitomycin C resistance was transferred to irs1SF cells by transforming them with an expression cDNA library and obtaining primary and secondary transformants. Functional cDNA clones were recovered from a cosmid library prepared from a secondary transformant. Transformants also showed partial correction of sensitivity to cisplatin and γ-rays, efficient correction of chromosomal instability, and substantially improved plating efficiency and growth rate. The *XRCC3* cDNA insert is ≈2.5 kb and detects an ≈3.0-kb mRNA on Northern blots. The cDNA was mapped by fluorescence *in situ* hybridization to human chromosome 14q32.3, which was consistent with the chromosome concordance data of two independent hybrid clone panels.**

DNA repair is a universal process in living cells that maintains the structural integrity of chromosomal DNA molecules in the face of damage arising from environmental insults, as well as from normal metabolic processes. Considerable progress has been made in identifying genes in human cells that determine DNA-repair pathways, especially nucleotide and base excision repair. Nucleotide excision repair (NER) is responsible for removing most types of bulky chemical adducts and the major photoproducts arising from UV radiation. The excision product is an oligonucleotide 27–29 bases in length (1). Base excision repair acts on smaller lesions including (m)ethylated, oxidized, and depurinated bases, as well as nonligatable breaks, and results in a repair patch of one or several nucleotides (2, 3). The use of mutant cell lines assigned to genetic complementation groups has been the main approach to identifying the individual enzymatic steps of the NER pathway (4).

In rodent cells a wide variety of mutants showing sensitivity to ionizing radiation have been reported, and many of these mutants have complex phenotypes showing sensitivity to other kinds of DNA-damaging agents (5). The analysis of these mutants and their complementing genes holds considerable promise for identifying components of mammalian DNA repair that remain poorly understood. Mutants isolated on the basis of hypersensitivity to one agent have often shown much greater sensitivity to other agents. An example is the CHO mutant irs1SF that was isolated as a moderately (2-fold) x-ray-sensitive mutant (6) but was found to have cross-sensitivity to UV radiation (2.5-fold), ethyl methanesulfonate (2.3-fold), camptothecin (5-fold), and the cross-linking agents mitomycin C (MMC), cisplatin, nitrogen mustard, and melphalan (all 20- to 60-fold) (refs. 6 and 7 and results presented here). irs1SF was also reported to have ≈50% reduced efficiency of both single-strand break rejoining and repair replication, elevated spontaneous chromosomal aberrations (10–24% abnormal cells), hypomutability in response to x-rays, but a normal baseline for sister chromatid exchange (6). We describe here the cloning of a cDNA sequence that corrects x-ray and cross-linking sensitivities, as well as spontaneous chromosomal aberrations of irs1SF, and we have localized the corresponding gene to human chromosome 14q32.3.

MATERIALS AND METHODS

Cell Culture and Mutagen Exposure. Wild-type CHO AA8, irs1SF, and transformants (TFs) were cultured in monolayer or suspension at 37°C as described (8). Cell survival curves in the presence of MMC or cisplatin were obtained by exposing exponentially growing 10-ml cultures of 10^5 cells to the drug at 37°C for 1 h in suspension. Treatment was terminated on ice, and the cells were centrifuged and resuspended in fresh medium for plating. Plating efficiency was assessed in triplicate 10-cm dishes (300 cells each), and survival was measured by using triplicate dishes with various inocula. Cells were exposed to ^{137}Cs γ-rays in 15-ml polystyrene tubes at 10^4 cells per ml in 10 ml of medium at a dose rate of 2.83 Gy/min. Cells were kept on ice before and after exposure until they were plated. Dishes were incubated sufficiently long to allow the majority of colonies to be clearly macroscopic in size—plates exposed to high doses were incubated longer than those exposed to low doses. For chromosome aberration analysis, colcemid was added to 0.1 μg/ml to cultures 4 h prior to harvest. The cells were then fixed three times in methanol/acetic acid (3:1, vol/vol), dropped onto slides and air dried. After aging for several days, the slides were stained in 5% Giemsa and coded.

DNA Transfections. The human cDNA expression library in vector pEBS7 (9) was isolated by using Qiagen column chromatography (Qiagen, Chatsworth, CA). Calcium phosphate/DNA precipitates containing 15 μg (fraction 2, 2–4 kb) of this DNA were used to transfect irs1SF cells in 10-cm dishes (4×10^6 cells per dish) as described (10). Cells were then incubated for a 48-h expression period (approximately two doubling times for irs1SF; see Table 1), trypsinized, and plated at 1×10^6 cells per dish in 25 ml of medium containing 15 nM MMC (Sigma) and 70 μg of hygromycin B (Calbiochem) per ml. Secondary transfections were done by using precipitates containing 25 μg of high molecular weight genomic DNA (from

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: MMC, mitomycin C; NER, nucleotide excision repair; TF, transformant; FISH, fluorescence *in situ* hybridization.

†Present address: Laboratory of Radiobiology and Environmental Health, University of California, San Francisco, CA 94143-0750.
§Biology and Biotechnology Research Program, L452, Lawrence Livermore National Laboratory, P.O. Box 808, Livermore, CA 94551-0808.

TF F2.b1) per dish with 2×10^6 irs1SF cells. Electroporation was used to test cosmid and plasmid clones for correcting activity; 6×10^6 irs1SF cells were electroporated (BRL Cell Porator) with 10 μg of cosmid DNA or 5 μg of pXR3 plasmid (see below) DNA at 230 V/1600 μF.

Cosmid Library Construction and Screening. Vector SuperCos 1 was prepared according to the manufacturer's suggestions (Stratagene). Genomic DNA from secondary transformant 2T.4 was prepared as described (11) and ligated to SuperCos 1 arms. The ligation mixture was packaged according to the manufacturer's protocol by using Gigapack II Gold packaging extract (Stratagene). Packaged cosmids were used to infect host bacteria DH5αMCR, which were then spread onto filters (Whatman HATF045) on 15-cm dishes containing Luria broth and 50 μg of kanamycin sulfate (Boehringer Mannheim) per ml at 8×10^4 colony-forming units per dish. Colonies were lysed, and the DNA was fixed to the filters as described (8). PCR primers 5′-CTCTCGGAGGGCGAAGAAT and 5′-AGATGTTGGCGACCTCGTAA were used to synthesize a 616-bp hygromycin gene fragment, which was labeled by using random primers (Boehringer Mannheim) and used to probe the cosmid library.

Cosmid Analysis. Cosmid DNAs digested with restriction enzymes were separated by electrophoresis through a 0.8% agarose gel and analyzed by Southern blotting as described (11) with minor modifications. Oligonucleotides 5′-CTAGAGAACCCACTGCTTAACTGGC and 5′-TGTCACACCACAGAAGTAAGGTTCC (located 5′ and 3′, respectively, of the cloning site in vector pEBS7) were ^{32}P-labeled according to the manufacturer's protocol (Boehringer Mannheim), passed through a Worthington G-50 spin column (Cooper Biomedical), and used to probe the membranes. Hybridization was for 6 h at 40°C followed by washing the filters three times in 2× SSC/1% SDS at room temperature for 5 min, two times in 2× SSC/1% SDS at 55°C for 30 min, and one time in 2× SSC at room temperature for 5 min (1× SSC is 0.15 M NaCl/0.015 M sodium citrate, pH 7.0).

Production and Chromosome Content Analysis of Somatic Cell Hybrids. The methods used to fuse irs1SF cells with human lymphocytes and select for complementing hybrids were similar to those used for the CHO mutant UV20 (12). MMC-resistant hybrids (1SHL series) were analyzed by a combination of isozyme and Southern blot analyses of 42 chromosomally assigned protein and DNA markers representing all autosomes and the X chromosome as described (13) to determine which human chromosome was associated with MMC resistance.

Mapping of Isolated cDNA with Somatic Cell Hybrid Panels. Southern blot hybridization with the cloned cDNA (pXR3) as the probe against the genomic DNA of a 17-member hybrid clone panel informative for all human chromosomes was performed. Hybrids and methods were described (14), and the same probe and methods were used on blots containing DNA from a subset of MMC-resistant 1SHL hybrids.

Regional Assignment of Isolated cDNA by Fluorescence *in Situ* Hybridization (FISH). FISH was performed on normal human lymphocytes using pXR3 as the probe. Two-color FISH was also performed by competitive cohybridization with labeled cDNA probe along with differentially labeled chromosome 14-specific probe generated by inter-Alu PCR (15) from hybrid MHR14 [monochromosomal for human chromosome 14 (16)]. Signal detection and amplification were performed for biotin-labeled probes by using fluorescein isothiocyanate conjugated avidin and anti-avidin antibody from Oncor. Digoxigenin-labeled probes were detected with rhodamine-tagged anti-digoxigenin antibody (Oncor) and rabbit anti-sheep antibody as required for amplification as per manufacturer's instructions. Slides were counter stained with propidium iodide/antifade or 4′,6-diamidino-2-phenylindole (DAPI)/antifade, examined with a Nikon fluorescence microscope equipped with a multiple-pass filter, and photographed without image processing onto Kodak Ektachrome 160 slide film as described (17).

RESULTS

Cloning a Functional cDNA That Confers MMC Resistance to irs1SF. We exploited the MMC sensitivity of mutant irs1SF to clone the complementing cDNA by transfecting cells with a cDNA expression library in pEBS7, which carries the gene for hygromycin resistance and the cytomegalovirus viral promoter for the cDNA (9). Since pEBS7 may not replicate episomally in hamster cells, it was used as an integrating vector. Transfected irs1SF cells were selected in medium containing 70 μg of hygromycin per ml and 15 nM MMC. From the equivalent of 4.7×10^5 hygromycin-resistant TFs, two colonies showing significant MMC resistance were isolated. Genomic DNA was isolated from one primary TF (F2.b1) and used in a secondary transformation to obtain four MMC-resistant clones from 2.7×10^8 transfected cells selected in MMC and hygromycin. Transformants were analyzed by Southern blotting, and secondary TF 2T.4 showed a single *Bam*HI restriction fragment when probed with the hygromycin gene, while the primary TF F2.b1 contained multiple bands (results not shown). Genomic DNA from TF 2T.4 was used to construct a cosmid library, which was probed with a 616-bp fragment of the hygromycin gene. Since the hygromycin gene is located <1.5 kb from the cDNA insert in vector pEBS7, it served as a linked marker for detecting cosmids likely to carry the functional cDNA. From 1.4×10^6 cosmid clones screened, seven positives were purified. All four positive cosmids that were tested by electroporation into irs1SF conferred resistance to MMC at a frequency of $2–5 \times 10^{-4}$ per viable cell (results not shown; background frequency $<10^{-7}$).

To obtain a small restriction fragment carrying the functional cDNA, we digested one cosmid (XR3-18) with several restriction enzymes and tested the fragmented DNAs for correcting activity. Several digests were active, including that from *Eag* I digestion, which gave a transfection frequency of 6×10^{-5} per viable cell (results not shown). We also probed the digested DNAs on Southern blots with oligonucleotide primers flanking the cDNA insert (results not shown). A 4.1-kb *Eag* I fragment was found to possess correcting activity when subcloned into plasmid pSL1180 (Pharmacia) to give construct pXR3, which retains the cytomegalovirus promoter of pEBS7. On the basis of the location of *Eag* I sites in the pEBS7 vector sequence, we deduced that the cDNA insert was ≈2500 bp in length. A Northern blot, prepared by using a 2.2-kb *Sac* I–*Bam*HI subfragment of the *Eag* I fragment, showed the presence of a band at ≈3.0 kb in human poly(A)$^+$ RNA but not total RNA (Fig. 1, lanes 1 and 2), which was consistent with the possibility that the complete open reading frame of the cDNA

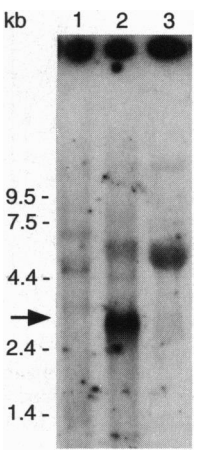

FIG. 1. Northern blot of human and mouse RNA. The blot was probed with a 2.2-kb *Sac* I–*Bam*HI fragment isolated from the 4.1-kb *Eag* I fragment of cosmid XR3-18. Lane 1, human total RNA; lane 2, human poly(A)$^+$ RNA; lane 3, mouse poly(A)$^+$ RNA. The position of the 3-kb hybridizing fragment in human poly(A)$^+$ RNA is marked by an arrow.

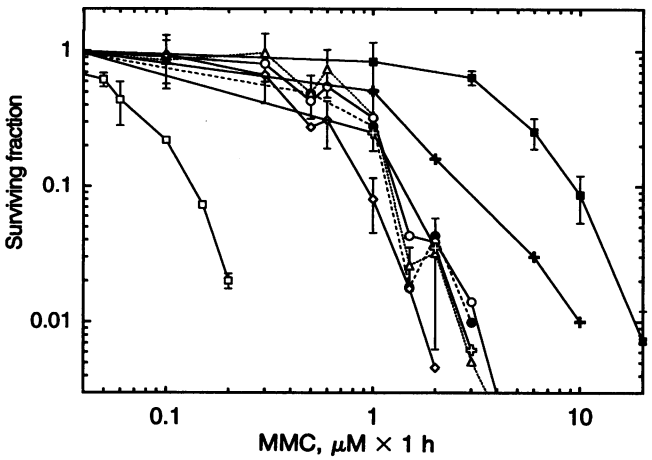

FIG. 2. Correction of sensitivity to MMC in transformants and hybrids of irs1SF. □, irs1SF; ◇, primary TF F2.b1; ○, secondary TF 2T.4; △, cosmid TF CXR3; ●, plasmid TF PXR3; ■, wild-type AA8; and open and closed crosses refer to hybrids 1SHL-20 and 1SHL-16, respectively. Three to five experiments were done for all lines except the plasmid TF (two experiments) and the hybrids (single experiment).

was present. In mouse poly(A)$^+$ RNA (Fig. 1, lane 3), a band was observed at 5.5–6.0 kb.

Degree of Correction of irs1SF Transformants. TFs were tested for correction of cell survival in response to three agents. Correction, defined in terms of dose-reduction factor, was assessed by comparing the dose of the agent which results in a 10% survival rate of the TFs with that of mutant and wild-type AA8 cells (18). All TFs showed partial correction with all agents. With MMC, correction ranged from 0.07 (7%) for the primary TF to 0.14 for a pXR3 plasmid TF, line PXR3 (Fig. 2). We also tested two MMC-resistant somatic cell hybrid lines (see next section), which had values of 0.13 and 0.28. Thus, the correction efficiency in hybrids, which should be carrying an intact gene, was not appreciably better than in several TFs. With cisplatin, correction was somewhat higher than with MMC, ranging from 0.17 for the primary TF to 0.29 for the cosmid TF (Fig. 3). With γ-rays, correction appeared even higher, ranging from 0.21 for the primary TF to 0.42 for the cosmid TF (Fig. 4).

The growth properties of the TFs were also substantially improved as shown in Table 1. Plating efficiency approached that of AA8 cells, which suggested that there might be a

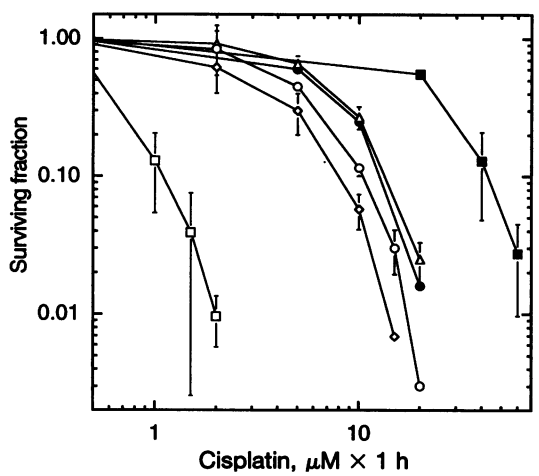

FIG. 3. Correction of sensitivity to cisplatin in transformants. The cell lines analyzed are identified by the symbols as defined in the legend to Fig. 2. Error bars represent standard deviations for two or three experiments.

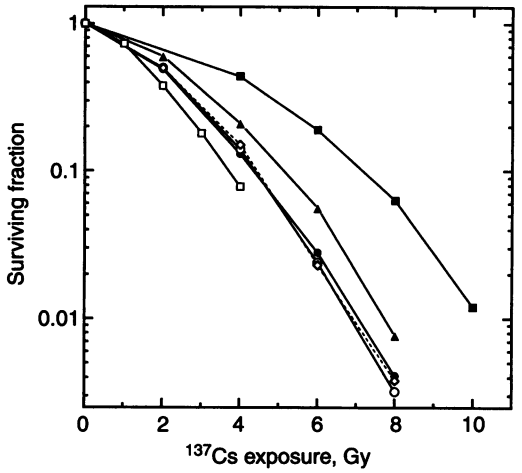

FIG. 4. Correction of sensitivity to ^{137}Cs γ-rays in transformants. The symbols are as defined in the legend to Fig. 2; one experiment is shown.

reduction in spontaneous chromosomal aberrations. Table 2 confirms this prediction and shows that the high level of chromosomal aberrations in irs1SF was corrected from 72% in the primary TF to 100% in the cosmid TF, determined on the basis of the percentage of cells having aberrations other than gaps. It is noteworthy that chromatid gaps, as opposed to true breaks, remained elevated in TFs. This very efficient correction of spontaneous breaks and exchanges contrasts with the much lower correction values measured by survival curves in response to cross-linking agents and γ-rays.

Chromosomal Localization of *XRCC3*. Fusion of irs1SF with human lymphocytes resulted in the selection of 28 independent MMC-resistant, 1SHL hybrid clones. The expectation was that every hybrid should contain the human chromosome that carried the gene conferring MMC resistance, which we defined as *XRCC3* (for x-ray repair cross-complementing). All hybrids retained one of the two markers for human chromosome 14 (*CKB* at 14q32.3). The other marker for chromosome 14 (*NP* at 14q11.2) was present in all hybrids but one (1SHL4). All other chromosomes segregated randomly. Therefore, *XRCC3* was provisionally assigned to chromosome 14, likely distal to 14q11.2. Unlike our mapping studies with other genes, we were unable to obtain sensitive subclones from resistant hybrids grown under nonselective conditions. In view of the data in Table 1, we interpret this result as likely due to a selective growth disadvantage for TFs that have lost *XRCC3*.

To determine whether pXR3 is a cDNA representing the *XRCC3* gene defined in the complemented hybrids, it was used as probe on Southern blots containing *Bam*HI-digested genomic DNA of a 17-member hybrid clone panel informative for all human chromosomes (14). The cDNA identified a human-specific 8-kb *Bam*HI restriction fragment (data not shown). Concordant presence or absence of this band with chromosome 14 was observed for all hybrids except one (6%

Table 1. Improved plating efficiency and growth rate of transformants

Cell line	Plating efficiency*	Growth rate†
irs1SF	0.45 ± 0.03	22.5 ± 0.8
Primary TF (F2.b1)	0.75 ± 0.05	17.0 ± 0.2
Secondary TF (2T.4)	0.79 ± 0.05	17.0 ± 0.5
Cosmid TF (CXR3)	0.82 ± 0.08	16.7 ± 0.7
Plasmid TF (PXR3)	0.80 ± 0.04	17.8 ± 0.4
Wild-type AA8	0.84 ± 0.09	12.3 ± 0.6

*Mean ± SD of four to six experiments.
†Values represent the doubling time in h (mean ± SD of six measurements in suspension culture).

Table 2. Correction of spontaneous chromosomal aberrations in *XRCC3* cDNA transformants

Cell line	Abnormal cells* (excluding gaps)†	Chromatid breaks	Chromatid exchanges	Chromosome breaks	Chromosome exchanges (dicentrics and rings)	Chromatid gaps	Chromosome gaps	% correction of abnormal cells (excluding gaps)‡
irs1SF	27	16	13	4	0	37	8	0
Primary TF (F2.b1)	9	7	1	1	2	20	2	72
Secondary TF (2T.4)	4	0	0	3	1	11	1	92
Cosmid TF (CXR3)	2	1	0	0	1	19	2	100
Wild-type AA8	2	1	0	0	1	6	1	100

*A total of 100 cells were scored for each line.
†Gaps are defined as chromosomal discontinuities that are less than the width of a chromatid.
‡Percentage correction was calculated by using the formula: $[100 - (A_{TF} - A_{AA8})] \times 100/(A_{irs1SF} - A_{AA8})$, where A_{TF}, A_{AA8}, and A_{irs1SF} are the percentage of abnormal cells in transformant, AA8, and irs1SF cells, respectively.

discordance). Discordances between the fragment and all other human chromosomes ranged from 24% to 65%. A second experiment was performed by using the same probe on blots containing DNA from seven 1SHL hybrids (including the exceptional hybrid 1SHL4) that all had some portion of human chromosome 14 and in which other human chromosomes were only randomly present. All hybrids had the human *Bam*HI fragment (data not shown). Therefore, pXR3 is viewed as a cDNA derived from the mRNA of the gene on human chromosome 14 responsible for providing the intermediate repair proficiency in the 28 1SHL hybrids. When the cDNA was used as probe in FISH experiments, it mapped to the end of the q-arm of chromosome 14 (14q32.3) (Fig. 5). This assignment was consistent with all the data from both hybrid panels. Thus, we conclude that pXR3 represents *XRCC3*, which maps to human chromosome 14q32.3.

DISCUSSION

The field of mammalian DNA repair has progressed rapidly as methods developed for gene cloning (19). For NER, eight genes have been isolated by transfection/complementation of hamster or human repair-deficient mutant cell lines, usually by transfecting hamster cells with genomic sequences and then screening cosmid libraries. A functional *XPC* cDNA was obtained by transfecting human XP-C cells with the pEBS7 library used here (20), and, additionally, the *FAC* cDNA was isolated by a very similar approach with Fanconi anemia cells (21). A full-length *FAC* cDNA sequence was obtained, but the *XPC* sequence, while efficiently correcting UV sensitivity, proved to be missing 160 bp of the open reading frame (22). While pEBS7 replicated episomally in the human cells that were used for cloning, in our study it behaved as an integrating plasmid in CHO cells. The incomplete correction we saw for several end points could be due to truncation of our *XRCC3* sequence, interspecies differences between human and hamster, or a dominant-negative effect of mutant allele(s). Further studies will be needed to resolve this issue, but the fact that the two somatic cell hybrid lines overlapped the transformants for MMC resistance (Fig. 2) argues that partial correction is not simply the consequence of an incomplete gene product.

A particularly interesting finding with our cDNA clone is the differential correction seen for different end points. Correction for survival after damage with the two cross-linking agents was relatively low, with the maximum values for transformants being 17% and 29% for MMC and cisplatin, respectively. The fact that the correction for MMC in the primary transformant was only 7% is noteworthy and illustrates that high levels of

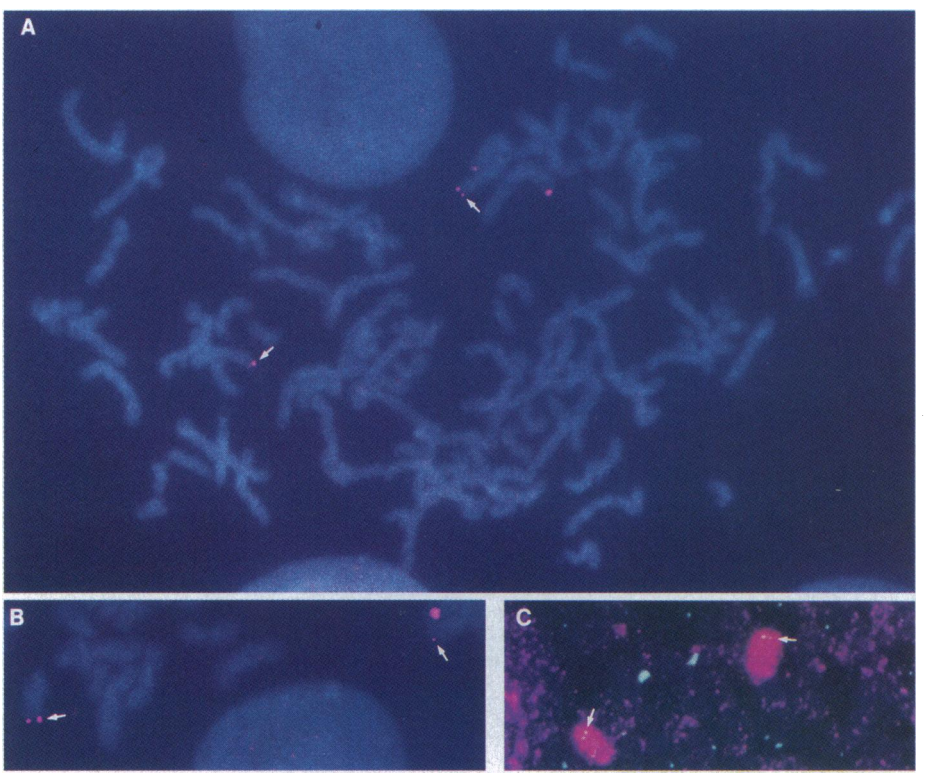

FIG. 5. FISH using pXR3 cDNA probe hybridized to chromosomes from normal human lymphocytes. (*A*) Digoxigenin-labeled probe visualized with rhodamine red fluorescence on 4′,6-diamidino-2-phenylindole-stained (blue) chromosomes of a full metaphase indicates the specificity of the hybridization (paired red dots indicated by arrows) to the end of a D-group chromosome. (*B*) Same as in *A*, except showing a partial metaphase with hybridization (arrows) showing clearer resolution at the end of the chromosome. (*C*) Two-color FISH on a partial metaphase. Chromosome 14 probe was visualized with the digoxigenin/rhodamine system (red), while the cDNA was labeled with biotin and visualized with fluorescein (normally green but appears yellow on a red background). The two red chromosome 14s each have a pair of yellow dots indicating the location of *XRCC3*. Unfortunately the distortion of the chromosomes in this preparation does not allow one to appreciate the position of the gene at the very end of the chromosome, as seen in *A* and *B*.

correction are not always necessary for cloning the relevant cDNA. The correction seen with γ-rays (up to 42%) further suggests that the multiple sensitivities of irs1SF to different kinds of DNA damage are likely due to mutations in the alleles of one genetic locus that affects the cellular response to diverse agents. It was surprising that the correction for both spontaneous chromosomal aberrations and plating efficiency was very high [up to 100% for true breaks and exchanges (Table 2) and up to 95% for plating efficiency (Table 1)]. This dramatic difference in correction seen for the different end points suggests that the XRCC3 protein has more than one role—e.g., one role in repairing strand breaks and another role in repairing base damage. The correction for a putative function related to strand-break repair appears more efficient, given the higher corrections seen for γ-ray survival and spontaneous chromosomal aberrations. The function associated with repairing base damage from cross-linking agents (which also produce monoadducts) and γ-rays seems less efficiently corrected.

It is difficult to understand the multiple mutagen sensitivities of irs1SF as a direct repair defect in terms of our current knowledge of excision repair. NER acts on a multitude of base damage, especially bulky adducts, and base excision repair acts on oxidative damage, (m)ethylation, abasic sites, nonligatable breaks, and other lesions. Besides its low sensitivity to agents involving these pathways—e.g., UV and x-rays—irs1SF is extremely sensitive to cross-linking agents. The repair of cross-links seems to require certain excision repair proteins (see below) and other, unidentified proteins, as implied by the phenotypes of NER-proficient mutants such as irs1, V-H4, and V-C8 (5). The defect in irs1SF may lie outside excision repair pathways, perhaps operating at the level of the interaction of the DNA replication machinery with lesions.

The chromosomal instability of irs1SF could be due to a defect in completing DNA replication that causes spontaneous DNA breaks, followed by misrepair (chromosomal exchanges) or lack of repair (chromosomal breaks). The sensitivity to mutagens could be interpreted as an exacerbation of this defect by increasing the probability that replicated regions will result in chromosomal discontinuities, perhaps due to replication delay associated with inefficient bypass of lesions. A defect in replication would be consistent with our observation that the *XRCC3* gene strongly affects the growth rate of irs1SF. It would be interesting to determine whether the S phase of irs1SF is prolonged. A possible defect in a mitotic checkpoint function, as proposed for the p53 protein (23), seems less likely since this alteration would not be expected to cause a prolonged cell cycle.

The extreme sensitivity of irs1SF to cross-linking agents described here and elsewhere (6, 7) argues for a very efficient cellular mechanism(s) for processing or removing such lesions. In addition to the *XRCC3* gene identified here, the cloned *ERCC1* and *ERCC4* NER genes (11, 24) appear necessary for repairing cross-links (25). Complementation group analysis of rodent mutants (26–28) and human Fanconi anemia mutants (29) suggests that many more related genes remain to be identified. Hypersensitivity to cross-link damage is the hallmark defect associated with Fanconi anemia, which, like irs1SF, is also characterized by chromosome instability. Among the four reported complementation groups (29), only the *FAC* gene is cloned (21). The *FAC* cDNA, which maps to chromosome 9q22.3 (29), was found to correct the chromosomal instability of Fanconi anemia group C cells as well as their hypersensitivity to MMC and diepoxybutane (30). Thus, there is a strong parallel between the complementing activities of the cDNAs of *XRCC3* and *FAC*. The *FAC* gene is the only other cloned gene that we are aware of that plays a major role in chromosome stability. The possibility that *XRCC3* is involved in one of the complementation groups of Fanconi anemia can be examined.

We thank Dr. Karen Fu for extensive efforts to obtain hybrid subclones that had regained MMC sensitivity, Kerry Brookman for assistance with the Northern blot, and Dr. Christine Weber for valuable discussions. This work was done under the auspices of the U.S. Department of Energy by the Lawrence Livermore National Laboratory under Contract W-7405-ENG-48 and was funded in part by National Institutes of Health Grants GM32833, CA52461, and CA34936 and a gift from Mr. Kenneth D. Muller.

1. Huang, J. C., Svoboda, D. L., Reardon, J. T. & Sancar, A. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 3664–3668.
2. Lindahl, T., Satoh, M. S. & Dianov, G. (1995) *Philos. Trans. R. Soc. London* **347,** 57–62.
3. Dianov, G., Price, A. & Lindahl, T. (1992) *Mol. Cell. Biol.* **12,** 1605–1612.
4. Hoeijmakers, J. H. (1993) *Trends Genet.* **9,** 173–177.
5. Collins, A. R. (1993) *Mutat. Res.* **293,** 99–118.
6. Fuller, L. F. & Painter, R. B. (1988) *Mutat. Res.* **193,** 109–121.
7. Caldecott, K. & Jeggo, P. (1991) *Mutat. Res.* **255,** 111–121.
8. Weber, C. A., Salazar, E. P., Stewart, S. A. & Thompson, L. H. (1988) *Mol. Cell. Biol.* **8,** 1137–1146.
9. Peterson, C. & Legerski, R. (1991) *Gene* **107,** 279–284.
10. Thompson, L. H., Brookman, K. W., Jones, N. J., Allen, S. A. & Carrano, A. V. (1990) *Mol. Cell. Biol.* **10,** 6160–6171.
11. Thompson, L. H., Brookman, K. W., Weber, C. A., Salazar, E. P., Reardon, J. T., Sancar, A., Deng, Z. & Siciliano, M. J. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 6855–6859.
12. Thompson, L. H., Mooney, C. L., Burkhart-Schultz, K., Carrano, A. V. & Siciliano, M. J. (1985) *Somatic Cell Mol. Genet.* **11,** 87–92.
13. Chen, D. J., Marrone, B. L., Nguyen, T., Stackhouse, M., Zhao, Y. & Siciliano, M. J. (1994) *Genomics* **21,** 423–427.
14. Stallings, R. L., Olson, E., Strauss, A. W., Thompson, L. H., Bachinski, L. L. & Siciliano, M. J. (1988) *Am. J. Hum. Genet.* **43,** 144–151.
15. Liu, P., Siciliano, J., Seong, D., Craig, J., Zhao, Y., de Jong, P. J. & Siciliano, M. J. (1993) *Cancer Genet. Cytogenet.* **65,** 93–99.
16. Mares, A., Ledbetter, S. A., Ledbetter, D. H., Roberts, R. & Hejtmancik, J. F. (1991) *Genomics* **11,** 215–218.
17. Marlton, P., Claxton, D. F., Liu, P., Estey, E., Beran, M., Le Beau, M. M., Testa, J. R., Collins, F. S., Rowley, J. D. & Siciliano, M. J. (1995) *Blood* **85,** 772–779.
18. Cleaver, J. E. (1990) *Carcinogenesis* **11,** 875–882.
19. Sancar, A. (1994) *Science* **266,** 1954–1956.
20. Legerski, R. & Peterson, C. (1992) *Nature (London)* **359,** 70–73.
21. Strathdee, C. A., Gavish, H., Shannon, W. R. & Buchwald, M. (1992) *Nature (London)* **356,** 763–767.
22. Masutani, C., Sugasawa, K., Yanagisawa, J., Sonoyama, T., Ui, M., Enomoto, T., Takio, K., Tanaka, K., van der Spek, P. J., Bootsma, D., Hoeijmakers, J. H. J. & Hanaoka, F. (1994) *EMBO J.* **13,** 1831–1843.
23. Kuerbitz, S. J., Plunkett, B. S., Walsh, W. V. & Kastan, M. B. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 7491–7495.
24. van Duin, M., de Wit, J., Odijk, H., Westerveld, A., Yasui, A., Koken, M. H. M., Hoeijmakers, J. & Bootsma, D. (1986) *Cell* **44,** 913–923.
25. Hoy, C. A., Thompson, L. H., Mooney, C. L. & Salazar, E. P. (1985) *Cancer Res.* **45,** 1737–1743.
26. Jones, N. J., Stewart, S. A. & Thompson, L. H. (1990) *Mutagenesis* **5,** 15–23.
27. Overkamp, W. J. I., Rooimans, M. A., Neuteboom, I., Telleman, P., Arwert, F. & Zdzienicka, M. Z. (1993) *Somatic Cell Mol. Genet.* **19,** 431–437.
28. Jones, N. J. (1994) *Mutagenesis* **9,** 477–482.
29. Strathdee, C. A., Duncan, A. M. & Buchwald, M. (1992) *Nat. Genet.* **1,** 196–198.
30. Stavropoulos, D. J., Allingham-Hawkins, D. J., Tomkins, D. J. & Buchwald, M. (1994) *Am. J. Hum. Genet.* **55,** Suppl., A119.