Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 4176–4179, June 1987
Genetics

Microcell-mediated transfer of a single human chromosome complements xeroderma pigmentosum group A fibroblasts

(UV radiation/DNA repair/human disease/gene transfer)

ROGER A. SCHULTZ†, PAUL J. SAXON†, THOMAS W. GLOVER‡, AND ERROL C. FRIEDBERG†

†Department of Pathology, Stanford University School of Medicine, Stanford, CA 94305; and ‡Departments of Pediatrics and Human Genetics, University of Michigan, Ann Arbor, MI 48109

Communicated by A. Dale Kaiser, March 16, 1987 (received for review November 1, 1986)

ABSTRACT **Chromosomes from an immortalized aneuploid human fibroblast cell line were randomly tagged with the selectable marker *neo* by transfection with the plasmid pSV2neo. Somatic cell fusions between transfected human cells and mouse A9 cells generated pools of G418-resistant human–mouse hybrid clones containing various numbers of human chromosomes. Microcell-mediated chromosome transfer from the hybrid pools to xeroderma pigmentosum complementation group A (XP-A) cells in culture and selection for G418-resistant colonies resulted in the identification of XP cells with enhanced resistance to ultraviolet radiation. Screening of subclones from selected pools of human–mouse hybrids facilitated the identification of hybrids containing a single *neo*-tagged human chromosome. Transfer of this chromosome to XP-A cells (but not to XP-F or XP-C cells) results in enhanced resistance to ultraviolet light and enhanced excision repair capacity. The identification of a single human chromosome that complements the phenotype of XP-A cells in culture provides the potential for genetic mapping of the complementing gene and for its isolation by molecular cloning.**

Many genes from prokaryotes and from lower eukaryotes have been cloned by complementation of appropriate mutants. The identification and isolation of human genes by complementation of mutant human cells has been less successful. Standard techniques for DNA-mediated gene transfer are less efficient in human cells, and for cells with nonselectable phenotypes complementation assays may be sufficiently laborious to preclude screening total genomic DNA on a routine basis. Some mutant phenotypes offer the potential for positive selection, thus greatly facilitating the identification of rare transformants. A notable example is the autosomal recessive human disease xeroderma pigmentosum (XP). Cells from afflicted individuals are defective in the excision repair of pyrimidine dimers and are extremely sensitive to killing by ultraviolet (UV) radiation (1). The phenotype of marked UV sensitivity is expected to offer amenability to positive selection by direct transfer of genomic DNA from normal human cells. However, attempts to unambiguously demonstrate such complementation have failed (2–5). The limitations to phenotypic complementation of XP cells by DNA transfection are not understood, but they are likely to include parameters such as the efficiency with which the XP complementation group A (XP-A) gene is stably integrated and the level of resistance this gene imparts to cells homozygous for the mutant gene in question. The latter issue is particularly important, since the frequency of reversion of XP-A cells to enhanced UV resistance (UV^R) correlates inversely with the level of UV^R selected (4, 5).

In view of the uncertainties mentioned above, we have explored complementation procedures that are independent of stable integration into the genome of recipient cells and that offer the potential for extension to gene mapping and gene cloning strategies. We present an application of the technique of microcell-mediated chromosome transfer (MCT) (6) and demonstrate that it permits the rapid and efficient screening of human chromosomes for complementation of the mutant phenotype of XP-A cells. Human chromosomes from an aneuploid cell line designated VA-13 were randomly tagged with the selectable marker *neo* and were transferred to XP-A cells by MCT. Recipients were identified by selection for G418 resistance ($G418^R$) and colonies were secondarily screened for enhanced UV^R. Chromosome transfers were facilitated by maintaining *neo*-tagged human chromosomes in a limited series of pooled human–mouse hybrid cells. A complementing pool was identified and was used to establish independent human–mouse hybrid lines bearing the same single *neo*-tagged human chromosome. Transfer of this chromosome to XP-A cells results in significantly enhanced UV^R and increased levels of DNA repair synthesis. The complementing chromosome is rearranged and has not yet been definitively identified. These results constitute a critical first step toward chromosomal assignment, subchromosomal mapping, and molecular cloning of a gene required for excision repair of DNA in human cells.

MATERIALS AND METHODS

Cell Lines and Growth Conditions. The simian virus 40 (SV40)-transformed fibroblast cell line VA-13 (subclone 2RA) was used as a source of donor human chromosomes and as a normal control for complementation studies. The hypoxanthine phosphoribosyltransferase-negative ($HPRT^-$) mouse cell line A9 was used to generate human–mouse hybrids. Recipient cell lines for chomosome transfer included SV40-transformed fibroblasts from XP complementation groups A (XP12RO and XP20SO), C (XP4PA-SV), and F (XP2YO-SV). All cell lines were grown in Eagle's minimal essential medium (MEM) with 2× nonessential amino acids, antibiotics, and 10% fetal bovine serum (complete MEM). XP-F cells required 15% fetal bovine serum.

Generation of Human–Mouse Hybrid Pools. Chromosomes from VA-13 cells were randomly tagged with the plasmid pSV2neo using the calcium phosphate transfection procedure (7). A population of 532 $G418^R$ transformants was pooled and propagated in culture for 10 days. Various numbers of VA*neo* cells ($0.5–5.0 \times 10^5$) and mouse A9 cells ($0.5–3.0 \times 10^5$) were mixed and seeded in 100-mm dishes. One or 2 days later, cells were fused by removing the medium, treating them with 50% polyethylene glycol (PEG) in Dulbecco's modified Eagle's medium (DMEM) for 1 min and washing twice with serum-

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: $G418^R$, G418 resistance; MCT, microcell-mediated chromosome transfer; UV^R, ultraviolet resistance; XP-A, xeroderma pigmentosum complementation group A.

free DMEM at 37°C. Cells were grown for 48 hr in complete MEM before selection with G418 (400 μg/ml) and ouabain (2 μM). Human and mouse chromosomes were differentiated in mitotic spreads of hybrid cells following G-11 staining as described (8).

MCT. Microcells were generated and chromosomes were transferred by modification of a described technique (9). Human or A9 mouse cells were exposed to Colcemid for 48 hr at 0.02 or 0.05 μg/ml, respectively, and microcells were prepared and selected as described (9). Microcells were mixed in suspension with trypsinized recipient cells and allowed to agglutinate for 20 min in the presence of phytohemagglutinin P (25 μg/ml) (Difco) and Polybrene (25 μg/ml) (Sigma). Fusion was actuated by the addition of 50% PEG in serum-free medium. PEG was removed and cells were plated in 100-mm dishes or 75-cm^2 flasks with an excess of complete MEM. Selection for recipient cells was initiated 24–48 hr later in the presence of 250 μg or 400 μg of G418 per ml for human and rodent recipient cells, respectively.

Analysis of XP-A Complementation. G418^R cells were clonally propagated and seeded into two 60-mm dishes in complete MEM lacking G418. Cells in one dish were exposed to 2 J of UV radiation per m^2 at a dose rate of 0.5 J·m^{-2}·sec^{-1}. Fresh complete medium was added and plates were quantitatively evaluated for the extent of cell killing at 48–72 hr. UV sensitivity was quantitatively determined by measuring the colony-forming ability of cells after exposure to various doses of UV radiation. Unscheduled DNA synthesis was measured as described (10).

Analysis of the Complementing Chromosome. The complementing human chromosome present in mouse A9 cells was analyzed by G-11 and trypsin Giemsa banding and by Southern hybridization using the chromosome 11-specific probe APOA1 (11).

RESULTS

Establishment of Human–Mouse Hybrid Pools. Transfection of VA-13 cells with the plasmid pSV2neo was extremely efficient and yielded 532 randomly tagged colonies. These were pooled and expanded briefly to minimize enrichment of specific clones (VA*neo* pool). Mouse A9 cells undergo efficient multinucleation after exposure to Colcemid and typically form microcells carrying small numbers of chromosomes (12). We therefore generated human–mouse hybrids containing randomly tagged human chromosomes by whole-cell fusion between the VA*neo* pool and A9 cells, and we used these hybrids as a source for MCT to XP-A recipients. Unfused human and rodent cells were counterselected by their sensitivity to killing by ouabain and G418, respectively.

Ninety colonies from 8 of 12 cell fusion experiments were propagated as 8 independent pools designated A, C, J1, J2, J4, K1, K3, and K4. Assuming a random distribution of the *neo* marker in the transfected VA-13 line and a random representation of tagged human chromosomes in the hybrids, these pools are expected to represent the entire human genome as selectable chromosomes in mouse A9 cells. The hybrids were expanded for 12 weeks to allow segregation of unselected chromosomes and cells from several pools were examined by G-11 staining to determine the number of human chromosomes retained. Although some metaphase spreads showed the presence of a single human chromosome, the majority contained more than one and occasional hybrids contained as many as 16 human chromosomes.

Complementation of XP-A Cells by MCT. In initial experiments *neo*-tagged human chromosomes were transferred from individual human–mouse hybrid pools to XP-A recipient cells. G418^R colonies were selected and screened for enhanced resistance to killing by UV radiation. Of six pools tested, one yielded UV^R XP-A cells. With this pool (K3), complementation was observed in two of seven G418^R colonies evaluated (Table 1). This pool was reexamined after growth under selective conditions for a further 8 weeks, with the expectation that hybrids would segregate additional untagged human chromosomes. MCT from this pool (K3*) showed that 12 of 19 G418^R colonies were also UV^R (Table 1).

Complementation from the K3 and K3* Pools. Qualitative evaluation of XP-A cells that received chromosomes from the K3 or K3* hybrid pools showed similar levels of enhanced UV^R, but none were as resistant as the parental VA-13 fibroblasts. This was confirmed by quantitative measurements of cell killing after exposure of one of the complemented cell lines (K3-2-12) to UV radiation at a variety of doses (Fig. 1). Quantitative analysis of DNA repair synthesis (unscheduled DNA synthesis) in K3-2-12 cells also demonstrated substantial but incomplete complementation of excision repair capacity (data not shown).

The K3 pool was originally derived from three different human–mouse hybrid cell lines. The observation that MCT from this pool results in UV resistant XP-A cells at a frequency of ≈30% (Table 1) is consistent with the notion that only one of the hybrids in this pool contains the complementing chromosome. However, at this stage we could not distinguish between the possibilities that complementation was conferred by a single human chromosome tagged with the selectable marker, by a cotransferred untagged human chromosome, or by cotransferred rodent material. To determine whether rodent DNA was transferred to the XP-A line the complemented clone K3-2-12 was examined by dot-blot hybridization using radiolabeled mouse genomic DNA as a probe. Within the limits of sensitivity of these experiments, K3-2-12 contains no detectable mouse DNA (<200 kilobases per human genome equivalent). We therefore conclude that complementation of the XP-A line is effected by a human chromosome. Evidence in support of this conclusion, and the simultaneous demonstration that the complementing chromosome also carries the *neo* marker, comes from segregation analysis. A number of complemented XP-A cells were grown under nonselective conditions and independent clonal isolates were examined for G418^R and UV^R. In most cases, the two phenotypes cosegregated at a very high frequency, although occasional (presumably highly stable) clones failed to segregate either marker (Table 2). Rare colonies were identified that segregated the UV^R determinant without loss of resistance to G418. However, the reciprocal segregation pattern was not observed (Table 2).

Human–Mouse Hybrids Carrying a Single Complementing Human Chromosome. To directly demonstrate that the UV^R determinant and the *neo* marker are on the same chromosome, we established a hybrid line carrying a single *neo*-tagged chromosome. The K3* pool was subdivided and subclones were examined for transfer of complementing material and for their content of human chromosomes. Of six subclones initially examined, all contained more than one

Table 1. Phenotypic complementation of XP-A cells by MCT

Human–mouse hybrid	Origin	Human chromosomes per metaphase spread	Complementation (UV^R/G418^R)
K3	Random pool	ND	2/7
K3*	Random pool	2–6	12/19
K3SUB1	Subclone	2–3	2/2
K3SUB6	Subclone	2–4	2/2
K3SUB1A9-1	MCT (A9 to A9)	1	31/36
K3SUB1A9-3	MCT (A9 to A9)	1	2/2
212A9-1	MCT (XP to A9)	1	3/3

ND, not done.

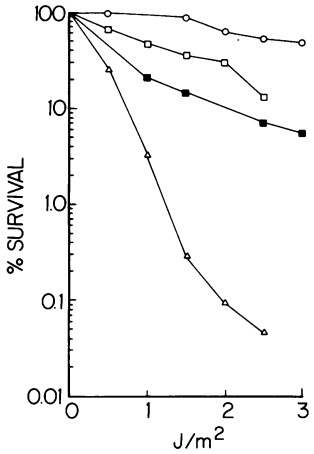

FIG. 1. Quantitative UV sensitivity of complemented XP-A clones (squares) relative to the XP-A parental cell line XP12RO (△) and the wild-type chromosome donor VA (○). □, Clone K3-2-12 isolated following MCT from the K3 pool; ■, clone XPA91-2 isolated following MCT from K3SUB1A9-1.

human chromosome. Two of these (K3SUB1 and K3SUB6) consistently transferred both UV^R and $G418^R$ to XP-A recipient cells (Table 1). Examination of seven additional K3* subclones failed to identify hybrids with only a single human chromosome, and these were not tested further.

Human chromosomes from K3SUB1 were passaged to the mouse A9 line by MCT. Two of the hybrids generated (K3SUB1A9-1 and K3SUB1A9-3) were shown by G-11 staining to contain a single human chromosome (Table 1). In a separate experiment, the complemented XP-A clone K3-2-12 was used as a donor to return the *neo*-tagged chromosome to mouse A9 cells by MCT. After selection in G418, a clone (212A9-1) containing a single human chromosome was identified (Table 1). G-11 staining of metaphase spreads indicates that this chromosome is indistinguishable from that present in K3SUB1A9-1 (Fig. 2) and K3SUB1A9-3. All three human–mouse hybrids containing single human chromosomes complement the phenotype of XP-A cells following MCT. The XP-A line XP12RO is complemented at high efficiency (Table 1). A second XP-A cell line (XP20SO) derived from a different individual was also complemented by the human chromosome present in K3SUB1A9-1. In contrast, six $G418^R$ clones from XP-F failed to exhibit enhanced UV^R relative to the parental control following MCT from K3SUB1A9-1. Negative results were also obtained with a single $G418^R$ XP-C clone.

Cytogenetic analysis shows that the complementing chromosome is rearranged. The rearranged chromosome is readily detected in metaphase spreads of the VA-13 parent donor line and has cytogenetic characteristics of a translocation involving chromosome 11. This result was confirmed by demonstrating positive hybridization with the chromosome 11-specific DNA probe APOA1 (data not shown). However, MCT from a human–mouse hybrid containing chromosome

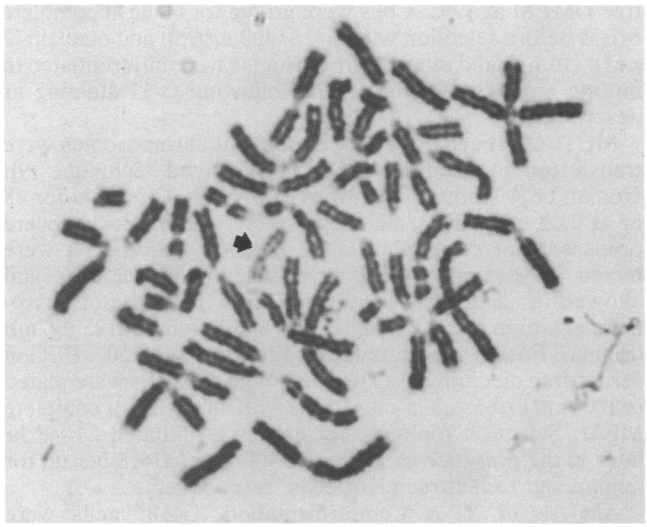

FIG. 2. G-11 staining of the human–mouse hybrid K3SUB1A9-1 bearing a single human chromosome (arrow), which complements XP-A cells.

11 from a pseudodiploid human cell line did not result in complementation of XP-A cells, and it is probable that the complementing gene is normally present on another chromosome, which is rearranged with chromosome 11 in the VA-13 cell line.

Phenotypic Characterization of Complemented XP-A Cells. For quantitative analysis of complementation, we selected a single $G418^R$ UV^R clone (XPA91-2) isolated by transfer of the tagged human chromosome from the human–mouse hybrid K3SUB1A9-1. Like K3-2-12, XPA91-2 cells are significantly more UV resistant than XP-A cells, but not as UV resistant as the parent VA-13 donor line (Fig. 1). The excision repair capacity of XPA91-2 was evaluated by measuring unscheduled DNA synthesis. Most XPA91-2 cells exhibit excision repair proficiency equivalent to that observed in wild-type cells (Fig. 3). However, some cells show background levels of unscheduled DNA synthesis comparable to that observed in XP-A (Fig. 3). Southern hybridization did not reveal the presence of rodent DNA in XPA91-2 cells, consistent with earlier observations on the UV-resistant line K3-12-2 (see above). Nonetheless, we cannot definitively exclude the possibility that the transferred human chromosome contains an undetectable amount of mouse DNA, including a complementing gene.

DISCUSSION

The present studies demonstrate that MCT offers a powerful approach to the complementation of a mutant phenotype such as XP, in which standard DNA transfection protocols have been unsuccessful (2–5). This experimental approach should be generally applicable to other recessive mammalian

Table 2. Segregation of $G418^R$ and UV^R from XP-A clones complemented by MCT

	XP-A clone examined							
Phenotype	K3-2-12	K3N3	K3N11	K3N12	K3N13	K3N14	K3N15	K3N17
$G418^R$ UV^R	1	5	5	0	0	0	0	1
$G418^S$ UV^S	76	0	1	6	6	5	6	4
$G418^R$ UV^S	4	0	0	0	0	1	0	0
$G418^S$ UV^R	0	0	0	0	0	0	0	0

Complemented XP-A clones were grown in the absence of selection for 4 weeks, seeded at low density, and propagated for 2–3 weeks to form colonies. Individual colonies were isolated and independently tested for UV^R and $G418^R$. $G418^S$ and UV^S denote G418 and UV sensitivity.

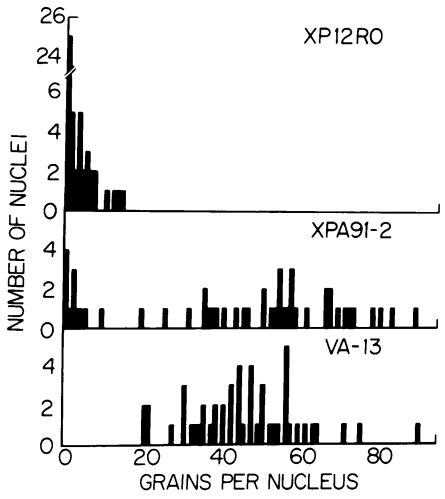

FIG. 3. Unscheduled DNA synthesis in XP-A parental cells (XP12RO), wild-type cells (VA-13), and the complemented XP-A clone XPA91-2. Cells were grown and assays were performed in the presence of G418.

mutant phenotypes, including those that are not readily selectable. Workers in several laboratories have recently reported preliminary efforts to establish libraries of rodent cells carrying cytogenetically characterized single human chromosomes tagged with a selectable marker (9, 13). However, these libraries are not yet representative of the entire human genome. To circumvent this limitation, we undertook the alternative approach of transferring tagged human chromosomes from random pools of human–mouse hybrid cells. We selected an immortalized human line as the chromosome donor for initial studies, because this line is efficiently transformed by plasmid pSVneo carrying a selectable marker. Using these pools, we were able to complement the phenotype of XP-A cells in two independent trials (both from the same pool) out of a total of 96 $G418^R$ recipients examined. This represents a high frequency event well suited to an initial screen of the human genome. Secondary screening of the relevant subpopulation from this pool and selection of complemented cells that cosegregate the UV^R and $G418^R$ determinants facilitated the establishment of cell hybrids bearing single human chromosomes that reproducibly complement the phenotype of XP-A cells after MCT.

The use of a primary selection that is independent of the complemented phenotype requires no *a priori* quantitative assumptions concerning the level of phenotypic complementation. Since a relatively small number of chromosome transfers is required to screen pools of randomly tagged chromosomes, the logistics of screening under various levels of stringency are manageable. In this regard, it is instructive to note that complementation of the UV sensitivity of XP-A cells was always at lower levels than that observed in normal cells. This observation underscores the concern that screening XP cells (and perhaps other human cells with selectable phenotypes) for normal phenotypic levels can result in a failure to detect complementation. In populations of complemented cells, the overall level of unscheduled DNA synthesis was also less than wild type. However, in individual cells with enhanced unscheduled DNA synthesis, the level of repair synthesis was indistinguishable from that in normal cells. This suggests that some cells carrying the complementing chromosome do not express the XP-A gene, or they do so in a regulated manner. We also considered the possibility that these results reflect instability of the complementing chromosome in XP-A cells with resultant loss of the complementing gene in a fraction of the cell population. However, our segregation analyses do not support this view. Although some clones do exhibit instability of the complementing chromosome, we have identified several in which the chromosome is highly stable and the latter do not show greater levels of UV^R.

The single human chromosome present in the three complementing hybrid cell lines isolated has been identified in the aneuploid parent donor by cytogenetic analysis. The chromosome represents a rearrangement involving human chromosome 11. However, the complementing gene is apparently normally present on a different chromosome. Identification of the chromosome carrying the complementing gene should be facilitated by generating human–mouse hybrid cell lines carrying single chromosomes from diploid human cells. Independent of the chromosome of origin, the use of carefully selected (rearranged or normal) complementing chromosomes and noncomplementing deletion derivatives is expected to facilitate molecular cloning of the XP-A gene by subtractive enrichment for sequences to probe established cosmid and/or genomic human libraries.

We thank Dr. Eric J. Stanbridge for the human–mouse hybrid containing chromosome 11 and Dr. S. K. Karathanasis for the APOA1 probe. We also thank Constance Stein for technical help and our colleagues in the laboratory for helpful discussions and suggestions on the manuscript. This study was supported by contract DEAT03 76EV70032 from the U.S. Department of Energy and Grant CA 44247 from the Public Health Service. P.J.S. is supported by a training grant in Tumor Biology (CA 09151) from the Public Health Service.

1. Friedberg, E. C. (1985) *DNA Repair* (Freeman, New York), pp. 506–525.
2. Takano, T., Noda, M. & Tamura, T.-A. (1982) *Nature (London)* **296**, 269–270.
3. Ganesan, A., Spivak, G. & Hanawalt, P. (1982) in *Manipulation and Expression of Genes in Eukaryotes*, eds. Nagely, P., Linnane, A. W., Peacock, W. J. & Pateman, T. A. (Academic, Sydney, Australia), pp. 45–54.
4. Royer-Pokora, B. & Haseltine, W. A. (1984) *Nature (London)* **311**, 390–392.
5. Schultz, R. A., Barbis, D. P. & Friedberg, E. C. (1985) *Somatic Cell Mol. Genet.* **11**, 617–624.
6. Fournier, R. E. K. & Ruddle, F. H. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 319–323.
7. Wigler, M., Pellicer, A., Silverstein, S., Axel, R., Urlaub, G. & Chasin, L. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 1373–1376.
8. Wyandt, H. E., Wysham, D. G., Minden, S. K., Anderson, R. S. & Hecht, F. (1976) *Exp. Cell Res.* **102**, 85–94.
9. Saxon, P. J., Srivatson, E. S., Leipzig, G. V., Sameshima, J. H. & Stanbridge, E. J. (1985) *Mol. Cell. Biol.* **5**, 140–146.
10. Barbis, D. P., Schultz, R. A. & Friedberg, E. C. (1986) *Mutat. Res.* **165**, 175–184.
11. Karathanasis, S. K., McPherson, J., Zannis, V. & Breslow, J. L. (1983) *Nature (London)* **304**, 371–373.
12. Fournier, R. E. K. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 6349–6353.
13. Athwal, R. S., Smarsh, M., Searle, B. M. & Deo, S. S. (1985) *Somatic Cell Mol. Genet.* **11**, 177–187.