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Isolation of Microcell Hybrid Clones Containing Retroviral Vector Insertions into Specific Human Chromosomes

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We sought an efficient means to introduce specific human chromosomes into stable interspecific hybrid cells for applications in gene mapping and studies of gene regulation. A defective amphotropic retrovirus was used to insert the gene conferring G418 resistance (*neo*), a dominant selectable marker, into the chromosomes of diploid human fibroblasts, and the marked chromosomes were transferred to mouse recipient cells by microcell fusion. We recovered five microcell hybrid clones containing one or two intact human chromosomes which were identified by karyotype and marker analysis. Integration of the *neo* gene into a specific human chromosome in four hybrid clones was confirmed by segregation analysis or by in situ hybridization. We recovered four different human chromosomes into which the G418 resistance gene had integrated: human chromosomes 11, 14, 20, and 21. The high efficiency of retroviral vector transformation makes it possible to insert selectable markers into any mammalian chromosomes of interest.

Hybrid rodent cells containing limited numbers of human chromosomes have numerous applications in the study of human genetics. Such hybrid cells have been used to determine the chromosomal locations of many isozyme loci (26) and, more recently, DNA restriction fragments (33). Used in conjunction with probes for species-specific repetitive DNA, hybrid cells are advantageous starting materials for the cloning of DNA sequences known to reside on particular chromosomes (5). Hybrid cells can also be used to study the effects of *trans*-acting factors on the expression of tissue-specific genes (20).

The most useful hybrids are those that retain very few human chromosomes. Such hybrids can be produced efficiently by microcell fusion (11). However, since rodent cells segregate human chromosomes in an uncontrolled manner, selective pressure is required to maintain the transferred chromosome. A few human chromosomes naturally carry selectable genes (e.g., thymidine kinase or dihydrofolate reductase), but the majority do not.

Defined translocations involving chromosomes that carry selectable genes can be used to fix other chromosomes in hybrid cells. For instance, we have taken advantage of the large number of Robertsonian translocations (centric fusions) available in mice to produce rat and hamster hybrid cells carrying specific murine chromosomes (13). However, the number of suitable human translocations that have been described is small.

An alternative approach is to introduce exogenous selectable markers into human chromosomes. Certain bacterial genes, including the *gpt* gene from *Escherichia coli* and the neomycin resistance gene (*neo*) from transposon Tn*5*, can confer selectable phenotypes to mammalian cells. The *neo* gene, which renders mammalian cells resistant to the antibiotic G418, is a dominant marker, so that recipient cells with recessive mutations are not required.

Several groups have transferred *gpt* or *neo* into human tumor cell lines via calcium phosphate coprecipitation and then used microcell fusion to construct human-rodent hybrid cells (1, 27, 30). The utility of this approach has been limited by the low efficiency of calcium phosphate-mediated DNA transfer. Human primary diploid fibroblasts are the donor material of choice for many applications because they are free of the chromosome abnormalities documented in malignant cells. Gene transfer mediated by calcium phosphate occurs at very low frequency (typically approximately 10^{-6}), and this process is too inefficient to introduce selectable genes into primary cells, which have limited proliferative capacity.

Recently Weis et al. (32) have reported the use of a retroviral vector to insert the *neo* gene into the murine histocompatibility locus. Gene transfer by means of defective retroviruses has several advantages over the calcium phosphate technique (22). It is extremely efficient, occurring at frequencies approaching 100%, which permits the recovery of a large number of integration events from a single infection. In addition, retroviral vectors integrate into chromosomes quasi-randomly, and the integrated vector sequence has precisely defined ends (rather than the tandem arrays often produced by calcium phosphate-mediated transfer), which facilitates analysis of the integration site. The high efficiency of this gene transfer technique permits the introduction of selectable genes into primary diploid human cells for use as chromosome donors.

Here we demonstrate the feasibility of this approach. We introduced the *neo* gene on a retroviral vector into the chromosomes of diploid human fibroblasts and then transferred these chromosomes via microcells into mouse recipients. We recovered four different hybrid lines that each retained a single human chromosome. In these monochromosomal microcell hybrids, the *neo* gene had inserted into human chromosome 11, 14, 20, or 21.

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MATERIALS AND METHODS

Cells and culture conditions. Primary diploid human fibroblasts were obtained from a sample of foreskin and propagated in F/DV medium (a 1:1 mixture of Dulbecco modified Eagle medium and Ham's F12 medium) supplemented with 15% fetal calf serum and 2 mM glutamine. 3T6 cells are an established line of mouse fibroblasts; these were maintained in F/DV medium plus 10% fetal calf serum.

Preparation of virus and infection of cells. Culture medium containing ZIP-Neo SV(X)1 virus (4) was harvested from Ψ-AM producer cells (6), filtered through a filter assembly (0.45-μm-pore size filter no. 4184; Gelman Sciences, Inc., Ann Arbor, Mich.), and incubated with human fibroblasts for 2 h at 37°C in the presence of 8 μg of Polybrene per ml. Infected cell clones were selected by growth in 2 mg of G418 per ml.

Microcell-mediated chromosome transfer. The infected fibroblasts were plated onto plastic bullets cut from tissue culture dishes and incubated in the presence of 10 μg of colcemid per ml for 48 h (20, 21). After micronuclei had formed in the cells, the bullets were placed back-to-back in centrifuge tubes containing 5 μg of cytochalasin B per ml in serum-free growth medium. They were centrifuged at 39,000 × *g* for 35 min at 28 to 32°C to enucleate the cells. The resulting preparation of microcells was suspended in a solution of 100 μg of phytohemagglutinin P per ml and applied to monolayers of recipient cells growing in 25-cm^2 tissue culture flasks. After 15 min of incubation at 37°C to permit agglutination, the cells and microcells were fused by 60 s of exposure to 44% (wt/wt) polyethylene glycol (molecular weight, 1,300 to 1,600). After an overnight incubation in nonselective medium, the recipient cells were plated into medium containing 500 μg of G418 per ml. Colonies appeared 14 days later. The cultures included G418-resistant donor fibroblasts that survived the enucleation procedure intact; these could be distinguished by their appearance from the microcell hybrids, which resembled 3T6 cells. Individual hybrid colonies were picked by using glass cloning rings and passaged through medium containing 500 μg of G418 per ml plus 3×10^{-6} M ouabain to eliminate any contaminating human donor cells. Subsequently the hybrids were maintained in 250 μg of G418 per ml.

Chromosome marker analysis. Microcell hybrids were screened electrophoretically for the presence of human isozymes of phosphoglucomutase-2, esterase-10, nucleoside phosphorylase, mannose phosphate isomerase, glucose phosphate isomerase, and adenosine deaminase. Cell extracts were subjected to electrophoresis on starch gels or cellulose acetate strips, followed by specific staining as described previously (26, 34). Filter hybridization was used to detect human alleles for argininosuccinate synthetase (2, 29), adenosine deaminase (23), nucleoside phosphorylase (16), and insulin (24) and for the arbitrary DNA markers G8 (18), 3.6/1.2 (15), pAW101 (9, 35), p267 (31), D20S2 (3), and D7S8 (pJ3.11) (7).

Cytogenetic analyses. Metaphase spreads were prepared for alkaline Giemsa staining and for Giemsa-trypsin banding as follows. Exponentially growing cells were exposed to 0.02 μg of colcemid per ml for 45 min. Mitotic cells, collected by selective detachment, were incubated for 35 min in 75 mM KCl at room temperature and fixed in three changes of methanol-glacial acetic acid (3:1, vol/vol).

For alkaline Giemsa staining the fixed cells were dropped onto dry microscope slides, which were hydrated the following day in double-distilled water (six changes over at least 2 h). The slides were stained for 3 to 7 min in a solution of 1.6% Giemsa stain (Fisher Scientific Co., Pittsburgh, Pa.) in 0.05 M sodium phosphate (pH 11.35) at 37°C.

For Giemsa-trypsin banding, the cells were dropped onto cold, wet slides, and these were aged at least 3 weeks before staining. A modified G-banding protocol was used (20). The stained metaphase chromosomes were photographed with a Zeiss photomicroscope III.

R-banded chromosomes were produced by staining with chromomycin A3 (0.5 mg/ml) for 20 min followed by counterstaining with distamycin A (0.1 mg/ml) for 4 min (9). After cover slips were mounted with glycerol, metaphase spreads were visualized through a standard fluorescein filter set on a Zeiss Universal fluorescence microscope with a Planapo 63× objective.

In situ hybridization. The chromosomal locations of some integrated ZIP-Neo SV(X)1 retroviruses were mapped by in situ hybridization of metaphase spreads with a tritium-labeled *neo* probe. The 1.4-kilobase (kb) *Bam*HI-*Hin*dIII fragment containing the neomycin phosphotransferase gene in pBRNeo plasmid was purified out of agarose and labeled by random hexamer-primed synthesis (10) to a specific activity of 1×10^8 cpm/μg (experiment 2) or 2×10^7 cpm/μg (experiment 1). In other experiments the probe was a 300-base-pair *Bam*HI restriction fragment containing the human repetitive DNA element Blur 8 (25), which was similarly purified and labeled to a specific activity of 2×10^7 cpm/μg. In all experiments, preparation of slides, hybridizations, and autoradiography were performed essentially as described previously (19). Chromosomes were identified after autoradiography by R-band staining through the liquid emulsion (see above).

RESULTS

The defective retrovirus ZIP-Neo SV(X)1 (4) was used to transduce the *neo* gene into human cells. This vector is derived from Moloney leukemia virus, an ecotropic murine retrovirus, and carries the *neo* gene driven by the promoter in the viral long terminal repeat. Amphotropic pseudotypes of ZIP-Neo SV(X)1 capable of infecting human cells were produced in the packaging cell line Ψ-AM (6), yielding a stock of amphotropic defective virus free of contamination with intact helper virus. The absence of replication-competent helper virus was critical for two reasons. First, a cell infected with intact virus would become immune to superinfection by the defective virus, thus decreasing the number of *neo* integrations that could be recovered. More important, infectious amphotropic retrovirus present in the human cell cultures could be transmitted to the recipient mouse cells during the chromosome transfer procedure, giving rise to G418-resistant cells that had not received human chromosomes.

Diploid human fibroblasts were infected at passage 10 with a high multiplicity of ZIP-Neo SV(X)1. G418-resistant cells from approximately 75 independent colonies were pooled to form a mass population. It was necessary to start with cells at a very early passage, because primary human fibroblasts lose the ability to form micronuclei (the basis of the microcell transfer technique) long before their capacity to divide is exhausted (21). The mass population of infected cells was induced to form micronuclei by prolonged exposure to a high concentration of the mitotic inhibitor colcemid. Micronuclei formed in 44% of the donor cells, with a few relatively large micronuclei in most of the micronucleate cells. The cells were enucleated by centrifu-

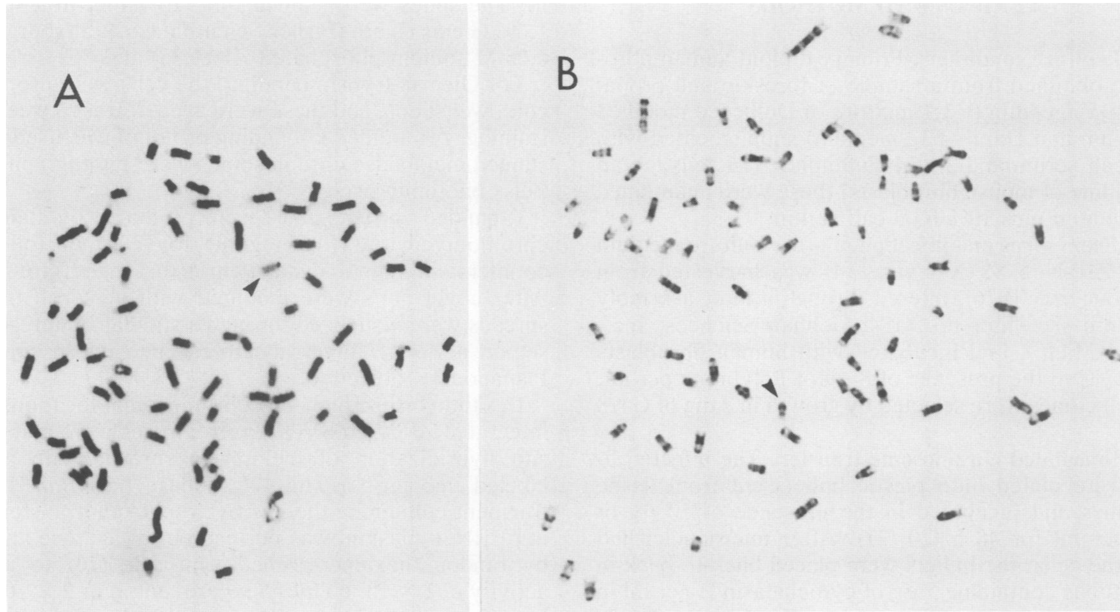

FIG. 1. Metaphase chromosomes of hybrid clone HDm-15. (A) Chromosomes stained with alkaline Giemsa. (B) G-banded chromosomes. Arrowheads, Human chromosome 14.

gation in the presence of cytochalasin B, and the resulting preparation of microcells was fused to mouse 3T6 cells. Hybrid cells were recovered after selection in G418.

Fifteen microcell hybrid clones were picked and screened for the presence of human chromosomes by staining metaphase preparations with alkaline Giemsa, which differentiates human and rodent chromosomes (14). Seven clones exhibited complex karyotypes with three or more human chromosomes retained at high frequency. This result was not unexpected, since the donor microcell preparation consisted predominantly of large microcells. An unexpected finding was that an additional six hybrid clones retained only fragments of human chromosomes, some translocated onto recipient mouse chromosomes. This relatively high frequency of chromosome fragmentation is probably characteristic of the 3T6 recipient cell line (see Discussion).

Two of the hybrid clones were karyotypically simple. One clone, HDm-5, initially contained both a D-group and an E-group human chromosome, but rapidly lost the smaller chromosome during serial cultivation in selective medium. We tested cell extracts for human isozyme markers corresponding to the three D-group chromosomes. This revealed the presence of human nucleoside phosphorylase (chromosome 14) and the absence of human esterase-10 (chromosome 13) and mannose phosphate isomerase (chromosome 15). We confirmed this identification by analysis of Giemsa-trypsin-banded karyotype preparations (Fig. 1).

Another simple hybrid, HDm-9, apparently contained only an F-group chromosome, which we identified as human chromosome 20 by karyotype and marker analysis (Table 1). However, we also found DNA sequences derived from human chromosomes 14 (pAW101, nucleoside phosphorylase) and 21 (p267) in clone HDm-9. These cells apparently contain submicroscopic fragments of both of these chromosomes.

Four of the more complex hybrids segregated human chromosomes after serial passage in culture, giving rise to populations with simpler karyotypes. The results of karyotype and marker analyses performed on these clones are also presented in Table 1. Clones HDm-4, HDm-18, and HDm-20 each retained a single human chromosome at high frequency, while clone HDm-15 retained two.

To confirm the karyotypic and isozyme analysis of these hybrids, we performed Southern blot analysis (28) on *Eco*RI-digested DNA from each hybrid using a cDNA of argininosuccinate synthetase as a probe. This cDNA hybridizes to 18 *Eco*RI bands in human genomic DNA which correspond to a minimum of 11 argininosuccinate synthetase-related genes in the human genome. Fifteen *Eco*RI bands have been assigned to single human chromosomes (2), and three bands have been assigned to one of two human chromosomes. Thus, the presence or absence of argininosuccinate-synthetase-hybridizing bands in genomic DNA from somatic cell hybrids indicates the presence or absence of 10 distinct human chromosomes. For example, our karyotype data predicted that in an *Eco*RI digest of DNA from cell line HDm-15, the argininosuccinate synthetase probe should reveal 1.6- and 4-kb bands, since these bands have been assigned to chromosome 11q. As the autoradiogram in Fig. 2A (lane 4) shows, the 1.6-kb *Eco*RI fragment can be seen after 3 days of autoradiography. The 4-kb and also an expected 9-kb band from chromosome 4 comigrated with cross-hybridizing mouse bands. The DNA from cell line HDm-20 should contain a 3.4-kb *Eco*RI band located on chromosome 7, but this band also comigrated with a cross-hybridizing mouse fragment of the same size (panel B). There are no other human-specific argininosuccinate synthetase bands seen in the DNA from any of the other cell lines, thus confirming the karyological data. Comparing panels A and B, however, we demonstrate the importance of doing short and long autoradiography after Southern blotting and hybridization: in the short exposure, weakly hybridizing bands can be seen which upon longer exposure are occluded by closely migrating, more strongly hybridizing bands, and conversely, only after long exposures will some weakly hybridizing bands (e.g., 1.6-kb band in lane 4) be visible. In

TABLE 1. Summary of karyotype and human chromosome marker analyses

Clone	Human chromosomes retained (fraction of cells)	Human markers detected[a] (corresponding human chromosome)	Site of *neo* integration
HDm-4	20 (0.84)	PGM-2 (4)	20p1.2-2[b]
	4 (0.28)	G8 (4p)	
		3.6/1.2 (4p)	
		D20S2 (20)	
		pAS (9 kb) (4)	
HDm-5	14 (0.81)	NP (14)	14[c]
	E group (0.25)	pAW101 (14)	
HDm-9	20 (0.90)	p267 (21)	ND[d]
	14	ADA (20)	
	21	D20S2 (20)	
		pAW101 (14)	
		pNP (14)	
HDm-15	21 (0.65)	p267 (21)	21q2.2[b]
	11 (0.41)	PGM-2 (4)	
	4 (0.34)	3.6/1.2 (4p)	
		Insulin (11p)	
		pAS (1.6 and 4 kb) (11)	
		pAS (9 kb) (4)	
HDm-18	11 (0.87)	pAS (9 kb) (4)	11q1.4-2.2[b]
	4 (0.47)	PGM-2 (4)	
		G8 (4p)	
		3.6/1.2 (4p)	
		Insulin (11)	
		pAS (1.6 and 4 kb) (11)	
HDm-20	7 (1.00)	D7S8 (7)	ND[c,e]
	4 (0.17)	PGM-2 (4)	
		G8 (4p)	
		3.6/1.2 (4p)	
		pAS (9 kb) (4)	
		pAS (3.4 kb) (7)	

[a] Cell extracts were tested for the following human isozymes as described in the text: phosphoglucomutase-2 (PGM-2); nucleoside phosphorylase (NP); adenosine deaminase (ADA); esterase-10 (chromosome 13 marker); mannose phosphate isomerase (chromosome 15 marker); glucose phosphate isomerase (chromosome 19 marker). pAS, cDNA probe corresponding to the argininosuccinate synthetase gene; pNP, cDNA probe corresponding to the nucleoside phosphorylase gene.

[b] Determined by in situ hybridization.

[c] Determined by segregation analysis.

[d] ND, Not determined.

[e] See Results.

somatic cell hybrids, weakly hybridizing bands may frequently be the result of low copy number per cell of the chromosome or chromosome fragment containing that sequence.

Finally, we confirmed the site of the *neo* integration in four of these clones. For clones HDm-4, HDm-15, and HDm-18, we accomplished this by in situ hybridization of a tritium-labeled *neo* probe to metaphase chromosome preparations. In the first experiment, in clone HDm-15 we observed 4.1% (16 of 395) of the total grains over human chromosome 21 in 75 metaphase spreads, although the chromosomes 21 represented only 0.6% of the total chromosome length in each cell counted. In the same experiment 4.3% (29 of 661) of the total grains were observed over human chromosome 11 in 90 metaphase spreads examined from HDm-18, although the chromosomes 11 represented only 1.3% of the total chromosome length in each cell counted. In contrast, we observed random background numbers of grains over the other human chromosomes present in each of these cell lines: 1% of grains over chromosome 11 in HDm-15 (2% of total chromosome length) and 1.2% of grains over chromosome 4 in HDm-18 (1.6% of total chromosome length). In the second in situ experiment on metaphase spreads from clone HDm-4, we used a *neo* probe with higher specific activity, resulting in an increase in the background number of hybridizing grains but with a concomitant increase in specific signal. We observed 10% (16 of 160) of grains over human chromosome 20, representing 0.4% of the total chromosome length, as opposed to 3.1% (5 of 160) of grains over chromosome 4, representing 0.5% of the total chromosome length. In both experiments a clear clustering of grains was seen over specific regions of individual human chromosomes (Fig. 3), localizing the ZIP-Neo SV(X)1 integration sites to chromosome 21q2.2 in HDm-15, to chromosome 11q1.4-2.2 in HDm-18, and to chromosome 20p1.2 in HDM-4.

For clones HDm-5 and HDm-20 we carried out a segregation analysis. A sample of each cell line was propagated briefly under nonselective conditions to permit segregation of the *neo* gene, and then subclones were tested individually for concordant loss or retention of G418 resistance and the human chromosome identified in the original hybrid (Table 2). For cell line HDm-5, there was complete concordance between the segregation of *neo* and that of human chromosome 14 in eight subclones, and we infer that in HDm-5, as expected, the human chromosome present at highest frequency contains the integrated marker. However, for hybrid HDm-20, the segregation of *neo* was not concordant with that of either human chromosome 7 or 4, although these were only human chromosomes evident in this clone (Fig. 4A). To see whether we could detect a human chromosome fragment that was not resolved by alkaline Giemsa staining, we carried out in situ hybridization to metaphase chromosomes of HDm-20 using radiolabeled Blur 8 DNA as the probe. This more sensitive technique revealed a small amount of human DNA translocated onto a mouse chromosome (Fig. 4B), which may contain the *neo* vector.

DISCUSSION

The use of a defective retrovirus as a highly efficient gene transfer vector enabled us to introduce the dominant, selectable gene *neo* into specific human chromosomes, employing primary diploid fibroblasts as the starting material. From a single infection and a single microcell transfer we were able to recover *neo* integrations into four different human chromosomes, including two of the smallest.

Although G-11- and R-band staining of metaphase spreads from the hybrids indicated that each appeared to carry only one or a few identifiable human chromosomes, it was important to be able to screen further for the presence of other unrecognized fragments. Among the six hybrids we examined, two contained human chromosome fragments that were not visible in chromosome preparations stained with alkaline Giemsa. The fragment in clone HDm-20 is very small, but the portion of human chromosome 14 in clone HDm-9 may be much larger if the two chromosome 14 markers detected in it are present on a continuous fragment. These two markers have been regionally localized, and the distance between them represents more than half of the long arm of the chromosome (8).

Fragments may be retained in a hybrid by translocating to chromosomes of the host complement or as small centromeric fragments. They may arise in any type of hybrid,

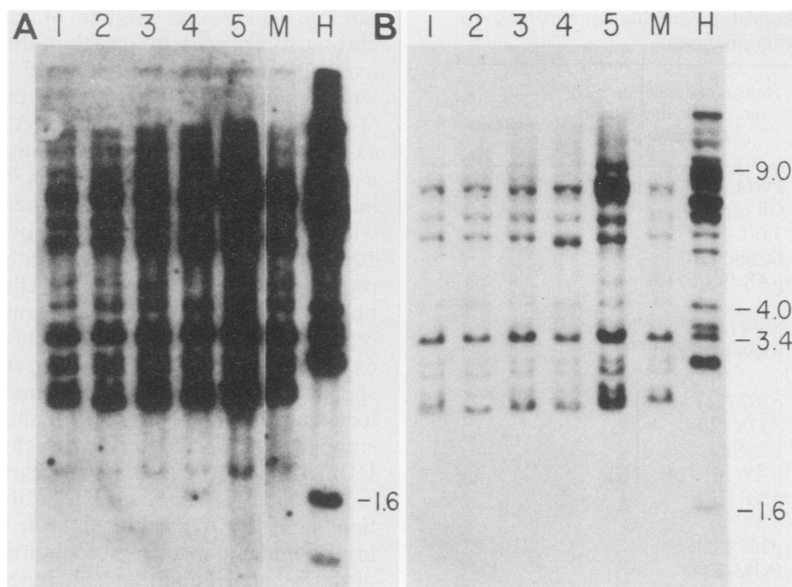

FIG. 2. Southern blot of *Eco*RI-digested DNAs (10 μg per lane) hybridized for 36 h with 10^7 cpm of [^{32}P]dCTP-labeled argininosuccinate synthetase cDNA probe in 10 ml of hybridization solution containing 50% formamide. The blot was washed at 55°C in 150 mM NaCl–15 mM sodium citrate (final wash). Autoradiography was for 3 days (A) or 12 h (B). Lanes: H, HeLa; M, NIH 3T3; 1, HDm-4; 2, HDm-5; 3, HDm-9; 4, HDm-15; 5, HDm-20 (overloaded). Numbers show kilobases.

including those produced by fusing intact cells; careful characterization is the only way to avoid potential errors resulting from unrecognized material. We found that the tendency of chromosomes to undergo degradation after microcell-mediated chromosome transfer depends on the recipient cells and can vary considerably even among closely related cell lines. In some cases, of course, hybrid cells with chromosome fragments can be useful for regional mapping provided the identity of the fragments can be determined.

Identifying all the human fragments in a given hybrid by standard isozyme or single-copy DNA probe screening is prohibitively tedious, but can be approached by using a DNA probe which recognizes a large family of related sequences located on numerous human chromosomes. Using the argininosuccinate synthetase cDNA probe, the presence or absence of regions of 10 different human chromosomes can be determined on one Southern blot. Here we were able to confirm the presence of three human chromosomes in three hybrids and to exclude the presence of those three and nine other chromosomes from each of the six hybrids analyzed. This method does not depend on the expression of specific genes in the hybrid, as isozyme analysis does, nor does it require many individual Southern blots.

Since we observed fragments and translocations in a significant proportion of our 3T6 hybrids, we made an effort to confirm the chromosomal location of the *neo* gene in those that apparently retained only whole human chromosomes. This turned out to be important, because we found that in clone HDm-20 neither of the intact human chromosomes contained the selectable marker. It seems most likely that the *neo* gene in HDm-20 is integrated into the small human

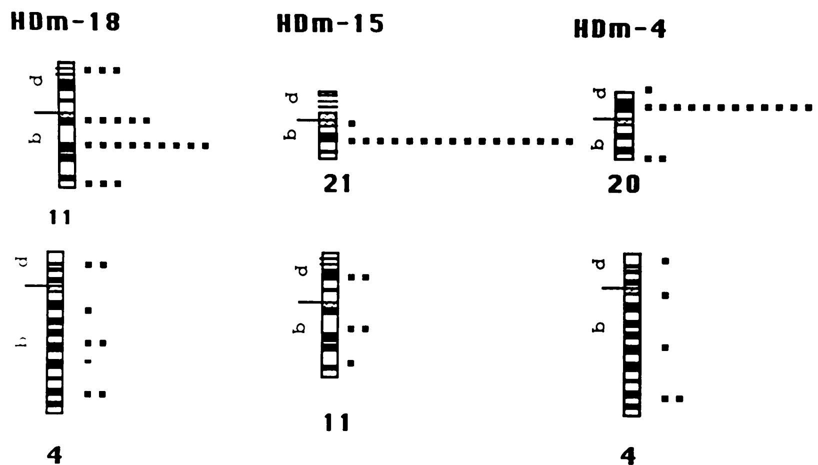

FIG. 3. Distribution of grains over human chromosomes in hybrid cell lines HDm-15, HDm-18, and HDm-4. Each dot represents one grain.

TABLE 2. Localization of the *neo* integration site in clones HDm-5 and HDm-20 by segregation analysis

Clone (subclone)	Relative plating efficiency in G418	Human NP[a] expression	D7S8 retention	G8 retention
HDm-5	0.48	+		
HDm-5a	0.51	+		
HDm-5b	0.92	+		
HDm-5c	0.87	+		
HDm-5d	0.93	+		
HDm-5e	0.94	+		
HDm-5f	0.95	+		
HDm-5g	0.78	+		
HDm-5h	0.001	−		
HDm-20	0.70		+	+
HDm-20a	0.001		+	−
HDm-20b	0.001		−	−
HDm-20c	0.001		+	−
HDm-20d	0.001		+	+
HDm-20e	0.001		+	−
HDm-20f	0.001		−	+
HDm-20g	0.002		+	−
HDm-20h	0.17		+	−

[a] NP, Nucleoside phosphorylase.

chromosome fragment we found translocated onto one of the mouse chromosomes. Even though 3T6 is an aneuploid cell line, it is surprising that under these circumstances the *neo* gene was readily segregated when the cells were removed from selective medium, because this presumably involves the loss of the host chromosome to which it is attached.

One technical problem we encountered in these experiments was the tendency of the donor human fibroblasts to form relatively large micronuclei containing several chromosomes, with the result that most of the hybrids were initially rather complex. We have observed that the micronucleation capacity of primary human fibroblasts is greatly reduced after passage 17, and these donor cells were approaching this limit. Purification techniques, including filtration and sedimentation (12), can be used to remove intact nuclei and larger microcells from microcell preparations.

Microcell hybrids such as those we described here have obvious applications in human gene mapping. Since the human chromosomes are maintained in the cells by direct selective pressure, a major problem associated with typical somatic cell hybrids—the uncontrolled loss of chromosomes and a consequent requirement for continuous monitoring of the karyotype—is avoided. Construction of mapping panels of microcell hybrids to cover the entire human chromosome complement is now under way in several laboratories (1, 27).

A microcell hybrid with the *neo* gene inserted into a specific human chromosome can also be used to produce cells with defined chromosome fragments. The hybrid can be used as the donor for transfer of isolated metaphase chromosomes (22, 32), or it can be gamma-irradiated to introduce chromosome breaks and then rescued by fusion with an unirradiated partner (5). Both techniques result in the transfer of acentric chromosome fragments that are retained by virtue of the selectable marker. Rodent cells carrying human chromosome fragments are useful for establishing the subchromosomal location of human genes by deletion mapping (17). They are also extremely advantageous starting materials for cloning any human genes known to reside on the fragment, since the amount of extraneous human DNA in the cells is reduced to a minimum.

Another important use for microcell hybrids has been the detection and definition of gene loci that affect cell type-specific gene expression (20). Human chromosomes that carry dominant markers such as *neo* can be introduced, either intact or as fragments, into many different kinds of cultured cells. This permits studies of gene interactions in a variety of histiotypic and species backgrounds. If appropriate recipients with recessive mutations are available, it is possible to construct complex hybrids with specified karyotypes.

Finally, the efficiency of retroviral vectors permits the introduction of more than one exogenous marker into a given chromosome. This can be done by infecting a microcell hybrid with a second vector and then selecting for cotransfer of both markers into a secondary recipient. Peppering a

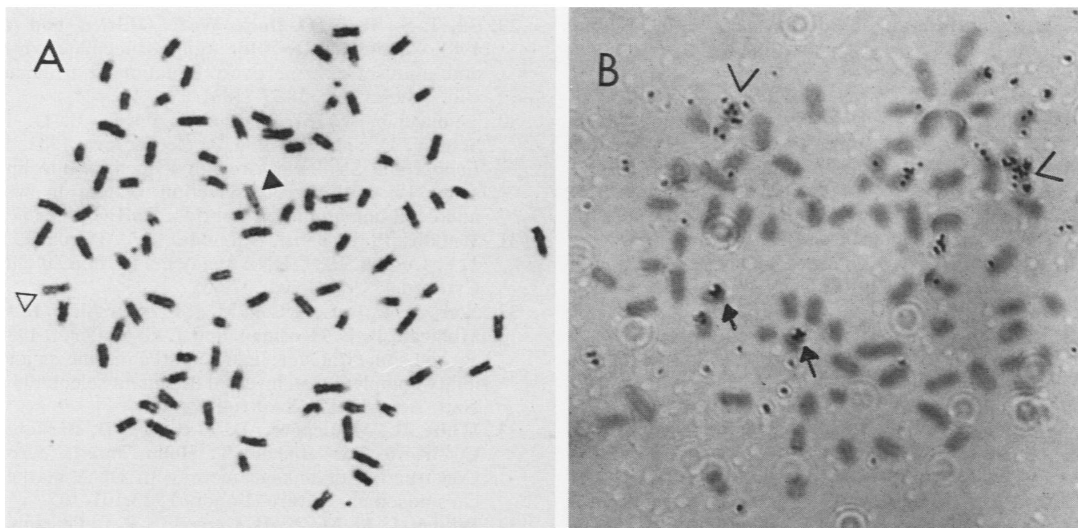

FIG. 4. Metaphase chromosomes of hybrid clone HDm-20. (A) Chromosomes stained with alkaline Giemsa. Symbols: ▶, human chromosome 7; ▷, human chromosome 4. (B) In situ hybridization of ^{3}H-labeled Blur 8 DNA to chromosomes. Symbols: >, human chromosome 7 previously identified by R-banding; arrows, human translocation to mouse chromosome(s).

chromosome with exogenous markers offers a means to recover a selectable gene integrated into a given region of interest, and it should also facilitate the physical mapping of the chromosome. The introduction of multiple markers could also be an approach to insertional mutagenesis.

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LITERATURE CITED

1. **Athwal, R. S., M. Smarsh, B. M. Searle, and S. S. Deo.** 1985. Integration of a dominant selectable marker into human chromosomes and transfer of marked chromosomes to mouse cells by microcell fusion. Somatic Cell Mol. Genet. **11**:177–187.
2. **Beaudet, A. L., T. S. Su, W. E. O'Brien, P. D'Eustachio, P. E. Barker, and F. H. Ruddle.** 1982. Dispersion of argininosuccinate-synthetase-like human genes to multiple autosomes and the X chromosome. Cell **30**:287–293.
3. **Bruns, G., J. Gusella, C. Keys, D. Housman, and P. Gerald.** 1982. Isolation of DNA segments from the human X. Cytogenet. Cell Genet. **32**:256.
4. **Cepko, C. L., B. E. Roberts, and R. C. Mulligan.** 1984. Construction and applications of a highly transmissible murine retrovirus shuttle vector. Cell **37**:1053–1062.
5. **Cirullo, R. E., S. Dana, and J. J. Wasmuth.** 1983. Efficient procedure for transferring specific human genes into Chinese hamster cell mutants: interspecific transfer of the human genes encoding leucyl- and asparaginyl-tRNA synthetases. Mol. Cell. Biol. **3**:892–902.
6. **Cone, R. D., and R. C. Mulligan.** 1984. High-efficiency gene transfer into mammalian cells: generation of helper-free recombinant retrovirus with broad mammalian host range. Proc. Natl. Acad. Sci. USA **78**:6349–6353.
7. **Cooper, D. N., B. A. Smith, H. J. Cooke, S. Niemann, and J. Schmidtke.** 1985. An estimate of unique DNA sequence heterozygosity in the human genome. Hum. Genet. **69**:201–205.
8. **Cox, D. R., and T. Gedde-Dahl, Jr.** 1985. Report of the committee on the genetic constitution of chromosomes 13, 14, 15, and 16. Eighth International Workshop on Human Gene Mapping. Cytogenet. Cell Genet. **40**:206–241.
9. **Donlon, T. A., M. Litt, S. R. Newcom, and R. E. Magenis.** 1983. Localization of the restriction fragment length polymorphism *D14S1* (pAW-101) to chromosome 14q32.1 32.2 by *in situ* hybridization. Am. J. Hum. Genet. **35**:1097–1106.
10. **Feinberg, A. P., and B. Vogelstein.** 1983. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. **132**:6–13.
11. **Fournier, R. E. K.** 1981. A general high-efficiency procedure for production of microcell hybrids. Proc. Natl. Acad. Sci. USA **78**:6349–6353.
12. **Fournier, R. E. K.** 1982. Microcell-mediated chromosome transfer, p. 309–327. *In* J. W. Shay (ed.), Techniques in somatic cell genetics. Plenum Publishing Corp., New York.
13. **Fournier, R. E. K., and J. A. Frelinger.** 1982. Construction of microcell hybrid clones containing specific mouse chromosomes: application to autosomes 8 and 17. Mol. Cell. Biol. **2**:526–534.
14. **Friend, K. K., S. Chen, and F. H. Ruddle.** 1976. Differential staining of interspecific chromosomes in somatic cell hybrids by alkaline Giemsa stain. Somatic Cell Genet. **2**:183–188.
15. **Gilliam, T. C., P. Scambler, T. Robbins, C. Ingle, R. Williamson, and K. E. Davies.** 1984. The positons of three restriction fragment length polymorphisms on chromosome 4 relative to known genetic markers. Hum. Genet. **68**:154–158.
16. **Goddard, J. M., D. Caput, S. R. Williams, and D. W. Martin.** 1983. Cloning of human purine-nucleoside phosphorylase cDNA sequences by complementation in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **80**:4281–4285.
17. **Gusella, J. F., C. Jones, F.-T. Kao, D. Housman, and T. Puck.** 1982. Genetic fine-structure mapping in human chromosome 11 by use of repetitive DNA sequences. Proc. Natl. Acad. Sci. USA **79**:7804–7808.
18. **Gusella, J. F., N. S. Wexler, P. M. Conneally, S. L. Naylor, M. A. Anderson, R. E. Tanzi, P. C. Watkins, K. Ottina, M. R. Wallace, A. Y. Sakaguchi, A. B. Young, I. Shoulson, E. Bonilla, and J. B. Martin.** 1983. A polymorphic DNA marker genetically linked to Huntington's disease. Nature (London) **306**:234–238.
19. **Harper, M. E., and G. F. Saunders.** 1981. Localization of single copy DNA sequences on G-banded human chromosomes by *in situ* hybridization. Chromosoma **83**:431–439.
20. **Killary, A. M., and R. E. K. Fournier.** 1984. A genetic analysis of extinction: *trans*-dominant loci regulate expression of liver-specific traits in hepatoma hybrid cells. Cell **38**:523–534.
21. **McNeil, C. A., and R. L. Brown.** 1980. Genetic manipulation by means of microcell-mediated transfer of normal human chromosomes into recipient mouse cells. Proc. Natl. Acad. Sci. USA **77**:5394–5398.
22. **Nelson, D. L., J. H. Weis, M. J. Przyborski, R. C. Mulligan, J. G. Seidman, and D. E. Housman.** 1984. Metaphase chromosome transfer of introduced selectable markers. J. Mol. Appl. Genet. **2**:563–577.
23. **Orkin, S. H., P. E. Daddona, D. S. Shewach, A. F. Markham, G. A. Bruns, S. C. Goff, and W. N. Kelley.** 1983. Molecular cloning of human adenosine deaminase gene sequences. J. Biol. Chem. **258**:82–84.
24. **Owerbach, D., G. I. Bell, W. J. Rutter, and T. B. Shows.** 1980. The insulin gene is located on chromosome 11 in humans. Nature (London) **286**:82–84.
25. **Rubin, C. M., C. M. Houck, P. L. Deininger, T. Friedman, and C. W. Schmid.** 1980. Partial nucleotide sequence of the 300-nucleotide interspersed repeated human DNA sequences. Nature (London) **284**:372–374.
26. **Ruddle, F. H., and R. P. Creagan.** 1975. Parasexual approaches to the genetics of man. Annu. Rev. Genet. **9**:407–486.
27. **Saxon, P. J., E. S. Srivatsan, G. V. Leipzig, J. H. Sameshima, and E. J. Stanbridge.** 1985. Selective transfer of individual human chromosomes to recipient cells. Mol. Cell. Biol. **5**:140–146.
28. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.
29. **Su, T.-S., H.-G. O. Bock, W. E. O'Brien, and A. L. Beaudet.** 1981. Cloning of cDNA for argininosuccinate synthetase mRNA and study of enzyme overproduction in a human cell line. J. Biol. Chem. **256**:11826–11831.
30. **Tunnacliffe, A., M. Parker, S. Povey, B. O. Bengtsson, K. Stanley, E. Solomon, and P. Goodfellow.** 1983. Integration of *Ecogpt* and SV40 early region sequences into human chromosome 17: a dominant selection system in whole cell and microcell human-mouse hybrids. EMBO J. **9**:1577–1584.
31. **Watkins, P., R. Tanzi, G. Landes, J. V. Tricoli, T. B. Shows, and J. F. Gusella.** 1984. DNA segments for human chromosome 21. Cytogenet. Cell Genet. **37**:602.
32. **Weis, J. H., D. L. Nelson, M. J. Przyborski, D. D. Chaplin, R. C. Mulligan, D. E. Housman, and J. G. Seidman.** 1984. Eukaryotic chromosome transfer: linkage of the murine major histocompatibility complex to an inserted dominant selectable marker. Proc. Natl. Acad. Sci. USA **81**:4879–4883.
33. **White, R., M. Leppert, D. T. Bishop, D. Barker, J. Berkowitz, C. Brown, P. Callahan, T. Holm, and L. Jerominski.** 1985. Construction of linkage maps with DNA markers for human chromosomes. Nature (London) **313**:101–105.
34. **Wijnen, L. M. M., K.-H. Grzeschik, P. L. Pearson, and P. Meera Khan.** 1977. The human *PGM-2* and its chromosomal localization in man-mouse hybrids. Hum. Genet. **37**:271–278.
35. **Wyman, A. R., and R. White.** 1980. A highly polymorphic locus in human DNA. Proc. Natl. Acad. Sci. USA **77**:6754–6758.