Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 4879–4883, August 1984
Genetics

Eukaryotic chromosome transfer: Linkage of the murine major histocompatibility complex to an inserted dominant selectable marker

(DNA transfection/*H-2* genes/genomic mapping)

JOHN H. WEIS*, DAVID L. NELSON†, MARY JO PRZYBORSKI†, DAVID D. CHAPLIN*, RICHARD C. MULLIGAN‡, DAVID E. HOUSMAN†, AND J. G. SEIDMAN*

*Department of Genetics, Harvard Medical School, Boston, MA 02115; and †Center for Cancer Research, Department of Biology and ‡Whitehead Institute for Biomedical Research, Massachusetts Institute of Technology, Cambridge, MA 02139

Communicated by Philip Leder, April 16, 1984

ABSTRACT **We have developed an approach for genetic analysis of the murine *H-2* complex that has broad general applicability to the study of eukaryotic genome organization. We have used a retroviral vector to introduce a selectable marker into the mouse genome close to the major histocompatibility complex (*MHC*). Chromosomal segments containing large portions of the *MHC* from these donor cells have been transferred both to hamster and monkey cell recipients. The procedure involved the following steps. First, a murine cell line was multiply infected with a defective recombinant murine leukemia virus that contains the neomycin-resistance gene (a gene that confers resistance to G418). In this way, the neomycin-resistance gene was introduced at multiple sites in the mouse genome. Second, metaphase chromosomes, prepared from this infected cell population, were transferred to hamster cell recipients. Third, two G418-resistant transferents were identified that expressed murine H-2 antigens on their cell surface. These transferents were shown to contain a large segment of the murine *MHC* (*H-2K* and *I* regions) by DNA hybridization. The neomycin-resistance gene and the mouse *MHC* genes must be physically linked in these cells since they could be co-transferred from the hamster cells to monkey cells. Fourth, the murine cell carrying the neomycin-resistance gene near the MHC was identified from the original donor cell population. This cell will serve as a useful source of chromosome fragments for analysis of larger portions of the MHC. This series of steps can serve as a paradigm for the first steps in a detailed genetic analysis of any specific region of a mammalian genome to which one or more genes have already been mapped.**

Efforts to determine the genetic organization of large regions of the mammalian genome have been limited by the complexity of the DNA of each species. The largest segment of a mammalian chromosome that has been mapped with precision is the DNA coding for the mouse major histocompatibility complex (MHC). Within this locus, contiguous DNA segments of 65 (*H-2K* region), 270 (*I* region), and 280 (*S* region) kilobases (kb) have been isolated and characterized (for review, see refs. 1 and 2). The *MHC* is favorable for genetic mapping because of the large number of partially homologous coding regions within the complex and the existence of a large number of congeneic mouse strains. Nevertheless, the three major clusters of DNA segments that have been mapped thus far have not been directly connected by DNA mapping methods nor have they been molecularly linked to the flanking *H-2D* and *Tla/Qa* gene clusters (1). To approach the problem of mapping DNA segments approaching 10^6 base pairs long, we felt it would be necessary to develop new methods for genetic mapping. Somatic cell genetic techniques involving manipulation of an entire chromosome do not give resolution at the desired level of complexity since an average mouse or human chromosome contains $\approx 10^8$ base pairs of DNA. A technique that seemed promising was the methodology originally developed by McBride and Ozer (3) and extended by Ruddle and co-workers (4) for the transfer of intact segments of metaphase chromosomes to recipient cells of another species. We reasoned that the development of cell lines of this type could allow molecular analysis of 10^6 to 10^7 base pairs of DNA. Furthermore, previously developed techniques involving the use of interspersed repeated DNA sequences as hybridization probes to DNA isolated from somatic cell hybrids to identify cloned DNA segments in recombinant DNA libraries derived from such hybrids could be used to carry out detailed mapping of such cells (5).

A significant problem in the creation of such hybrid cells is that, as originally described by McBride and Ozer (3) and Ruddle and co-workers (4), metaphase chromosome transfer involves the use of a selectable marker to retain the transferred chromosome segment. Such markers occur naturally in only a few locations in a mammalian genome. To develop a general approach involving chromosome transfer methodology, we proposed to insert a selectable marker at many different sites within a mammalian genome (6).

We have chosen the murine *MHC* as the focus of our initial studies because recipient cells carrying and expressing the murine MHC can be directly identified by antibody screening tests (7). We have used the strategy outlined in Fig. 1 to isolate chromosome segments encoding portions of the murine MHC in monkey and hamster recipient cells.

MATERIALS AND METHODS

Cell and Culture Media. The MD26 cell line, a murine T-cell hybridoma line formed by fusion of BW5147 (*H-2^k* cells) with BALB/c T cells (*H-2^d*), was used as the chromosomal donor (8). It is easily infected by murine retroviruses. The CHTG49 cell line is a Chinese hamster fibroblast line (9). CV-1 cells are from a monkey fibroblast line (6). The cell line Ψ2 5-3 produces a defective recombinant murine leukemia virus whose genome includes the gene for neomycin resistance (ref. 10; unpublished work).

Cells were grown in either RPMI (MD26) or Dulbecco's modified Eagle's (Ψ2 5-3, CHTG, and CV-1) medium supplemented with 10% fetal calf serum as described (6). Cells were selected for expression of the neomycin-resistance gene by adding G418 (Geneticin; GIBCO 1 mg/ml), an analogue of neomycin capable of killing eukaryotic cells in culture, to the media (11).

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: MHC, major histocompatibility complex; kb, kilobase(s).

Virus Infection. Culture supernatants from the Ψ2 5-3 cell line containing defective retrovirus particles that encode a neomycin-resistance gene in their genome were removed from 5×10^7 cells every 24 hr. The culture supernatants were filtered through 0.45-μm filter (Nalgene) and Polybrene was added to 10 μg/ml. MD26 cells (1×10^6) were incubated for 7 days with the Ψ2 5-3 culture supernatant. Fresh culture supernatant was added every 24 hr. G418 was added to the culture on the 5th day to select for cells containing the virus. Virtually all of the MD26 cells were infected since all of the cells were G418 resistant.

Chromosome Transfers. Chromosomes were isolated and transfected essentially as described (3, 4, 6). Briefly, virus-infected cells were mitotically blocked with Colcemid (0.1 μg/ml) for 16 hr causing the chromatin to condense. After washing with cold phosphate-buffered saline, cells were suspended in cold lysis buffer (10 mM Hepes, pH 7.1/3 mM $CaCl_2$) and held at 4°C for 15 min. The cells were lysed in a Dounce homogenizer and centrifuged for 7 min at $150 \times g$; the pellet was discarded. Chromosomes were isolated by centrifuging the supernatant for 25 min at $1500 \times g$ at 4°C. The pellet was suspended in cold lysis buffer and centrifuged for 7 min at $150 \times g$; the supernatant was recovered. Again chromosomes were isolated by centrifuging the supernatant for 25 min at $1500 \times g$. The pellet was suspended in transfection buffer (25 mM Hepes, pH 7.1/134 mM NaCl/5 mM KCl/5 mM glucose/0.7 mM Na_2HPO_4), brought to 125 mM $CaCl_2$, and plated on either CHTG49 or CV-1 cells. Usually chromosomes from 1×10^8 cells were plated onto 1×10^7 recipient cells. Transfected fibroblasts were selected for G418 resistance 48 hr after transfection.

Southern Blotting and Radioimmunoassay. Restriction fragments encoding murine genes were identified by Southern blot analysis as described (12–14). DNA probes specific for restriction fragments bearing *H-2* (13) and *I-A* (14) genes were isolated from the cloned genes. The DNA probe used for identifying the end of the murine *I* region closest to the telomere (the distal end) was isolated from cosmid 7.1 [provided by L. Hood (15)]. Probes for the murine *S* region genes, complement component 4 (C4), and factor B have been described (2). The α-globin pseudogene probe (α-4) was described by Leder *et al.* (16).

Cells were analyzed for the expression of new H-2 antigens by radioimmunoassay (17) using procedures described previously. About 10^5 cells were allowed to react with a monoclonal antibody (15.5.5) specific for the H-$2K^d$ and H-$2D^k$ antigens [available from the American Type Tissue Culture Collection (18)]. The cells were washed and allowed to react with ^{125}I-labeled staphylococcal protein A (New England Nuclear). The cells were washed again and the amount of labeled protein A bound to the cells was measured in a gamma counter.

RESULTS

Isolating a Mouse Chromosome with an Inserted Neomycin-Resistance Gene Linked to the Murine *MHC*. The purpose of the series of experiments described here was to separate a fragment of murine chromosome 17 containing the *MHC* from other murine chromosomes in a cell of a different species. The basis of this approach was to place a selectable marker near the *MHC* so that cells containing this chromosome region can be selected in the appropriate media. The approach that we have taken is outlined below and in Fig. 1.

The first step involved the generation of a population of cells that contained a neomycin-resistance gene integrated randomly throughout their genomes. Specifically, the murine T-cell hybridoma MD26 was multiply infected with the defective retrovirus MSV-neo that carries the neomycin-resistance (*neo*^{r}) gene. This defective retrovirus is derived from a Ψ2 helper cell so that no infectious virus was present in the virus stock (10). Retroviruses appear to integrate randomly in the genome of the cell (19), so the population of infected *neo*^{r} cells consisted of a series of cells in which the *neo*^{r} gene had integrated in multiple locations throughout their genomes (6). MD26 cells were multiply infected with MSV-neo for 7 days and selected for G418 resistance. At least 90% of the MD26 cells were infected and contained at least three *neo*^{r} genes (data not shown; ref. 6). Given a large enough population of infected cells, it was likely that a *neo*^{r} gene had integrated into one cell's genome near the *MHC*.

The next step involved transferring chromosomal fragments from the population of G418-resistant MD26 cells to recipient hamster cells. The transfer method we used (see

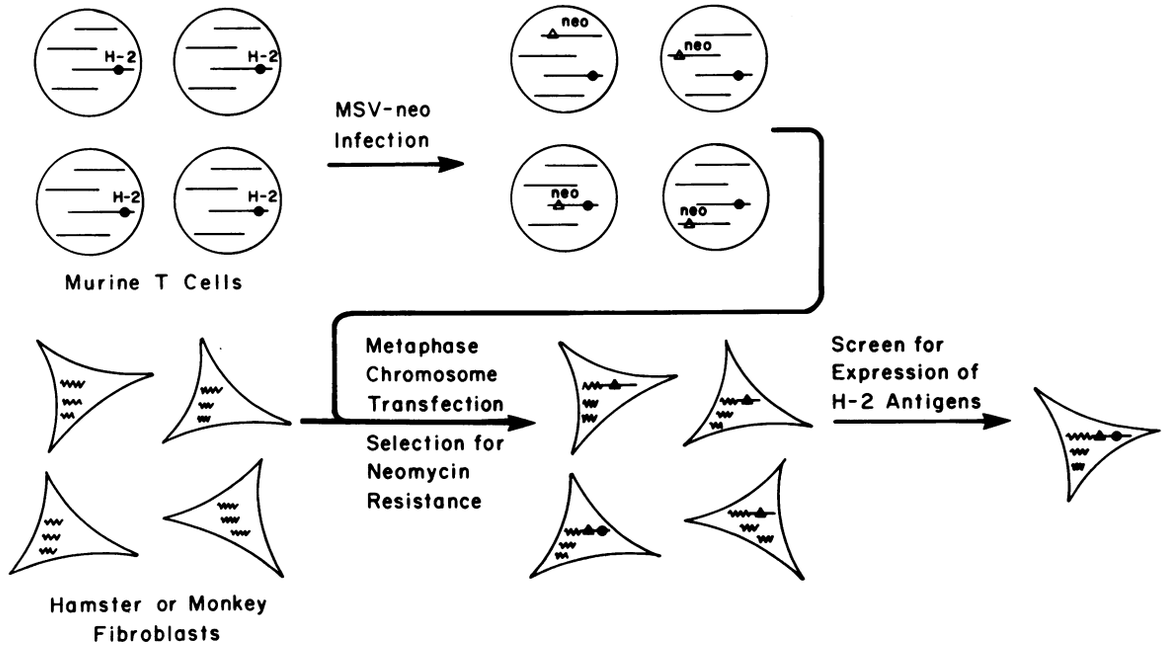

FIG. 1. Procedure for isolation of a chromosome-recipient cell bearing the *MHC*. Cell clones were selected for resistance to the neomycin analogue G418 and analyzed for expression of murine H-2 antigens. ●, The murine *MHC*; △, the neomycin-resistance gene.

Table 1. Expression of H-2 antigens on transfected hamster fibroblasts

Cell type	NSA	15.5.55
MD26	470	1600
CHTG	430	450
CHMD D2	450	3400
CHMD E1	480	5600

Transfected hamster fibroblast cell lines were examined for expression of H-2 antigens by radioimmunoassay. Duplicate samples of 50,000 cells were analyzed with nonspecific antisera (NSA) and a monoclonal antibody specific for H-2K^d and H-2D^k (15.5.55).

Materials and Methods and ref. 6 for details) involved isolation of chromosomes from Colcemid-treated cells followed by transfection of these chromosome fragments into Chinese hamster recipient (CHTG49) cells. The cells receiving a fragment of chromosome bearing the *neo*^{r} gene were selected by growth in the presence of G418. The number of *neo*^{r} chromosome-recipient cells required to ensure that *neo*^{r} has been transferred with fragments representing an entire genome equivalent is determined by the size of the chromosomal fragments maintained in the recipient cell. We have estimated that chromosome-recipient cells made in this way normally receive about 1.0% of the donor genome (6). We expected that we would have to screen about 100 chromosome-recipient cells to find the desired cell.

Finally, the *neo*^{r} chromosome-recipient cell that contained the murine *MHC* was identified by its ability to express an H-2 antigen. That is, hamster cells do not normally express the same H-2 antigens on their surface as the murine T-cell MD26. The MD26 cell is a hybrid cell and expresses H-2 antigens of the *d* and *k* haplotypes, so recipient cells might express either H-2^d or H-2^k antigens. Thirty cloned chromosome-recipient cell lines were screened for expression of the H-2K^d or H-2D^k antigens. Two cells lines were identified, CHMD D2 and CHMD E1, that bound a monoclonal antibody specific for these two H-2 antigens (Table 1).

Mapping the Murine Chromosome Fragments in CHMD D2 and E1 Cells. The chromosome-transfer procedure introduces chromosome fragments of various sizes into recipient cells (6). It was not possible, therefore, to predict the precise amount of murine DNA that had been transferred to the CHMD D2 and E1 cells. One method of estimating the amount of murine DNA in the chromosome-recipient cells involves hybridization of a DNA probe bearing a reiterated sequence found in mouse DNA, but not in hamster DNA, to the DNA of the chromosome-recipient cells (6). These experiments indicated that the CHMD E1 cell contains ≈0.5% of the entire mouse genome while the CHMD D2 cell contains about 0.3% of the mouse genome (data not shown).

To map precisely the boundaries of the transferred chromosomal fragment, we have taken advantage of the results of classical genetics. Over the past 20 years the order of the genes on murine chromosome 17 and in the *MHC* has been determined (Fig. 2*A*). More recently, DNA probes specific for *H-2*, *I-Aβ*, and *C4* genes have been isolated (1, 12–14). *H-2* DNA probes identify a family of about 30 genes encoded in the *H-2K*, *H-2D*, and *Tla/Qa* (distal to *H-2D*) regions (Fig. 2*A*; ref. 1). However, the DNA probes specific for the *I-Aβ* and the *C-4* genes hybridize to only one *I* and two *S* region-derived restriction fragments. The DNA of the murine *H-2K*, *I*, and *S* regions has been cloned by cosmid-walking procedures (1, 2, 20, 21) and the sizes of the cloned regions are indicated in Fig. 2*B*.

To identify the portions of the murine *MHC* that were transferred to the CHMD D2 and E1 cells, their DNAs were digested and hybridized to *H-2* (Fig. 3*A*) and *I-Aβ* (Fig. 3*B*) probes as well as to a fragment derived from the telomeric end of the cloned *I* region segment (Fig. 3*C*). The results allow several conclusions about the structure of the chromosomal fragments that have been transferred to the CHMD D2 and E1 cells.

First, these results confirm that the neomycin-resistance gene has integrated in the *H-2*^{d} chromosome rather than the *H-2*^{k} chromosome. That is, the MD26 donor cell contains the genes of both the *H-2*^{k} and *H-2*^{d} alleles and thus the *I-Aβ*-chain gene probe hybridizes to two restriction fragments in the *Bam*HI digests of MD26 DNA (Fig. 3*B*). However, the *I-Aβ*-chain gene probe finds only the single *Bam*HI fragment in the digests of CHMD D2 and E1 DNAs that is found in *Bam*HI digests of BALB/c (*H-2*^{d}) DNA (Fig. 3*B*). Thus, both chromosome-recipient cells, CHMD D2 and E1, have received the *I-Aβ*-chain allele of the *H-2*^{d} haplotype and not the *I-Aβ* gene fragment derived from the *H-2*^{k} allele.

Second, only two *H-2*-hybridizing fragments are identified in the digests of CHMD D2 and E1 DNA (Fig. 3*A*). Since these cells are expressing the H-2K^d antigen, one of these two fragments must encode the *H-2K*^{d} gene. Two groups (20, 21) have shown that there are two *H-2* genes encoded within 20 kb of one another at the *K* end of the *MHC* (Fig. 2*B*). These results also confirm the conclusion drawn by Winoto *et al.* (23) that there are only two *H-2* genes at the *K* end of the *MHC*.

Third, one boundary of the fragments transferred into the chromosome-recipient cells is defined by these experiments. The *I-Aβ* gene is present in both the CHMD D2 and E1 cells (Fig. 3*B*). However, a probe derived from the distal end of

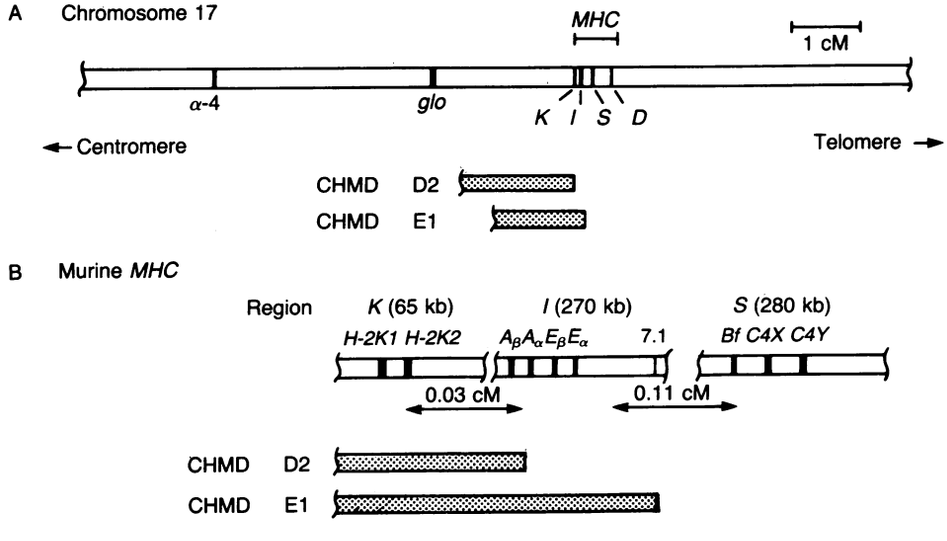

FIG. 2. Genetic organization of murine chromosome 17 and its relationship to the chromosome fragments found in the CHMD D2 and E1 cells. (*A*) Relative positions of the α-globin pseudogene (α-4), glyoxylase (*glo*), and the *H-2K*, *I*, *S*, and *H-2D* regions of the *MHC* (1, 2, 20–22). (*B*) Genes that have been mapped to the regions of the *MHC* and sizes of the cosmid clusters that are available in each of the regions. Distances between the regions measured in terms of frequency of recombination (centimorgans, cM) are also shown.

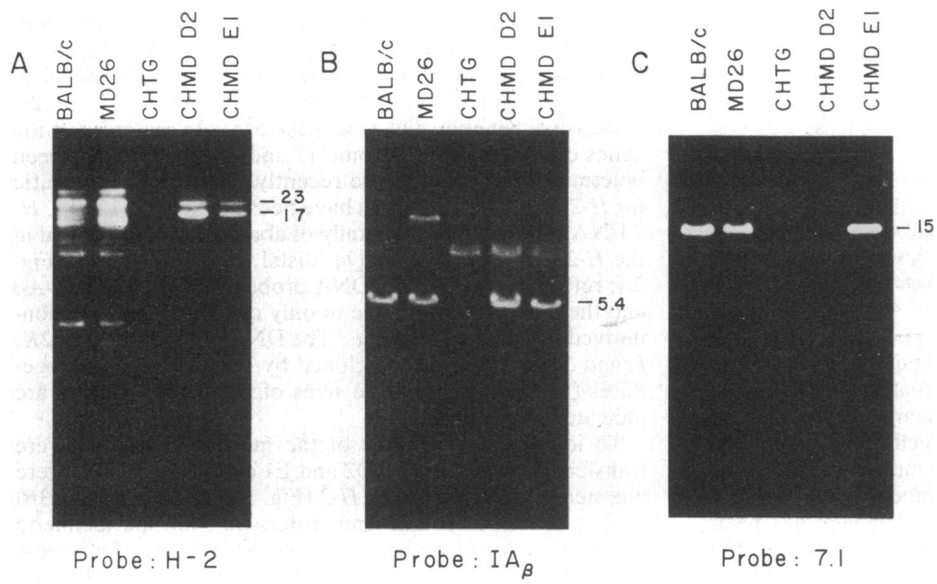

FIG. 3. Southern blot analyses of *Eco*RI-restricted DNA from BALB/c mouse liver, MD26 cells (donor cells), CHTG (hamster recipient cells), and the CHMD D2 and E1 chromosome-recipient cells using probes specific for *H-2* genes (*A*), the *I-Aβ* gene (*B*) and the telomeric end of the cloned *I* region segment (*C*). Sizes of the fragments (kb) are indicated.

the cloned *I* region hybridized to DNA derived from the CHMD E1 cells but not to DNA from the CHMD D2 cells (Fig. 2*C*). Thus, the "distal" boundary of the CHMD D2 chromosomal fragment falls within the cloned segment between the *I-Aβ* gene and the end of the cloned *I* region segment (Fig. 2). The distal boundary of the CHMD E1 chromosomal fragment lies between the cloned *I* region segment (Fig. 2) and the *S* region because its DNA hybridizes to the *I* region-derived probe but not to murine *S* region probes (data not shown).

Co-transfer of the *neo*^{r} Gene and the *MHC* from Hamster Cells to Monkey Fibroblasts. To determine further the physical association between the neomycin-resistance gene and the *MHC*, chromosomes were isolated from CHMD D2 and E1 cells and transferred to the monkey fibroblast cell line CV-1 as well as to CHTG49. Nineteen G418-resistant secondary transferents were isolated. None of the *neo*^{r} chromosome-recipient monkey fibroblast lines expressed a detectable H-2K^d antigen on the cell surface. However, analysis of the DNA of these chromosome recipient monkey fibroblast lines by Southern blotting (Fig. 4) showed that they contained murine *H-2* and *I-A* genes. That is, *Bam*HI digests of the CVQD2C3 cell DNA, a CV-1 cell transfected with chromosomes from the CHMD D2 cell line, contained the same *H-2* hybridizing fragments that were present in digests of CHMD D2 and MD26 DNA (Fig. 4*A*). Furthermore, the CVQD2C3 cell contains the murine *I-Aβ* gene (Fig. 4*B*). Thirteen secondary chromosome transferents were analyzed; all of them contained the murine *H-2*-bearing restriction fragments. The reason why murine *H-2* gene products are not detected on the surface of the chromosome-recipient monkey cells remains unclear; nevertheless, these cells do contain both the *neo*^{r} gene and the murine *H-2K*^{d} gene. Thus, the murine *H-dK*^{d} gene is closely linked to the *neo*^{r} gene in the CHMD D2 and E1 cell lines.

Identification of the Murine Cell Containing the *neo*^{r} Marker Near the *MHC*. The chromosome-recipient cells CHMD E1 and D2 have received only a portion of the murine *MHC*—the *H-2K* and *I* regions. To study the organization of the entire MHC, it would be necessary to isolate the entire *MHC* on a chromosome fragment. One could imagine screening more recipient cells until the desired cell was obtained. Another approach is to identify the *neo*^{r} MD26 cell that donated the chromosome fragments in the CHMD E1 and D2 cells. The donor *neo*^{r} MD26 cells should have a *neo*^{r} gene in precisely the same location in its genome as the *neo*^{r} gene in the genomes of the CHMD E1 and D2 cells. This donor might be better used as a source of chromosome fragments containing the entire *MHC*.

As a first step in identifying the donor cell, we characterized the restriction fragments encoding the *neo*^{r} gene in the CHMD D2 and E1 cells. Southern blot analyses of *Bgl* II- and *Eco*RI-digested CHMD D2 and E1 DNA revealed that the *neo*^{r} gene was on identical restriction fragments in these two cells (Fig. 5). Apparently the two chromosome-recipient cells received their chromosomes from the same *neo*^{r} MD26 donor cell.

Since the donor cell had independently donated chromosome fragments to two recipient cells, we hypothesized that this particular donor cell might comprise a relatively large fraction of the donor cell population. To test this hypothesis, the DNA from the donor cell population (8 weeks after infection) was isolated, digested with *Eco*RI, and hybridized to the *neo*^{r} gene probe. A series of discrete bands was identified (data not shown) and one of these bands corresponded to the 8.4-kb fragment found in the *Eco*RI digests of CHMD D2 and E1 DNAs. We assumed that the particular donor cell

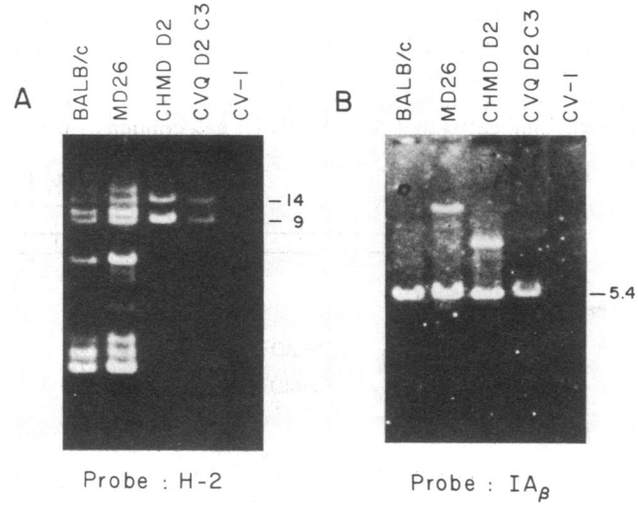

FIG. 4. Visualization of restriction fragments bearing *H-2* and *I-Aβ*-chain genes in donor and recipient cell DNAs. Ten-microgram samples of DNA from BALB/c liver, MD26 cells, chromosome-recipient hamster CHMD D2 cells, the chromosome-recipient monkey fibroblast cell line CVQD2C3, and monkey fibroblast cell line CV-1 were digested with *Bam*HI (A) or *Eco*RI (*B*) and hybridized to the indicated probes. Sizes of the fragments (kb) are shown.

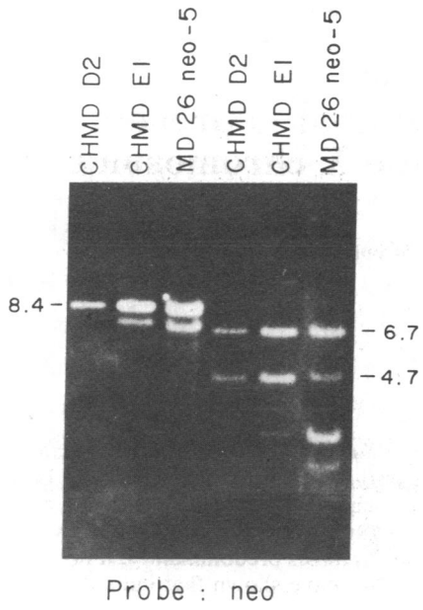

FIG. 5. Southern blot analyses using *Eco*RI (*Left*) and *Bgl* II (*Right*) of DNAs from the chromosome-recipient cells CHMD D2 and E1 as well as from the donor cell, MD26 neo-5, using the neomycin-resistance gene-specific probe derived from bacterial plasmid pCK7 (24). Sizes of the fragments (kb) are shown.

in which the *neo*^{r} gene was closely linked to the murine *MHC* comprised a significant fraction of the *neo*^{r} MD26 donor cell population. Individual cloned lines of the *neo*^{r} MD26 donor cell population were isolated. DNAs from 20 of these cloned lines were characterized by hybridization to the *neo*^{r} gene probe. One cell line, MD26 neo-5, was identified whose DNA produced restriction fragments that co-migrated with the fragments derived from the CHMD E1 and D2 cells (Fig. 5). We assumed that the *neo*^{r} gene is closely linked to the *MHC* in this cell line. Recently, we have demonstrated that the H-2K^d and neo^r phenotypes co-transfer at high frequency from this cell to recipient hamster cells (data not shown). Thus, this cell does indeed have a *neo*^{r} gene closely linked to the *MHC*.

DISCUSSION

Hamster and monkey cells bearing a fragment of murine chromosome 17 encoding a part of the murine *MHC* closely linked to a selectable marker (neomycin resistance) have been isolated. Specifically, we have demonstrated that the neomycin-resistance gene is closely linked to the *H-2K*^{d} gene. One end of the chromosome fragments in these cells can be precisely mapped because of the availability of DNA probes to genes encoded in the murine *MHC* (Fig. 2*B*). The chromosome fragments certainly contain genes that are known to be 0.03 centimorgans apart but do not contain genes that are 0.21 centimorgans further away. Since murine chromosome 17 has no genetic markers between the *MHC* and the glyoxylase gene (3 centimorgans), the other end of the chromosome fragment in these cells cannot be accurately mapped.

The method for transferring chromosomes used here appears to introduce 0.1–1% of the donor genome into the recipient cells (3, 4, 6). Thus, we expect that one should be able to co-transfer genes that are separated by as much as 20 centimorgans. The recipient cells described here have received less DNA than is required to encode genes 5 centimorgans apart. However, isolation of the parental infected MD26 donor cell will allow the creation of a series of CV-1 transferents, each containing various lengths of murine chromosome 17 in association with the neomycin-resistance gene. Cosmid libraries prepared from such cell lines will contain only a fraction of clones that possess mouse-specific sequences. Purification of the mouse-specific clones away from the monkey DNA-containing cosmids should allow chromosomal-walking experiments to progress at a faster pace than is currently realized.

We thank Dr. L. Hood for cosmid clone 7.1. This research was supported in part by National Institutes of Health Grants AI-18436 and AI-19148 to J.G.S. and ROI-GM27882, POI-CA26717, and ROI-CA17575 to D.E.H. Research was also funded in part by a grant from the Whittaker Foundation to J.G.S. and D.E.H. J.H.W. was a recipient of the postdoctoral fellowship from the American Cancer Society. D.D.C. is a fellow of the Jane Coffin Childs Memorial Fund for Medical Research.

1. Steinmetz, M. & Hood, L. (1983) *Science* **222**, 727–733.
2. Chaplin, D. D., Woods, D. E., Whitehead, A. S., Goldberger, G., Colten, H. R. & Seidman, J. G. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 6947–6951.
3. McBride, O. W. & Ozer, H. L. (1973) *Proc. Natl. Acad. Sci. USA* **70**, 1258–1262.
4. Miller, C. L. & Ruddle, F. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 3987–3990.
5. Gusella, J. F., Keys, C., Varsanji-Breiner, A., Kao, F.-T., Jonas, C., Puck, T. T. & Housman, D. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 2829–2833.
6. Nelson, D. L., Weis, J. H., Przyborski, M. J., Seidman, J. G. & Housman, D. E., *J. Appl. Mol. Biol.*, in press.
7. Klein, J. (1975) *Biology of the Mouse Histocompatibility Complex* (Springer, Berlin).
8. Kaufman, Y., Golstein, P., Pierres, M., Springer, T. & Eschar, Z. (1982) *Nature* (*London*) **300**, 357–360.
9. Athwal, R. S. & McBride, I. W. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 2943–2947.
10. Mann, R., Mulligan, R. C. & Baltimore, D. (1983) *Cell* **33**, 153–159.
11. Southern, P. J. & Berg, P. (1982) *J. Mol. Appl. Genet.* **1**, 327–341.
12. Southern, E. (1975) *J. Mol. Biol.* **98**, 503–511.
13. Evans, G. A., Margulies, D. H., Camerini-Otero, D., Ozato, K. & Seidman, J. G. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 1994–1998.
14. Robinson, R., Germain, R. N., McKean, D. J., Mescher, M. & Seidman, J. G. (1983) *J. Immunol.* **131**, 2025–2031.
15. Steinmetz, M., Minard, K., Horvath, S., McNicholas, J., Strelinger, J., Wake C., Long, E., Mach, B. & Hood, L. (1982) *Nature* (*London*) **300**, 35–42.
16. Leder, A., Swan, D., Ruddle, F., D'Eustachio, P. & Leder, P. (1981) *Nature* (*London*) **293**, 196–200.
17. Evans, G. A., Margulies, D. H., Shykind, B., Seidman, J. G. & Ozato, K. (1982) *Nature* (*London*) **300**, 755–757.
18. Ozato, K., Mager, N. & Sachs, D. (1980) *J. Immunol.* **124**, 533–540.
19. Varmus, H. E. & Swanstrom, R. (1982) in *RNA Tumor Viruses*: Molecular Biology of Tumor Viruses, eds. Weiss, R., Teich, R., Varmus, H. E. & Coffin, J. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 369–519.
20. Mellor, A. L., Golden, L., Weiss, E., Ballman, H., Murt, J., Simpson, E., James, R. F. C., Townsend, A. P. M., Taylor, P. M., Schmidt, W., Fedozo, J., Geben, K., Santamonia, M., Altfield, J., Festenstein, H. & Flavell, R. A. (1982) *Nature* (*London*) **298**, 529–534.
21. Steinmetz, M., Winoto, A., Minard, K. & Hood, L. (1982) *Cell* **28**, 489–498.
22. D'Eustachio, P., Fein, B., Michaelson, J. & Taylor, B. A. (1984) *J. Exp. Med.* **159**, 958–963.
23. Winoto, A., Steinmetz, M. & Hood, L. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 3425–3429.
24. Rao, N. R. & Rogers, S. G. (1979) *Gene* **7**, 79–82.