

CHROMOSOMAL LOCATION OF STRUCTURAL GENES
ENCODING MURINE
IMMUNOGLOBULIN λ LIGHT CHAINS
Genetics of Murine λ Light Chains*

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Immunoglobulin (Ig) molecules consist of two identical heavy (H) polypeptide chains and two identical light (L) polypeptide chains held together by noncovalent forces and disulfide bonds (1). The structural genes encoding these chains are grouped into at least three sets, one encoding H chains, one encoding L chains of the κ class, and one encoding L chains of the λ class (2). Three subclasses of λ polypeptide chains have been identified, λ_I , λ_{II} , and λ_{III} (3, 4; and T. Azuma, L. A. Steiner, and H. N. Eisen, personal communication). DNA fragments containing λ_I chain genes have been isolated using recombinant DNA techniques. There are three such genes, a $V_{\lambda I}$ gene encoding the amino-terminal 97 residues of the λ_I polypeptide, a $J_{\lambda I}$ gene encoding residues 98–114, and a $C_{\lambda I}$ gene encoding the remainder of the molecule. These genes occur in two configurations. In gametes (germline cells) and most nonlymphoid somatic cells, the V gene is separated from the J and C genes by an unknown but large distance. In B cells and myelomas committed to produce λ_I L chains, the V gene directly abuts the J gene. Splicing and excision events in the intervening chromosomal DNA would provide a simple mechanism for this rearrangement, and would require linkage in the germline DNA among $V_{\lambda I}$, $J_{\lambda I}$, and $C_{\lambda I}$. Less straightforward models that do not require linkage remain possible, however, and in any event no linkage is required among the various subclasses of λ chain genes (5).

A satisfactory account of the genetics of the λ L chain, however, needs to investigate these linkage relationships: what chromosome or chromosomes these genes are on and what other genes they are linked to. In the case of murine λ L chains, Mendelian genetic analyses have been uninformative, largely due to the lack of appropriate polymorphic markers. An alternative approach to the problem is provided by the recent development of nucleic acid hybridization techniques to detect DNA sequences in interspecific somatic cell hybrids. These techniques have been used previously to

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map L chains of the κ class to chromosome 6 in the mouse (6) and H chains to the distal half of chromosome 12 (7). Here we report results for the λ class of L chains.

Materials and Methods

Somatic cell hybrids were formed between the Chinese hamster cell line E36 and either peritoneal macrophages from A/HeJ mice (MACH hybrid series), fibroblasts from BALB/c fetal mice (BEM hybrid series), cells from a tissue culture-adapted subline of the Meth A murine fibrosarcoma (MAE hybrid series) (8), or cells from the murine cell lines CT11c (hybrid ECm4e) (9), or C1 1DH3 (hybrid R44-1) (10). Hybrid cell lines were grown in monolayer culture, in antibiotic-free Dulbecco's modified essential medium, high-glucose formulation (Grand Island Biological Co., Grand Island, N. Y.) supplemented with 10% fetal bovine serum. Cells were harvested by trypsinization and washed with 0.15 M NaCl.

To isolate high molecular weight cellular DNA, a washed cell pellet (either freshly harvested or harvested, stored at -70°C , and freshly thawed) was resuspended in 40 vol of 0.15 M NaCl, 0.01 M ethylenedinitrilotetraacetate, 0.01 M Tris, pH 7.8, lysed by the addition of sodium dodecyl sulfate to a final concentration of 0.2%, and incubated for 4–10 h at 37°C in the presence of 500 $\mu\text{g}/\text{ml}$ final concentration of *Streptomyces griseus* protease (type V; Sigma Chemical Co., St. Louis, Mo.). The digestion mixture was extracted sequentially with equal volumes of phenol (saturated with digestion buffer), phenol:chloroform:isoamyl alcohol (25:24:1), and chloroform:isoamyl alcohol (24:1). DNA was precipitated from the extracted aqueous phase by the addition of 2 vol of ice-cold absolute ethanol, and the precipitate was redissolved in sterile 0.1 mM ethylenedinitrilotetraacetate, 10 mM Tris, pH 8.0, and stored at 4°C .

To identify the mouse chromosomes present in each hybrid line, samples of the same cell population from which the DNA was extracted were analyzed karyotypically using the sequential Giemsa-Viokase-Hoechst 33258 technique, and were tested for the presence of 18 mouse isoenzyme markers whose chromosomal locations are known (11).

DNA samples were digested with *Hind*III restriction endonuclease (Bethesda Research Laboratories, Rockville, Md.) by incubating the DNA at a concentration of 0.2–0.4 $\mu\text{g}/\text{ml}$ for 7–10 h at 37°C with 1 U of the enzyme/ μg DNA, in a buffer consisting of 60 mM NaCl, 7 mM MgCl_2 , 6 mM 2-mercaptoethanol, and 10 mM Tris, pH 7.4. DNA was digested with the enzymes *Eco*RI and *Bam*HI (Bethesda Research Laboratories) under the same conditions, except that for *Eco*RI the buffer consisted of 50 mM NaCl, 5 mM MgCl_2 , 6 mM 2-mercaptoethanol, and 100 mM Tris, pH 7.5, and for *Bam*HI it consisted of 150 mM NaCl, 6 mM MgCl_2 , 6 mM 2-mercaptoethanol, and 6 mM Tris, pH 7.8. Digested DNA was fractionated by agarose gel electrophoresis and transferred to nitrocellulose filters as described previously (12). Cloned cDNA probe molecules corresponding to an entire λ_1 L chain (from the murine myeloma MOPC104E) and to an entire λ_{II} L chain (from the murine myeloma MOPC315), each inserted into the plasmid pBR322 at its *Pst*I restriction site (A. Bothwell, M. Paskind, R. C. Schwartz, G. Sonenshein, M. L. Gefter, and D. Baltimore, manuscript in preparation) were labeled with [^{32}P]deoxycytidine triphosphate to a $0.5\text{--}3.0 \times 10^8$ dpm/ μg sp act by nick translation in vitro (13). To carry out the hybridization reaction, the nitrocellulose filters were incubated for 3 h at 65°C with a "prehybridization" solution, and then for 8 h at 65°C with a hybridization solution containing the radiolabeled probe molecules at a concentration of 6 ng/ml, as described by Wahl et al. (14). Nonspecifically bound material was removed from the filters by washing them three times for 5 min at room temperature in 0.3 M NaCl, 0.03 M sodium citrate, and 0.1% sodium dodecyl sulfate, and three times for 30 min at 65°C in 0.015 M NaCl, 0.0015 M sodium citrate, and 0.1% sodium dodecyl sulfate. The filters were then subjected to autoradiography at -70°C using Kodak XR-5 film (Eastman Kodak Co., Rochester, N. Y.) and DuPont "Lightning" intensifying screens (Dupont Instruments, Wilmington, Del.).

Results

DNA extracted from mouse cells and from hamster cells was analyzed to identify species-specific DNA fragments containing λ L chain genes. In the case of the mouse,

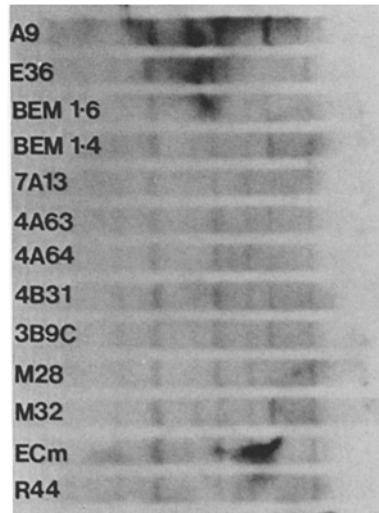


FIG. 1. λ L chain-specific DNA fragments. 30- μ g samples of mouse (A9), hamster (E36), and hybrid (BEM 1-6, BEM 1-4, MACH 7A13-3B3, MACH 4A63, MACH 4A64 A-1, MACH 4B31Az3, MACH 3B9C4-1, MAE 28A, MAE 32, ECm4e, and R44-1) DNA were digested with *Hind*III restriction endonuclease, fractionated by electrophoresis on a 1% agarose gel, and transferred to a nitrocellulose filter (12). Fragments containing λ L chain structural gene sequences were detected by hybridizing the DNA on the filters with radiolabeled probe molecules corresponding to an entire λ L chain. The filters were then washed and subjected to autoradiography.

total genomic DNA purified from the cell line A9 was digested with the restriction endonuclease *Hind*III, the resultant DNA fragments were fractionated according to size by agarose gel electrophoresis, and the fragments were transferred to nitrocellulose filters. The filters were hybridized with radiolabeled probe molecules corresponding to an entire λ L chain (Fig. 1), and fragments containing λ -specific DNA sequences were visualized by autoradiography. Four such fragments were seen, of sizes 11.6, 6.8, 5.2, and 3.9 kilobases (kb; one kilobase equals 1,000 nucleotide base pairs). The three largest of these fragments have been shown previously to contain the $J_{\lambda I} + C_{\lambda I}$, $V_{\lambda II}$, and $V_{\lambda I}$ genes, respectively (5). Genomic DNA purified from the hamster cell line E36 and analyzed in the same way yielded four fragments, of 21.5, 4.8, 3.8, and 2.9 kb, which were distinguished from the mouse fragments on the basis of size. To assess the sensitivity of this assay for the detection of the mouse-specific DNA fragments, mixtures of mouse and hamster DNA were digested, fractionated, and assayed in the same way. The mouse-specific fragments could be detected in mixtures of one part of mouse DNA with seven parts, by weight, of hamster DNA (data not shown).

To determine the distribution of the mouse-specific λ L chain DNA fragments in the mouse genome, 11 mouse \times hamster somatic cell hybrids were tested for the presence of these fragments (Fig. 1). Six of the hybrids were positive for all four mouse λ L chain-specific DNA fragments. The other five hybrids yielded no detectable mouse λ fragments at all. This result indicated that at least the $V_{\lambda I}$, $V_{\lambda II}$, and $C_{\lambda I}$ genes were grouped together on a single mouse chromosome.

To confirm that the mouse DNA fragments detected by our probes were authentic mouse λ L chain genes, DNA from two positive hybrids, MACH 4B31AZ3 and MAE 32, together with A9 and E36 DNA, was digested with each of the enzymes *Hind*III,

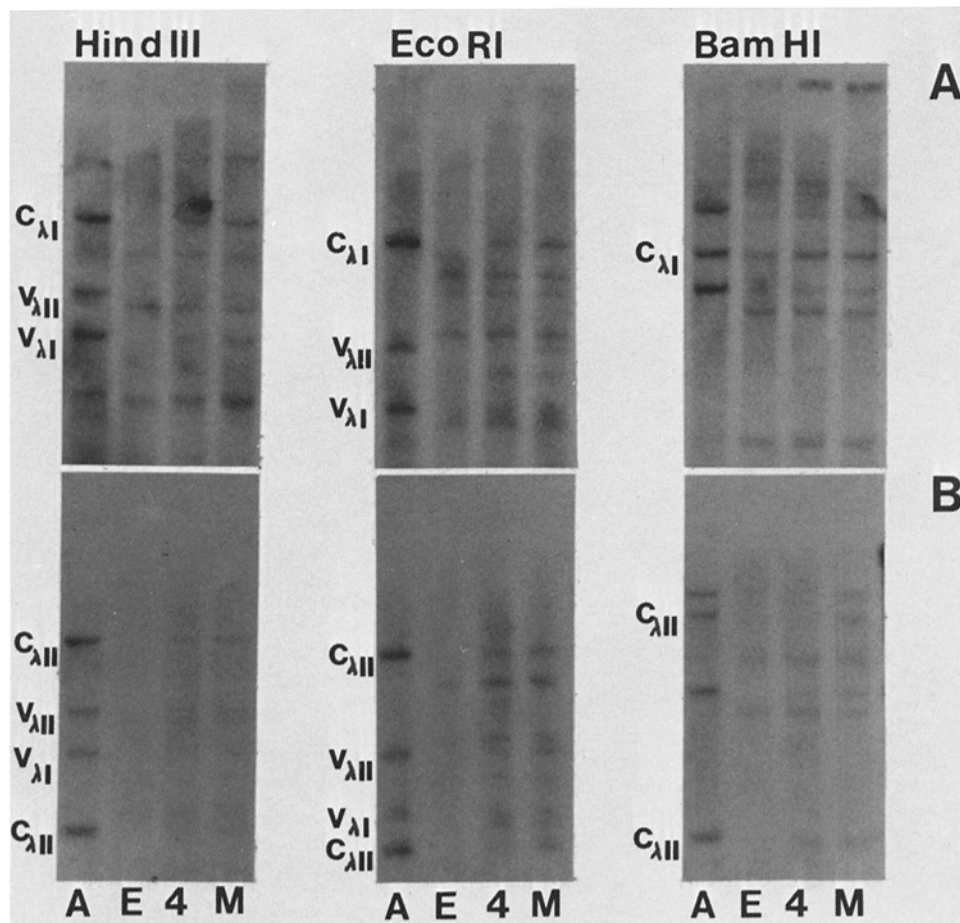


FIG. 2. Comparison of mouse and hybrid cell λ L chain-specific DNA fragments. DNA samples from mouse (A9), hamster (E36), and hybrid (MACH 4B31Az3; MAE 32) cell lines were digested with each of the enzymes *Hind*III, *Eco*RI, and *Bam*HI, fractionated as described in the legend to Fig. 1, and scored for the presence of fragments reactive with a λ _I-specific probe (A) or a λ _{II}-specific probe (B). Comparison of the resulting fragment patterns with those obtained in restriction mapping studies of cloned mouse genomic DNA sequences containing λ L chain genes (5; and A. Bothwell, P. D'Eustachio, F. H. Ruddle, and D. Baltimore, unpublished data) allowed identification of the specific genes contained in some of the DNA fragments, as noted.

*Eco*RI, and *Bam*HI. The digests were fractionated and transferred to nitrocellulose filters as before, and parallel filters were hybridized with λ _I and λ _{II} probe molecules (Fig. 2A and B). In all, 11 fragments reactive with the λ _I probe and 12 reactive with the λ _{II} probe were found. Seven DNA fragments containing $V_{\lambda I}$, $V_{\lambda II}$, and $C_{\lambda I}$ genes were identified on the basis of previous studies (5). In addition, restriction mapping experiments using a probe specific for the $C_{\lambda II}$ gene (data not shown) allowed the identification of six DNA fragments containing portions of this gene, as noted in Fig. 2. In all cases, the fragments present in mouse (A9) DNA were present without detectable rearrangement in the DNA from the two somatic cell hybrids. No rearranged fragments corresponding to those expected in cells committed to produce λ chains were found (5).

TABLE I
Hybrid Cell Lines Tested for Mouse λ L Chain Genes*

Mouse chromosome number	BEM 1-6	BEM 1-4	MACH 4A64 A1	MACH 3B9C4-1	MACH 7A13-3B3	MACH 4A63	ECm4c	MAE 28A	R44-1	MAE 32	MACH 4B31Az3
1	0.61	0.35	0.34	0.39	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	1.03	0.83	0.41	0.94	1.50	0.86	0.00	0.00	0.00	0.00	0.65
3	0.94	0.70	0.01	0.09	0.01	0.00	0.00	0.00	0.00	0.00	0.00
4	0.97	0.13	0.02	0.14	0.00	0.69‡	0.00	0.00	0.00	0.00	0.00
5	0.00	0.22	0.00	0.35	0.54	0.00	0.00	0.00	0.00	0.00	0.00
6	1.97‡	1.09	0.06	0.85	0.04	0.00	0.00	0.00	0.00	0.00	0.03
7	0.13	0.00	0.25	0.29	0.20	0.69	0.00	0.00	0.00	0.00	0.84
8	0.87	0.35	0.00	0.30	0.00	0.00	0.00	0.00	0.00	0.00	0.81
9	0.23	0.00	0.01	0.76	1.35	0.00	0.00	0.00	0.00	0.00	0.00
10	0.27	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
12	0.74	0.83	0.23‡	0.88‡	1.77	0.11	0.00	1.03‡	0.00	0.00	0.16
13	0.77	0.52	0.00	0.21	0.26	0.44	0.00	0.00	0.00	0.00	0.00
14	1.03	0.78	0.02	0.70	0.27	0.11	1.00‡	0.00	0.00	0.00	0.10
15	1.74	1.00	1.05	1.30	1.30	0.92	1.00‡	0.00	0.00	0.00	0.16
16	0.87	0.65	0.06	1.15	0.17	0.97	0.00	0.00	0.00	0.86	0.77
17	0.87	1.09	0.20	0.91	0.70	0.25	0.00	0.00	1.00‡	0.00	0.26
18	0.55	0.00	0.03	1.03	1.09	0.33	0.00	0.00	0.00	0.00	0.06
19	1.26	0.96	0.27	1.15	1.07	1.86	0.00	0.00	0.00	0.00	0.90
X	1.85‡	0.48	0.02	0.00	0.00	0.00	0.00	1.03‡	0.00	0.86	0.00
Number of cells karyotyped	31	23	101	33	33	36	9	31	18	100	31
Reaction with λ probe	+	+	-	+	-	+	-	-	-	+	+

* Mouse chromosomes were identified in metaphase spreads subjected to the sequential Giemsa-Viokase-Hoechst 33258 staining technique. The number shown is the mean number of copies of the chromosome per cell.

‡ Includes copies of the chromosome occurring in the form of translocations.

Karyotypic and isoenzyme analyses of the hybrid cell lines were carried out to assign this group of λ L chain genes to a particular mouse chromosome (Table I). Each of the hybrid cell populations retained a complete set of chromosomes from the hamster parental cell line E36, but only a subset of mouse chromosomes. Different cell populations retained different combinations of mouse chromosomes and to different extents. The combinations of mouse chromosomes present in this panel should allow unambiguous gene assignments to be made to mouse chromosomes 1, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, and X, and to the chromosome pair 2 + 19. In the present case, the only mouse chromosome present in all lines containing mouse λ L chain genes and absent from all lines lacking these genes was chromosome 16.

Discussion

To determine the chromosomal locations of λ L chain genes in the mouse, DNA restriction mapping techniques were used to identify and characterize DNA fragments containing these genes in mouse cells, hamster cells, and mouse \times hamster somatic cell hybrids. Specifically, the techniques allowed mouse DNA fragments corresponding to V_{HL} , V_{HII} , C_{HL} , and C_{HII} genes to be distinguished from each other and from the corresponding hamster fragments. 11 mouse \times hamster somatic cell hybrids were tested for the presence of these DNA sequences (Figs. 1 and 2). Six possessed the full set of mouse λ DNA fragments and five showed no mouse λ DNA fragments at all. This result indicated that at least these four λ genes are all on the same chromosome. Further, mouse chromosome 16 was the only one present in all of the positive hybrids

and absent from all of the negative ones, allowing the genes to be assigned to this chromosome (Table I). A third class of λ L chain polypeptides, λ_{III} , has recently been identified (T. Azuma, L. A. Steiner, and H. Eisen, personal communication). Preliminary experiments suggest that $C_{\lambda_{III}}$ DNA sequences may be closely linked to the C_{λ_I} and $C_{\lambda_{II}}$ genes we have already identified. Additional restriction mapping experiments will be necessary to confirm this finding and to order the various λ V and C genes with respect to one another.

The assignment of λ L chain genes to mouse chromosome 16, together with the previous assignments of H chain genes to chromosome 12 (7, 15), and κ L chain genes to chromosome 6 (6, 16), completes the mapping of Ig structural genes in the mouse at the chromosomal level. It represents the first systematic mapping of these genes in any species, and is consistent with earlier Mendelian (2) and somatic cell genetic (17) analyses that indicated that the λ , κ , and H chain gene clusters were autosomal and unlinked to one another.

The detailed arrangement of the λ L chain structural genes in our panel of somatic cell hybrids has an encouraging general implication for the use of these hybrids in gene mapping studies at the DNA level. Within the limits of resolution of our analyses, the λ DNA fragments in the somatic cell hybrids were the same as the ones in the mouse (Fig. 2). Neither the B cell-rearranged state nor any novel deletion or alteration was detectable in any of the hybrids we examined. Our previous analyses of mouse κ L chains (6) and H chains (7; and P. D'Eustachio and F. H. Ruddle, unpublished observations) have shown these sequences likewise to be faithfully preserved in their germline states in somatic cell hybrids. Clearly, the total amount of DNA sequence we have characterized in this way is a very small fraction of the murine genome. Nevertheless, the lack of any detectable change is consistent with the idea that introduced DNA sequences are preserved unaltered in conventional somatic cell hybrids, even in the absence of any identifiable selective pressure for the conservation of the genes in question.

In the course of this series of experiments, we have developed a panel of somatic cell hybrids that allows an 18-fold division of the mouse genome for gene mapping by the DNA hybridization techniques described here (Table I). The amount of DNA required for one determination, 30 μ g, is small, so that extensive comparative studies are possible with material obtained from a somatic cell hybrid population of moderate size. Given the appropriate probes, and assuming that the preservation of native gene order is a general phenomenon, a large number of murine genes can now be mapped unambiguously. In the long run, this panel may be of greatest use in the analysis of multigene families such as globins, immunoglobulins, actins, interferons, and ribosomal proteins, and in the analysis of genes of common evolutionary origin such as endogenous *src* gene homologues or α -fetoprotein and albumin. It should allow the dispersion of the members of such a family throughout the genome to be readily and accurately assessed. Further, because the assay is independent of gene expression, it can be applied not only to active structural genes, but also to pseudogenes and to the nontranscribed DNA sequences of regulatory function that flank structural genes, and should thus allow the evolution and distribution of these families of sequences to be systematically determined.

Summary

To determine the chromosomal localization of murine λ light (L) chain structural genes, DNA from a panel of 11 mouse \times hamster somatic cell hybrids was scored for

the presence of sequences homologous to cloned λ DNA probe molecules. Six of the hybrids had detectable λ_I and λ_{II} gene sequences. In all six, the full complement of murine sequences was present, and in its germline configuration. The remaining hybrids lacked any detectable murine λ L chain gene sequences. The only mouse chromosome present in all of the positive hybrids and absent from the negative ones was number 16, allowing the assignment of λ L chain structural genes to this chromosome. Together with the previous assignments of the κ L chain genes to chromosome 6 and heavy chain genes to chromosome 12, this finding completes the mapping of Ig structural genes in the mouse at the chromosomal level.

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References

1. Gally, J. A. 1973. Structure of immunoglobulins. *In* The Antigens. M. Sela, editor. Academic Press, Inc., New York. 161.
2. Mage, R., R. Lieberman, M. Potter, and W. D. Terry. 1973. Immunoglobulin allotypes. *In* The Antigens. M. Sela, editor. Academic Press, Inc., New York. 299.
3. Cesari, I. M., and M. Weigert. 1973. Mouse lambda-chain sequences. *Proc. Natl. Acad. Sci. U. S. A.* **70**:2112.
4. Dugan, E. S., R. A. Bradshaw, E. S. Simms, and H. N. Eisen. 1973. Amino acid sequence of the light chain of a mouse myeloma (MOPC-315). *Biochemistry.* **12**:5400.
5. Sakano, H., K. Hüppi, G. Heinrich, and S. Tonegawa. 1979. Sequences at the somatic recombination sites of immunoglobulin light-chain genes. *Nature (Lond.).* **280**:288.
6. Swan, D., P. D'Eustachio, L. Leinwand, J. Seidman, D. Keithley, and F. H. Ruddle. 1979. Chromosomal assignment of the mouse κ light chain genes. *Proc. Natl. Acad. Sci. U. S. A.* **76**:2735.
7. D'Eustachio, P., D. Pravtcheva, K. Marcu, and F. H. Ruddle. 1980. Chromosomal location of the structural gene cluster encoding murine immunoglobulin heavy chains. *J. Exp. Med.* **151**:1545.
8. Old, L. J., E. A. Boyse, D. A. Clark, and E. Carswell. 1962. Antigenic properties of chemically induced tumors. *Ann. N. Y. Acad. Sci.* **101**:80.
9. Fournier, R. E. K., and F. H. Ruddle. 1977. Stable association of the human transgenome and host murine chromosomes demonstrated with trispecific microcell hybrids. *Proc. Natl. Acad. Sci. U. S. A.* **74**:3937.
10. Smiley, J. R., D. A. Steege, D. K. Juricek, W. P. Summers, and F. H. Ruddle. 1978. A herpes simplex virus I integration site in the mouse genome defined by somatic cell genetic analysis. *Cell.* **15**:455.
11. Ruddle, N. H., B. S. Conta, L. Leinwand, C. Kozak, F. H. Ruddle, P. Besmer, and D. Baltimore. 1978. Assignment of the receptor for ecotropic murine leukemia virus to mouse chromosome 5. *J. Exp. Med.* **148**:451.
12. Scangos, G. A., K. M. Huttner, S. Silverstein, and F. H. Ruddle. 1979. Molecular analysis of chromosome-mediated gene transfer. *Proc. Natl. Acad. Sci. U. S. A.* **76**:3987.
13. Maniatis, T., A. Jeffrey, and D. G. Kleid. 1975. Nucleotide sequence of the rightward operator of phage λ . *Proc. Natl. Acad. Sci. U. S. A.* **72**:1184.
14. Wahl, G. M., S. Stern, and G. R. Stark. 1979. Efficient transfer of large DNA fragments from agarose gels to diazobenzyloxymethyl-paper and rapid hybridization by using dextran sulfate. *Proc. Natl. Acad. Sci. U. S. A.* **76**:3683.

15. Meo, T., J. Johnson, C. V. Beechey, S. J. Andrews, J. Peters, and A. G. Searle. 1980. Linkage analyses of murine immunoglobulin heavy chain and serum prealbumin genes establish their location on chromosome 12 proximal to the **T(5;12)3/H** breakpoint in band 12F1. *Proc. Natl. Acad. Sci. U. S. A.* **77**:550.
16. Gottlieb, P. D. 1974. Genetic correlation of a mouse light-chain variable region marker with a thymocyte surface antigen. *J. Exp. Med.* **140**:1432.
17. Valbuena, O., K. Marcu, C. M. Croce, K. Huebner, M. Weigert, and R. P. Perry. 1978. Chromosomal locations of mouse immunoglobulin genes. *Proc. Natl. Acad. Sci. U. S. A.* **75**: 2883.