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Chromosomal assignment of the mouse κ light chain genes

(somatic cell hybrids/DNA restriction/DNA·DNA hybridization)

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ABSTRACT **Mouse–hamster somatic cell hybrids containing a variable number of mouse chromosomes have been used in experiments to determine which mouse chromosome carries the immunoglobulin κ light chain genes. It has been shown by nucleic acid hybridization that the κ constant region gene and the genes for at least one variable region subgroup are on mouse chromosome 6. This somatic cell genetic mapping procedure appears to be general and can be applied to any expressed or silent gene for which an appropriate nucleic acid probe exists.**

The immunoglobulin molecule is a multichain structure composed of two identical heavy polypeptide chains and two identical light polypeptide chains held together by noncovalent forces and disulfide bonds. Light chains in turn can be divided into two classes, κ and λ, on the basis of homologies among their amino acid sequences. Heavy and light polypeptide chains are both composed of amino-terminal variable regions (V regions) whose sequences differ from one immunoglobulin molecule to the next, and carboxy-terminal constant regions (C regions) whose sequences are largely conserved (1, 2). Certain variations in the amino acid sequences of heavy and light chains are inherited as autosomal Mendelian codominant markers. The patterns of inheritance of these markers in humans, mice, and rabbits suggest that the genes coding for immunoglobulin chains fall into several unlinked clusters. One cluster directs the production of heavy chains, a second the production of κ light chains, and a third the production of λ light chains (3–5).

This number of clusters is a minimum estimate, however. At least in the case of murine light chains, the V region structural genes are located at some distance from the C region structural genes in the germ-line DNA. Indeed, there is no evidence that the two groups of genes need be on the same chromosome (6–8). In no case has the chromosomal location of any of these gene clusters been determined unequivocally. Analysis of the genetic control of immunoglobulin production *in vivo* and *in vitro* has demonstrated that the ability to produce κ chain V regions is controlled by a genetic locus on chromosome 6 in the mouse (9–11). Whether this marker corresponds to κ chain structural genes or to regulatory ones, however, cannot be determined from these experiments.

In order to determine the number and chromosomal locations of the immunoglobulin structural genes unambiguously, we have applied the techniques of nucleic acid hybridization to the analysis of somatic cell hybrids. Because these techniques allow direct analysis of the cell's genome, they are not affected by the cell's phenotype and can therefore be used to map existing panels of somatic cell hybrids (12, 13). In addition, the analytic procedure allows V region genes to be resolved from C region genes (7), facilitating the unambiguous mapping of each. Here, we report our results for the κ light chain of the mouse.

MATERIALS AND METHODS

Somatic cell hybrids were formed between the Chinese hamster cell line E36 and either the mouse cell line CT11C (hybrid line ECm4e) (14) or primary macrophages from A/HeJ mice (MACH hybrid series) or primary fibroblasts from BALB/c mice (hybrid line BEM 1-6) (15–17). Cloned sublines of these hybrids were grown in monolayer culture (in Dulbecco's modified Eagle's minimal medium supplemented with 10% heat-inactivated fetal calf serum) to yield 2- to 10-g quantities (wet weight) of each cell line. Washed cell pellets were stored at −96°C. To determine which mouse chromosomes were present, samples of each mass culture were scored for the presence of the murine form of each of 20 marker isozymes (16) and were subjected to karyotypic analysis by the combined trypsin/Giemsa banding and Hoechst staining techniques (18).

DNA was extracted from the frozen cell pellets with phenol/*m*-cresol (19), cleaved by *Eco*RI restriction endonuclease, and fractionated by RPC-5 chromatography (19). Fractions from the column were separated in a second dimension by electrophoresis in 1% agarose gels (20), and the DNA fragments were transferred to cellulose nitrate filters (21). Filters were air-dried and incubated overnight at 80°C. Just prior to hybridization they were soaked in 0.08% Ficoll/0.08% polyvinylpyrrolidone/0.08% bovine serum albumin/0.045 M sodium citrate/0.45 M sodium chloride, incubated at 70°C for 3 hr, and then hybridized against the appropriate ^{32}P-labeled nick-translated probe. The hybridization solution contained nick-translated [^{32}P]DNA (40–200 cpm/pg) at 10 ng/ml, 0.1% sodium dodecyl sulfate ($NaDodSO_4$), 0.09 M sodium citrate, 0.1 M sodium chloride, 0.08% Ficoll, 0.08% polyvinylpyrrolidone, 0.08% bovine serum albumin, and salmon sperm DNA at 100 pg/ml. After hybridization at 70°C for 12 hr, filters were removed and washed three times at 52°C for 30 min in 0.05% $NaDodSO_4$/1.5 mM sodium citrate/15 mM sodium chloride and then three times for 1 hr each in the same buffer without $NaDodSO_4$. Filters were air-dried, and radioactive ink spots were applied corresponding to the sample wells on the gel. The filters were then exposed at −60°C to Kodak X-RP film in cassettes equipped with Kodak regular intensifier screens.

[α-^{32}P]dCTP-Labeled DNA was prepared by nick-translation (22) of cloned mouse myeloma sequences. The two cloned sequences used in this study were: (*i*) K41C, a 500-base-pair DNA fragment derived from MOPC-41 light chain mRNA sequences that encodes amino acids 125–214 and the 3′ untranslated portion of the mRNA (7); and (*ii*) K41(V + C), a 600-base-pair fragment that contained DNA representing residues 48–214

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Abbreviations: V regions, variable regions; C regions, constant regions; $NaDodSO_4$, sodium dodecyl sulfate; kb, kilobase(s).

of the κ light chain expressed in MOPC-41 myeloma cells (7).

RESULTS

The DNA probes used in these studies were derived from murine mRNA and have been shown by restriction mapping and partial sequence analysis to correspond to portions of the V and C regions of mouse κ light chains (8, 23). Nevertheless, these probes reacted strongly with sequences in hamster genomic DNA. To resolve the reactive sequences in mouse from those in hamster, we used a two-dimensional mapping technique (7, 19, 20).

DNA from the hamster cell line E36 yielded a fragment of 4.7 kilobases (kb) when allowed to react with the C region probe (Fig. 1*A*, lanes 7–9). We assume that this fragment contains hamster κ chain C region sequences.

DNA extracted from A/HeJ mouse liver and digested with *Eco*RI yielded one prominent fragment of approximately 16 kb when it was fractionated in this way and allowed to react with the probe K41C (Fig. 1*B*, lanes 17–19). This fragment is assumed to contain the mouse κ C region gene. This interpretation is supported by additional independent evidence reported previously (7, 8, 23, 24). When the same DNA was allowed to react with the probe K41(V + C), additional fragments corresponding to the V region genes of the MOPC-41 subgroup were observed. Fragments indistinguishable from these were observed when DNA from BALB/c mouse liver or the mouse cell line CT11C was analyzed. On the basis of this reactivity pattern, the 16-kb fragment is assumed to contain the mouse κ chain C region gene.

To determine the chromosomal location of the κ chain structural genes in the mouse, we used this mapping procedure to analyze DNAs from somatic cell hybrids containing various combinations of mouse chromosomes and a complete hamster genome.

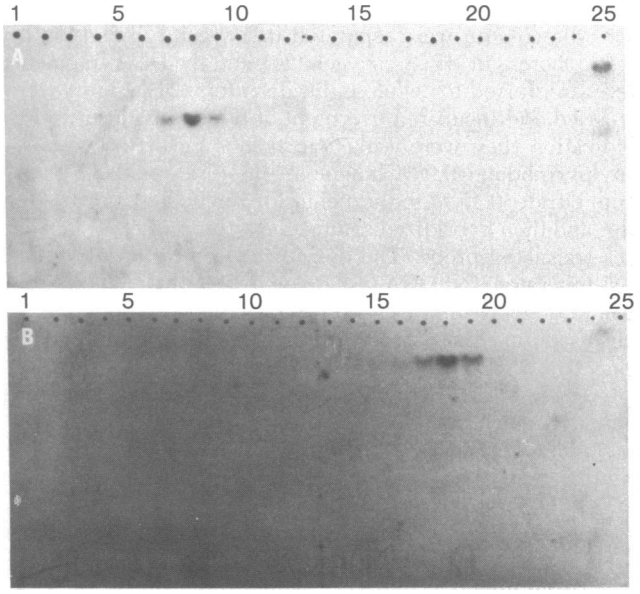

FIG. 1. Hybridization of *Eco*RI fragments of hamster cell line E36 DNA (*A*) or A/HeJ mouse spleen DNA (*B*) to mouse κ C region probe. DNA from E36 cells and A/HeJ spleen was fractionated by RPC-5 and then in a second dimension by agarose gel electrophoresis and stained with ethidium bromide. The fragments were transferred to nitrocellulose filters (Schleicher & Schuell) and hybridized to ^{32}P-labeled K41C C region probe (specific activity, 183 cpm/pg). The radioactive ink spots along the top of each filter correspond to the sample wells on the agarose gels. Lanes 1–24 (left to right) contained fractions 1–24 from the RPC-5 separations; lane 25 contained an *Eco*RI digest of phage λ DNA, included as a molecular weight marker.

Fig. 2 shows the pattern of ethidium bromide-stained DNA from hybrid cell line MACH 7A13 and an autoradiogram after filter hybridization of this DNA to a DNA probe containing MOPC-41 V and C region sequences [K41(V + C)]. The 4.7-kb hamster fragment in lanes 6–8 and the 16-kb mouse C region fragment in lanes 13–16 can be identified, as can at least six mouse V region fragments.

Subclones of MACH 7A13 (Table 1; Fig. 3) were derived and tested for the presence of the mouse 16-kb C region fragment. Subclones MACH 7A13-4A, MACH 7A13-5, and MACH 7A13-3b all contained the mouse C region fragment; MACH 7A13-5-4 did not. All clones, as expected, showed hybridization to the hamster 4.7-kb fragment, which was a convenient internal control for the reaction.

The only mouse chromosome present in all four positive lines and absent from the negative line MACH 7A13-5-4 was mouse chromosome 6. This was shown by karyotypic analyses and tests for the presence of the mouse form of the polymorphic enzyme triose phosphate isomerase, which is encoded on chromosome 6 in the mouse (25) (Table 1).

Further data from similar hybridization experiments with DNA from seven other independently derived clones showed correlation between the presence of the 16-kb C region fragment and only this one chromosome (Table 1).

To determine whether mouse κ chain V region genes were on the same chromosome as κ chain C region genes, DNA from 10 of the hybrid lines was analyzed by using K41(V + C) as a probe. Of the 10 lines tested, 6 gave positive reactions and 4 gave negative ones (Table 1). All six positive hybrids showed both murine V region and C region specific bands, and the negative hybrids lacked both sets of bands (compare Figs. 2 and 4).

DISCUSSION

We have described the use of cloned DNA probes in conjunction with a recently developed scheme for the fractionation of DNA fragments to map structural genes encoding mouse κ light chains to mouse chromosome 6. Specifically, analysis of DNAs extracted from each of 12 mouse–hamster somatic cell hybrids showed that mouse DNA sequences corresponding to V region

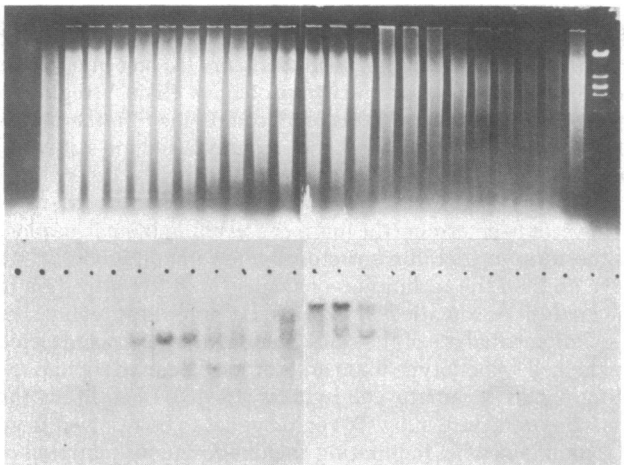

FIG. 2. Separation of *Eco*RI fragments of hamster–mouse hybrid cell line 7A13 DNA by agarose gel electrophoresis. (*Upper*) Stained with ethidium bromide. (*Lower*) Fragments were transferred to a nitrocellulose filter and hybridized to ^{32}P-labeled K41(V + C) probe. Last lane at right contained an *Eco*RI digest of phage λ DNA.

Table 1. Hybrid cell lines tested for mouse κ chain genes

Cell line	Reaction with probe* V + C	Reaction with probe* C only	No. of cells karyotyped	Mean number of copies per cell, by mouse chromosome number† 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	X
MACH 7A13	+	+	51	0.00	0.96	0.00	0.25	0.04	0.59	0.77	0.00	0.01	0.03	0.00	0.18	0.78	0.01	0.80	0.10	0.78	0.60	0.63	0.00
MACH 7A13-4A	ND	+	33	0.00	0.73	0.00	0.27	0.08	0.55	0.42	0.00	0.00	0.02	0.00	0.24	0.64	0.00	0.47	0.05	0.06	0.00	0.39	0.00
MACH 7A13-5	+	+	22	0.02	0.82	0.16	0.27	0.09	0.79‡	0.75	0.18	0.32	0.00	0.00	0.48	0.18	0.00	0.18	0.25	0.66	0.54‡	0.55	0.02
MACH 7A13-3b	+	+	32	0.00	0.83	0.20	0.13	0.08	0.14	0.64	0.16	0.50	0.06	0.00	1.08	0.42	0.16	0.23	0.08	0.77	0.45	0.64	0.03
MACH 7A13-5-4	−	−	12	0.00	1.42	0.00	0.21	0.33	0.00	0.58	0.00	1.04	0.00	0.00	1.79	0.50	0.00	0.71	0.00	0.21	1.00	0.42	0.00
MACH 4A6	+	ND	23	0.57	0.78	0.00	0.35	0.04	0.39	0.70	0.00	0.30	0.04	0.00	0.00	0.13	0.35	0.87	0.83	0.22	0.15	1.00	0.39
MACH 4A6-3	−	−	28	0.09	0.87	0.05	0.46	0.04	0.09	0.70	0.11	0.39	0.14	0.00	0.37	0.36	0.07	0.43	0.32	0.39	0.93	1.00	0.05
MACH 4A6-4	+	+	42	0.48	0.79	0.00	0.00	0.00	0.79	0.79	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.54	0.86	0.00	0.01	1.07	0.07
MACH 13-2-Az	−	−	23	0.00	0.65	0.00	0.07	0.00	0.00	0.83	0.00	0.02	0.00	0.00	0.00	0.02	0.00	0.74	0.00	0.09	0.78‡	0.78‡	0.00
MACH 4B31Az3	ND	−	16	0.00	0.12	0.00	0.00	0.00	0.00	0.85‡	0.06	0.00	0.00	0.00	0.00	0.03	0.06	0.12	0.12	1.65‡	0.59‡	0.50	0.00
ECm4e	−	−	9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00‡	1.00‡	0.00	0.00	0.00	0.00	0.00
BEM 1-6	+	+	31	0.61	1.03	0.94	0.97	0.00	1.97‡	0.13	0.87	0.23	0.27	0.00	0.74	0.77	1.03	1.74	0.87	0.87	0.55	1.26	1.85‡

* ND, not determined.

† Mouse chromosomes were identified in metaphase spreads prepared from hybrid cells and subjected to the combined trypsin-Giemsa and Hoechst staining techniques (18). The presence or absence of mouse chromosomes 1, 4, 5, 6, 7, 8, 9, 10, 11, 14, 17, and 18 was confirmed by testing cytoplasmic extracts of the hybrid cells for mouse isozyme variants mapped to these chromosomes (16). A typical cell had 0 or 1 copy of any given chromosome, although infrequently as many as 3 copies per cell were found.

‡ Includes copies of the chromosome occurring in the form of translocations. In all of these hybrids the translocated chromosomes were morphologically intact and all expected isozyme markers were found in the cell. We have therefore weighted the contribution of these translocation chromosomes equally with that of intact chromosomes in calculating the chromosome frequencies shown here.

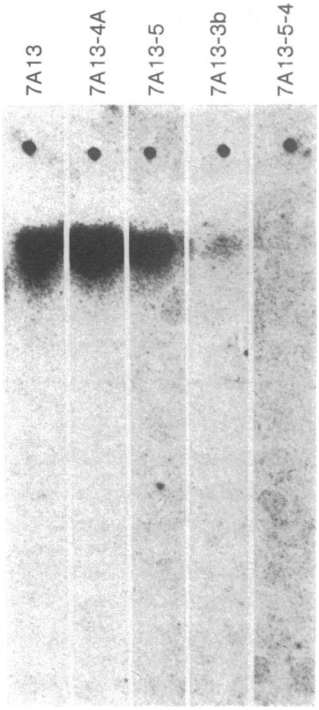

FIG. 3. Detection of κ chain C region gene in MACH 7A13 and clones derived from it. DNA from hybrid cell lines 7A13, 7A13-4a, 7A13-5, 7A13-3b, and 7A13-5-4 was digested with restriction endonuclease and separated as indicated in the legend to Fig. 1. Filters were hybridized to a ^{32}P-labeled κ chain C region segment, K41C.

sequences of subgroup K41 and to κ chain C region sequence were detectable if and only if the hybrid line had retained mouse chromosome 6. Other V region subgroups were not detected by these probes (7, 8) and their location therefore could not be established by these experiments. We assume, however, that all κ chain V region genes are linked and hence that they are all on chromosome 6 in the mouse. Because the procedure used is applicable not only to immunoglobulin genes but also to structural and regulatory elements in the mammalian genome generally, it is worthwhile to discuss several aspects of the mapping procedure itself in detail.

The first is the ability of the technique to resolve very closely related DNA fragments. Here we have applied the technique to resolve the V and C region sequences of mouse κ chain genes from each other and from their hamster homologues (Figs. 1–4). In preliminary experiments, we have found that human κ chain V and C region sequences also react with these probes and again that this technique allows the human DNA fragments to be distinguished from the murine ones. This high resolution is essential. We found that the liquid hybridization technique provided no reliable way to distinguish mouse–hamster DNA duplexes from mouse–mouse ones when mixtures of hamster and mouse DNA were tested for reactivity with a mouse κ chain DNA probe. This ambiguity may explain the discrepancy between our data and those of Valbuena *et al.* (26) who found no correlation between the presence of mouse κ chain DNA sequences in a somatic cell hybrid and the presence of mouse chromosome 6.

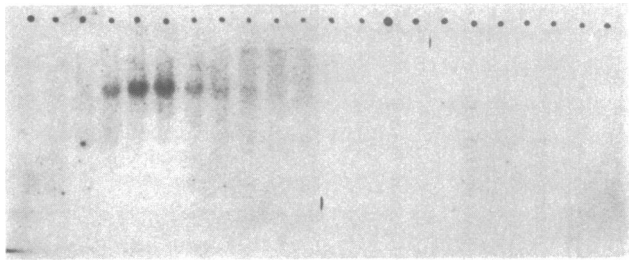

FIG. 4. Fragments of *Eco*RI-digested DNA from hybrid cell line 7A13-5-4 were analyzed by filter hybridization with ^{32}P-labeled V + C region sequences obtained by nick translation of K41(V + C).

Even when the liquid hybridization technique allows homologous DNA sequences from two species to be distinguished reliably, as in the case of human and murine globins (12, 13), the mapping technique can provide considerable additional information. For example, all known human variant globin genes are closely homologous to one another and are probably not distinguishable on the basis of the extent to which they hybridize with a globin DNA probe in solution. Nevertheless, Orkin *et al.* (27) have shown that a thalassemia variant globin gene gives a pattern of restriction endonuclease fragments that is readily distinguished from that of the normal gene. Wilson and Wilson (28) have shown further that, on the basis of the known nucleotide sequences of human α- and β- globins, almost half of all known variants can be expected to yield a set of restriction fragments different from the parental one.

A second key technical aspect of the mapping procedure is to obtain a suitable panel of mouse–hamster hybrid cell lines (Table 1). The property of these cell lines that makes them useful for mapping studies, of course, is the gradual loss of mouse chromosomes. However, this same property makes it virtually impossible to obtain genetically homogeneous hybrid cell populations. As shown in Table 1, even in recently cloned cell populations, the ideal case in which all of the cells have a copy or all lack a copy of any given mouse chromosome, is rarely found. In terms of detecting the gene of interest, this phenomenon presents no special problems. In the case of the cell line 7A13-3b, for example, the mouse κ chain C region DNA fragment was detected although only 14% of the cells in the population tested had a copy of chromosome 6. In terms of reproducibility, however, the problem is more severe. Even successive samples of the same cell population need not have the same genetic makeup. Because the amount of cellular DNA required for a single two-dimensional map is small, about 50 μg, we were able to generate enough cellular DNA in a single batch for as many as 200 independent two-dimensional maps. The DNA, before or after chromatographic fractionation, can be stored for at least 2 years without detectable degradation, so that this same set of specimens can be used to extend these mapping studies to other immunoglobulin structural genes and related DNA sequences in a systematic and internally controlled way.

In addition to allowing a standard clone panel to be established and used over a long period of time, this technique allows additional kinds of hybrid cell lines to be analyzed. Recently, it has become possible to generate somatic cell hybrids in which small, well-defined fragments of a chromosome of interest are introduced into a novel genetic background (refs. 14 and 29; L. Klobutcher, R. Church, and F. H. Ruddle, unpublished observations). Analysis of somatic cell hybrids containing such fragments should provide a general method of ordering genes on chromosomes and, potentially, of defining functional interactions among them (30). When the strategy of using DNA-based clone panels and restriction mapping techniques is extended to the analysis of such hybrids, it should be possible to define and analyze functional mammalian gene clusters at the molecular level.

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