

CHROMOSOMAL LOCATION OF THE STRUCTURAL GENE
CLUSTER ENCODING MURINE IMMUNOGLOBULIN
HEAVY CHAINS*

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At least three sets of genes determine the production of the heavy (H) chains of immunoglobulins (Ig). One set encodes the constant (C_H) regions of these chains, the second encodes their variable (V_H) regions, and the third consists of regulatory genes that affect the expression of the two structural gene sets. Genetic analysis of these sets has depended largely on the use of allotypic and idiotypic markers that behave as codominant structural polymorphisms of the Ig H chain. Breeding studies in rabbits and mice, and pedigree studies in human families, all indicate that V_H and C_H genes each form a tight cluster, and that the two clusters are spaced some distance apart on the same chromosome (1, 2).

The existence of regulatory genes has been proposed based on analyses of allotype expression in hyperimmune rabbits and mice. The bulk of the serum antibody produced by these animals expresses the one or two allelic variants expected for any given allotype. A subpopulation of antibodies is consistently detected, however, that expresses additional, latent allelic forms of the marker (3, 4). Recently, latent allotypic markers have also been detected on the surface Ig molecules of peripheral lymphocytes from nonimmunized rabbits (5). These data have been interpreted as showing that all animals carry most or all Ig structural genes and that the nominal allotype loci may correspond in fact to regulatory genes that determine which of the structural ones are to be expressed in an individual animal (3-5). Such an interpretation, however, calls into question the idea that V_H and C_H structural genes have been identified in any species.

Analyses of H chain production in vivo and in somatic cell hybrids in vitro suggest that it can be mapped to chromosome 12 in mice (6-8) and to chromosome 14 in humans (9). Whether this marker corresponds to structural or to regulatory genes, however, cannot be tested in these systems.

In an attempt to avoid these ambiguities, we have recently developed methods which use nucleic-acid-hybridization techniques to detect specific DNA sequences in somatic cell hybrids. Because these techniques allow direct analysis of the cell's genome, they are not affected by the cell's phenotype. We have previously used these procedures to map the V and C structural genes of κ -light chains to chromosome 6 in

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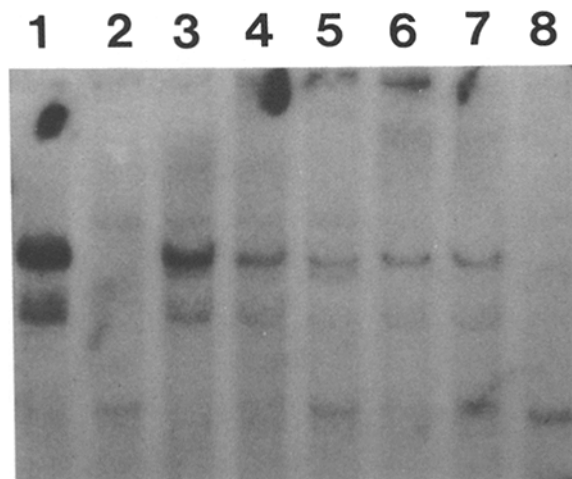


FIG. 1. Cy2b-specific DNA fragments. 30- μ g samples of mouse (A9, track 1), hamster (E36, track 2), and hybrid (BEM 1-6, track 3; MACH 4A64 A-1, track 4; MACH 3B9C4-1, track 5; MACH 7A13-3B3, track 6; MAE 28A, track 7; and ECm4e, track 8) cellular DNA were digested with *Hind*III restriction endonuclease, fractionated by agarose gel electrophoresis, and transferred to a nitrocellulose filter as described in Materials and Methods. Fragments containing Cy2b structural gene sequences were detected by hybridizing the DNA on the filter with a radiolabeled Cy2b probe, after which the filter was washed and subjected to autoradiography. DNA from bacteriophage λ , digested with *Hind*III and run in a parallel track, provided a molecular-weight standard.

the mouse (10). Here, we report our results for the set of genes encoding the C regions of murine H 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), primary fibroblasts from BALB/cJ fetal mice (BEM hybrid series), cells from the murine cell line CT11C (11) (hybrid ECm4e), or cells from a tissue-culture-adapted subline of the methylcholanthrene-induced Meth A murine fibrosarcoma (12) (MAE hybrid series). Hybrid cell lines were grown in monolayer culture to obtain large pools of cells for DNA extraction, analyzed karyotypically, and tested for the presence of mouse isoenzyme markers as described previously (10, 11, 13).

DNA was extracted from cells, digested with restriction endonucleases, fractionated by agarose gel electrophoresis, and transferred to nitrocellulose filters as described by Scangos et al. (14). Cloned cDNA probe molecules corresponding to residues 217 to 449 of the MPC 11 murine myeloma γ 2b heavy chain and to the entire C region of the 3741 murine myeloma μ -heavy chain, each inserted into the plasmid pMB9 ([15]; and K. Marcu, P. Tucker, O. Smithies, and F. Blattner. Unpublished data.) were labeled with [32 P]deoxycytidine triphosphate to a $0.5\text{--}1.5 \times 10^8$ dpm/ μ g sp act by nick-translation in vitro (16). To carry out the hybridization reaction, the nitrocellulose filters were incubated for 8 h with the radiolabeled probe molecules at a concentration of 6–10 ng/ml, as described by Wahl et al. (17). 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 two times for 30 min at 65° in 0.015 M NaCl, 0.0015 M sodium citrate, 0.1% sodium dodecyl sulfate. The filters were then subjected to autoradiography (14).

Results and Discussion

To identify and characterize the genomic DNA fragments that contained Ig H-chain structural genes, DNA purified from mouse and hamster fibroblastoid cell lines was analyzed (Fig. 1). When DNA fragments from the mouse cell line A9, prepared

TABLE I
Hybrid Cell Lines Tested for Mouse H-Chain Genes

Mouse chromosome number*	BEM 1-6	MACH 4A64 A1	MACH 3B9C4-1	MACH 7A13-3B3	MAE 28A	ECm4c
1	0.61	0.34	0.39	0.00	0.00	0.00
2	1.03	0.41	0.94	1.50	0.00	0.00
3	0.94	0.01	0.09	0.01	0.00	0.00
4	0.97	0.02	0.14	0.00	0.00	0.00
5	0.00	0.00	0.35	0.54	0.00	0.00
6	1.97‡	0.06	0.85	0.04	0.00	0.00
7	0.13	0.25	0.29	0.20	0.00	0.00
8	0.87	0.00	0.30	0.00	0.00	0.00
9	0.23	0.01	0.76	1.35	0.00	0.00
10	0.27	0.00	0.00	0.00	0.00	0.00
11	0.00	0.00	0.00	0.00	0.00	0.00
12	0.74	0.23‡	0.88‡	1.77	1.03‡	0.00
13	0.77	0.00	0.21	0.26	0.00	0.00
14	1.03	0.02	0.70	0.27	0.00	1.00‡
15	1.74	1.05	1.30	1.30	0.00	1.00‡
16	0.87	0.06	1.15	0.17	0.00	0.00
17	0.87	0.20	0.91	0.70	0.00	0.00
18	0.55	0.03	1.03	1.09	0.00	0.00
19	1.26	0.27	1.15	1.07	0.00	0.00
X	1.85‡	0.02	0.00	0.00	1.03‡	0.00
Number of cells karyotyped	31	101	33	33	31	9
Reaction with C γ 2b probe	+	+	+	+	+	-

* Mouse chromosomes were identified in metaphase spreads prepared from hybrid cells, and subjected to the combined trypsin-Giemsa- and Hoechst-staining techniques. The number shown is the mean number of copies of the chromosome per cell.

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

by digestion with the restriction endonuclease *Hind*III, were fractionated according to size by agarose gel electrophoresis, three fragments could be detected that hybridized with a probe corresponding to the C region of a γ 2b-H chain. These DNA fragments were 9, 6.0, and 5.7 kilobases in size (1 kilobase = 1,000 nucleotide base pairs). When DNA from the hamster cell line E36 was analyzed in the same way, fragments of 12 and 5.9 kilobases were detected. When mixtures of mouse and hamster DNA were analyzed, the 9-kilobase band, which was diagnostic for the presence of murine gene sequences, could be detected in a sample consisting of 1 part of mouse DNA plus 7-15 parts, by weight, of hamster DNA. Under the conditions used in these and the following experiments, >85% homology is needed for detectable hybridization to occur between the probe DNA molecule and a filter-bound DNA fragment (18). On the basis of the nucleotide and amino acid sequences so far determined for murine C_H region genes and polypeptides (15, 19, 20), this degree of homology to our C γ 2b probe would be expected only for C γ 2b and C γ 2a DNA sequences.

To determine the mouse chromosome from which these C γ 2b-specific fragments were derived, DNA samples isolated from six mouse \times hamster somatic cell hybrid lines were analyzed in the same way (Fig. 1; Table I). Each hybrid line contained a complete set of E36 chromosomes together with a smaller set of mouse chromosomes. Five of the six hybrids showed the mouse band, and all showed the 12-kilobase hamster diagnostic band. The only mouse chromosome present in all of the positive hybrids and absent from the negative one was chromosome 12, suggesting that the γ 2b-C_H region is encoded on this chromosome.

To confirm this assignment and to determine more precisely where on the chro-



FIG. 2. Murine chromosomes in MAE-series somatic cell hybrids. Chromosomes were stained using the trypsin-Giemsa technique (13). In each case, the mouse-derived marker chromosome is shown. The hybrid cells in addition contained a complete set of hamster (E36) chromosomes. For comparison, examples of mouse chromosomes X and 12 are shown.

mosome the gene was located, a group of mouse \times hamster hybrids containing only mouse chromosomes 12 and X on a hamster background was analyzed in more detail. These MAE hybrids (Fig. 2) were formed by fusing cells from the BALB/c methylcholanthrene-induced fibrosarcoma Meth A (12) with E36. Hybrid line MAE 28A contained a single complex mouse chromosome formed by the fusion of an entire mouse chromosome 12 and an entire X chromosome, together with a near-tetraploid set of E36 chromosomes. Hybrid line MAE 4 was similar except that the distal half of the 12 portion of the mouse complex chromosome had been lost. The breakpoint was at band 12C1/C2 in the nomenclature of Nesbitt and Francke (21). When MAE 28A cells were cultured in the presence of 30 $\mu\text{g}/\text{ml}$ of the drug 8-azaguanine (Sigma Chemical Co., St. Louis, Mo.), a subline, MAE 28A 8Ag^R, was derived that contained no detectable mouse chromosomes at all. When DNA samples from these three cell lines were scored for the 9-kilobase $\gamma 2\text{b}$ -specific mouse fragment, it was found only in line MAE 28A (Fig. 3A). This result confirms the assignment of the C $\gamma 2\text{b}$ structural gene to chromosome 12 in the mouse and suggests further that it is located in the distal half of the chromosome.

To test the linkage of the $\gamma 2\text{b}$ -H-chain structural gene to the μ -H-chain structural gene, DNA samples from this same group of MAE hybrids were analyzed using a probe corresponding to the C region of a mouse μ -chain (Fig. 3B). Mouse (A9) DNA digested with *Hind*III restriction endonuclease yielded fragments of 2.5 and 1.4 kilobases that reacted with the μ -probe. As before, these fragments could be detected only in DNA from the MAE 28A hybrid, and not in DNA from the MAE 4 or MAE 28A 8Ag^R lines. This result suggests that the C $\gamma 2\text{b}$ and C μ structural genes are clustered on the distal half of mouse chromosome 12. Preliminary experiments with a probe specific for the α -H chain suggest the same localization for the C α structural gene.

These results are consistent with those of Meo et al. (8), and extend them by unambiguously localizing the Ig C_H structural genes to a particular region of mouse chromosome 12. Because these structural genes can now be clearly distinguished from the putative regulatory ones (3-5), the functional analysis of these two kinds of genes can be extended to a molecular level. By generating and characterizing lymphoid somatic cell hybrids that carry mouse chromosome 12 and well-defined fragments of it, it should be possible to determine precisely the size of the C_H gene cluster, the functional roles of the genes in it, and the ways in which these genes interact to determine the production of Ig molecules in lymphoid cells.

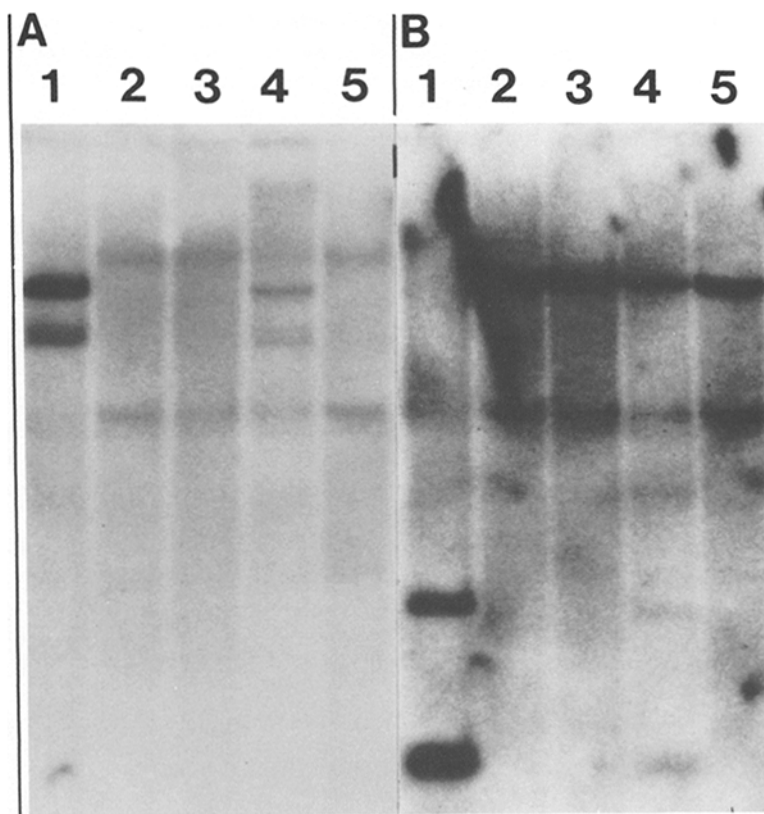


FIG. 3. C_H -specific DNA fragments in MAE-series hybrids. DNA samples from mouse (A9, track 1), hamster (E36, track 2), and hybrid (MAE 4, track 3; MAE 28A, track 4; and MAE 28A 8Ag^R, track 5) were fractionated exactly as described in the legend to Fig. 1 and scored for the presence of $C\gamma 2b$ -specific DNA fragments (A) or $C\mu$ -specific DNA fragments (B).

Summary

To determine the chromosomal location of mouse immunoglobulin heavy chain structural genes unambiguously, a panel of somatic cell hybrids was scored for the presence of DNA sequences homologous to $\gamma 2b$ -, μ -, and α -heavy-chain-constant region DNA probe molecules. The hybrids, formed between mouse and hamster cells, contained various combinations of mouse chromosomes plus a full set of hamster chromosomes. Hybrids that retained mouse chromosome 12 reacted with the probes, whereas hybrids that had lost the chromosome, or its distal half, failed to react. These results indicate that structural genes for the $\gamma 2b$ -, μ -, and α -heavy-chain-constant regions map to the distal half of this chromosome.

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