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Immunology

Cloned pairs of variable region genes for immunoglobulin heavy chains isolated from a clone library of the entire mouse genome

(recombinant phage/*Eco*RI* cloning/multiple V_H genes/spacer DNA)

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ABSTRACT **To investigate the organization of immunoglobulin genes, we have constructed a clone library containing 10^6 randomly generated fragments of mouse embryo DNA, corresponding to eight equivalents of the genome. The cloning method involved methylation of embryo DNA at *Eco*RI recognition sites, partial digestion by *Eco*RI* endonuclease activity, and direct ligation of the resulting large fragments to the λ phage vector Charon 4A. The library was searched for sequences homologous to a cloned complementary DNA copy of a μ heavy chain mRNA. Nine clones bearing variable heavy chain (V_H) sequences were isolated, representing at least eight distinct V_H genes. Thus, multiple related V_H genes are available in the genome to contribute to immunoglobulin diversity. Each of two clones carries a pair of V_H genes, one pair separated by 15 ± 1 kilobase pairs of mouse DNA and the other by 14 ± 2 kilobase pairs. This indicates that related V_H genes are clustered and may occur in a tandem array having a repeating unit of 14–16 kilobase pairs. The large spacer sequences between V_H genes cannot, however, be highly conserved.**

Immunoglobulins are composed of light and heavy (H) chains, each of which contains a variable (V) and a constant (C) region. The longstanding hypothesis (1) that V and C regions are encoded by separate genes in the germline has recently been confirmed by molecular cloning (2–9). The three independent families of immunoglobulin genes include one each for the κ and λ light chains and one for all types of heavy chains, such as μ, α, and γ. Indirect evidence that the mouse genome contains multiple V_κ and V_H genes has come from attempts to place known amino acid sequences (10) into "subgroups" on the basis of sequence homology. Because the sequences in different subgroups differ substantially, each subgroup presumably requires at least one germline V gene (11, 12). Direct evidence for multiple V_κ genes in the mouse genome has recently been obtained by cloning (5–7), but hitherto such evidence has not been available for the V_H genes. Classical genetic approaches suggest that the V_H genes are linked both to each other and to C_H genes (13), but such studies cannot establish the physical distance between genes nor provide any detailed picture of V gene organization. Molecular analysis of V gene linkage might demonstrate whether they are arranged as regular repeating units, as are the members of some other multigene families (12), and might reveal features of their organization relevant to the generation of immunoglobulin diversity.

Hitherto, individual immunoglobulin genes have been cloned in phage λ vectors by insertion of mouse DNA fragments generated by digestion with *Eco*RI (2–9). This approach has the limitation that any particular gene may occur in an *Eco*RI fragment too small to span a region of interest or too large to fit into a phage vector. This limitation can be overcome by cloning a set of large overlapping sequences. Maniatis *et al.* (14) have used this approach to construct "clone libraries" extensive enough to encompass an entire mammalian genome by attaching randomly fragmented DNA to the phage λ vector Charon 4A (15) with synthetic linkers. Because the clones in such a library have overlapping sequences, they are particularly appropriate to studies of gene linkage.

We report here the construction of a library of cloned segments of the mouse genome by an approach that obviates the need for linkers. As a start towards examining the organization of immunoglobulin heavy chain genes, we have searched this library with a cloned cDNA copy of a μ mRNA. At least eight distinct V_H genes are represented in the clones examined so far, demonstrating that multiple related V_H genes are available in the genome to contribute to immunoglobulin diversity. Each of two clones bears a pair of V_H genes, separated by a spacer DNA sequence some 40 times the length of the V_H genes. This indicates that related V_H genes are clustered and may be organized in a tandem array having a large repeating unit.

MATERIALS AND METHODS

Preparation of *Eco*RI* Fragments of Methylated Mouse Embryo DNA. Mouse embryos (13-day) were homogenized in liquid N_2 and digested with proteinase K (250 μg/ml) at 50°C in 5 vol of 0.5% Sarkosyl in 0.5 M EDTA at pH 8.0 (16). High molecular weight DNA [>100 kilobases (kb)] was then extracted (17) and, after centrifugation in CsCl (1.65 g/cm^3) at 35,000 rpm for 48 hr, extensively dialyzed against 10 mM Tris·HCl, pH 7.6/1 mM EDTA. The DNA (600 μg) was methylated to completion (14) at the *Eco*RI recognition sites by *Eco*RI methylase (the kind gift of T. Maniatis, purified according to ref. 18). The methylated DNA was extracted at pH 9.0 sequentially with phenol, $CHCl_3$, and ether and finally dialyzed for 2 days against three changes of Tris·HCl/EDTA.

Methylated DNA at 60 μg/ml in 33 mM Tris·HCl, pH 8.5/2.8 mM $MgCl_2$/0.8 mM EDTA was digested at 37°C with 1000 units of *Eco*RI per ml (Boehringer Mannheim) for the time required to generate a partial digest composed predominantly of 12- to 24-kb fragments (14 and 20 hr for two reactions). The reaction was followed by loading 5-μl samples onto a 0.5% agarose gel containing 1 μg of ethidium bromide per ml in electrophoresis buffer (72 mM Tris/60 mM NaH_2PO_4/2 mM EDTA, pH 7.8; ref. 19) and immediately running the gel at 5 V/cm for 1 hr, after which the DNA was visualized under 254-nm UV light. When sufficient digestion had been achieved, the reaction was terminated by addition of EDTA to 20 mM and DNA was extracted as above. The DNA fragments were fractionated by electrophoresis at 0.5 V/cm for 72 hr on a horizontal slab gel of 0.5% agarose (0.6 × 20 × 20 cm) in buffer

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Abbreviations: V, variable; C, constant; H, heavy; kb, kilobase(s).

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3 times the concentration used by Helling *et al.* (20). Fragments in the size range 14–24 kb were then eluted from the gel by ultracentrifugation (21) and freed from any contaminating material by centrifugation for 16 hr at 40,000 rpm (Beckman SW40 rotor) at 3°C on a 10–40% (wt/vol) sucrose gradient containing 0.2 M NaOAc/10 mM Tris·HCl, pH 7.6/1 mM EDTA. DNA sedimenting with or faster than the peak of UV absorbance was recovered by ethanol precipitation and washed once with 80% ethanol. The yield was 67 μg.

Cloning of Methylated *Eco*RI* Fragments in Charon 4A. The right and left *Eco*RI arms of Charon 4A (15) were purified from the internal fragments by centrifugation on sucrose gradients as above. Methylated *Eco*RI* fragments (160 μg/ml) were ligated (22) at 6°C to the vector arms (200 μg/ml) with *Escherichia coli* ligase (the gift of I. R. Lehman, purified according to ref. 23). The ligated DNA was packaged *in vitro* into phage by a modification (14) of the procedure of Sternberg *et al.* (24). With a packaging preparation that gave 4×10^7 plaques per μg of uncut vector DNA, the ligated DNA mixture yielded 1.3×10^5 plaques per μg of methylated *Eco*RI* fragments, compared to 3.3×10^5 plaques per μg for a preparation of 11- to 20-kb *Eco*RI fragments of mouse DNA. Only 0.6% of the plaques obtained from *Eco*RI* fragments were *lac*$^+$, indicating that >98% of the phage were recombinants (15). The packaging mixture was divided into 15 aliquots; a plate stock was prepared from each, generating 15 pools, each containing phage derived from 7×10^4 independent events.

Phage plaques were screened by the procedure of Benton and Davis (25) to identify those having sequences homologous to the μ cDNA insert from plasmid pH76μ17 (see *Results*). The probe was labeled by nick translation (26). Phage from positive plaques were purified through at least two cycles of low density plating with rescreening until >90% of the plaques were positive. Phage DNA was prepared from lysates of *E. coli* DP50 SupF essentially as described (14, 15).

Biological and Physical Containment. Work described here was conducted in facilities classified CII and CIII by the Australian Committee on Recombinant DNA (ASCORD) (both classified P3 on NIH guidelines) with EK2 host–vector systems, in compliance with both ASCORD and NIH guidelines for recombinant DNA research.

RESULTS

Mouse Embryo DNA Contains Multiple V_H Genes Related to a Cloned Sequence. In work to be reported elsewhere, we have cloned a cDNA copy of a μ heavy chain mRNA isolated from plasmacytoma HPC 76 (27). Briefly, the evidence that plasmid clone pH76μ17 bears a μ sequence is that it hybridizes strongly to cDNA made on purified HPC 76 μ mRNA but not to cDNA made on mRNA of five non-μ producing plasmacytomas. More definitively, pH76μ17 DNA specifically arrested translation of the μ mRNA into immunoprecipitable μ chain in the hybrid-arrest assay of Paterson *et al.* (28). Fig. 1 shows that pH76μ17 contains a copy of nearly the full length of the μ mRNA and thus includes a substantial portion of the V region. Fragment A, defined by the indicated sites, served as a specific V_{H76} probe.

To estimate how many V_H genes related to V_{H76} occur in the mouse genome, we digested mouse embryo DNA separately with endonucleases *Eco*RI and *Bam*HI, fractionated the resulting fragments by agarose gel electrophoresis, and detected sequences homologous to the V_{H76} region by the procedure of Southern (29), as shown in Fig. 2. At least eight or nine fragments were detected in each of the digests, although only the five to six most intense bands are visible in this figure. This suggests that the mouse genome contains at least this number of V_H genes with various degrees of homology to V_{H76}.

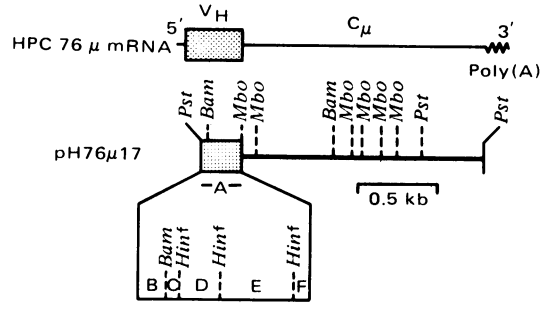

FIG. 1. Map of the μ cDNA segment in clone pH76μ17. V_H region is stippled. Construction, identification, and mapping of pH76μ17 and determination of its orientation with respect to H76 μ mRNA will be described elsewhere. The V-C boundary within pH76μ17 is within 20 base pairs of the indicated *Mbo* II site (unpublished results). Our V region probe (fragment A) is defined by the indicated *Pst* I and *Mbo* II restriction sites; fragments spanning the V region (B–F) are defined by the indicated *Bam*HI and *Hinf* restriction sites. The boundaries between the cDNA segment and the pBR322 vector (not shown) are defined by the left- and rightmost *Pst* I restriction sites shown.

Generation of a Set of Overlapping Clones that Should Represent the Entire Mouse Genome. We have taken advantage of the observations that the specificity of *Eco*RI endonuclease for the sequence G-A-A-T-T-C can be reduced to N_1-A-A-T-T-N_2 (where N_1 and N_2 can be any base) under so-

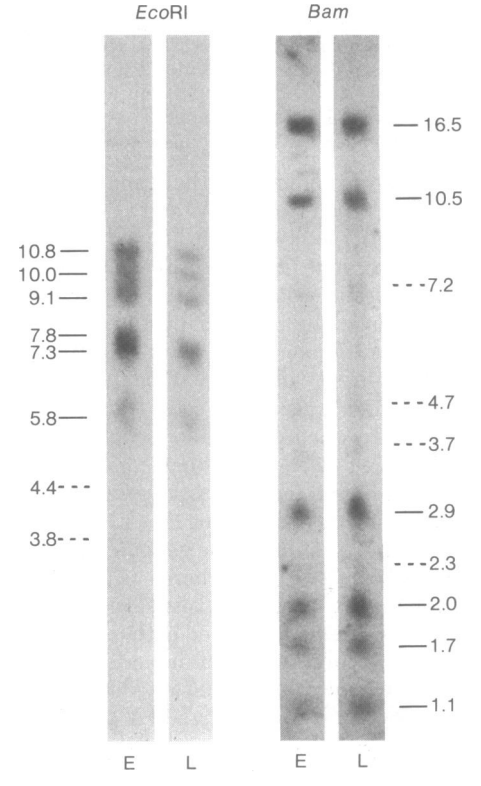

FIG. 2. Detection of V_{H76} genes in restriction endonuclease digests of mouse DNA. DNA (15 μg) from BALB/c embryos (E) or adult liver (L) was digested with *Eco*RI and with *Bam*HI, fractionated by electrophoresis on horizontal (30) slab gels of 0.7% agarose, and then transferred by blotting to cellulose nitrate filters (29, 31). The filters were hybridized with 1.5×10^6 cpm of nick-translated (26) ^{32}P-labeled V_{H76} probe per ml (fragment A in Fig. 1); autoradiographs are shown. Sizes of the fragments (given in kb) were determined from the mobilities of appropriate markers on the same gel. Weakly hybridizing fragments visible on the original autoradiograph are indicated with a dashed line. Pretreatment, hybridization, and washing of the filters was essentially as described by Jeffreys and Flavell (32), except for the use of poly(C) instead of poly(A) and the inclusion of denatured *E. coli* DNA (6.6 μg/ml) and 5 mM EDTA (pH 7.6). Autoradiography at −70°C (33) was for 14–21 days with flashed Kodak RPS-X-Omat film and an Ilford fast tungstate screen.

called *Eco*RI* ionic conditions and that *Eco*RI methylase blocks the standard *Eco*RI sites but not *Eco*RI* sites (34–36). Our approach to construction of a clone library (Fig. 3*A*) begins with methylation of high molecular weight DNA at *Eco*RI recognition sites to protect the DNA against subsequent cleavage at these sites by *Eco*RI. The methylated DNA is then partially digested by using *Eco*RI* conditions to produce fragments averaging about 20 kb in length. Protection of the *Eco*RI sites by methylation is crucial because even under *Eco*RI* conditions these sites are cleaved far more rapidly than other sites (36). Because *Eco*RI* recognizes the sequence N_1-A-A-T-T-N_2, potential *Eco*RI* sites should occur on average every 256 base pairs in DNA containing 50% G+C. Because only 1/80th of such sites need be cleaved to produce 20-kb fragments, partial products of this length should approximate to a randomly fragmented population, although randomness may be reduced somewhat by the marked effect of N_1 and N_2 on the rate of cleavage (36). An advantage of this approach is that *Eco*RI* fragments have cohesive termini (A-A-T-T) identical to those of *Eco*RI fragments (36) and, hence, can be ligated directly into the *Eco*RI-cut Charon 4A vector without recourse to linkers. A portion of the vector-insert joints should be cleavable by *Eco*RI because ligation of *Eco*RI* ends to *Eco*RI ends can produce an *Eco*RI site (i.e., G-A-A-T-T-C) if N_1 and N_2 contributed by the two ends happen to be G and C, respectively.

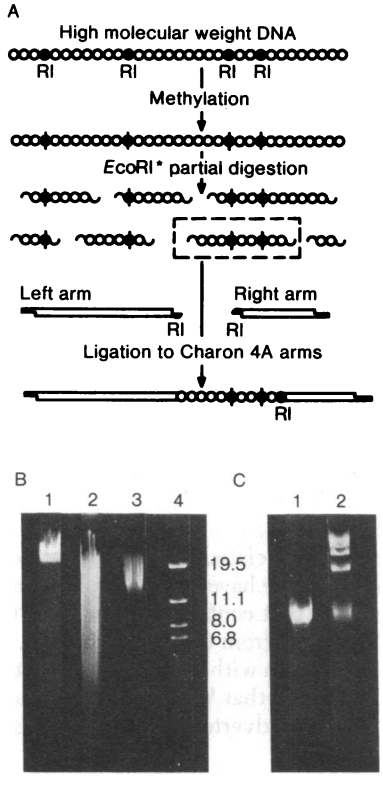

FIG. 3. Construction of overlapping clones by the methylase/*Eco*RI* procedure. (*A*) Schematic presentation. Filled circles depict *Eco*RI sites and open circles *Eco*RI* sites; filled circles with a vertical slash depict methylated *Eco*RI sites. Filled and open half-circles depict cohesive ends generated from *Eco*RI and *Eco*RI* sites, respectively. (*B*) Gel electrophoresis analysis of the products of reactions depicted above. Track 1, methylated mouse DNA (0.2 μg). Track 2, *Eco*RI* partial digest, 1.0 μg (from a pool of a 14-hr and a 20-hr digest). Track 3, 14- to 24-kb fragments (0.6 μg) purified by preparative agarose gel electrophoresis from the pooled digests shown in track 2. Track 4, *Eco*RI fragments of Charon 4A, with sizes indicated in kb. Electrophoresis was at 1 V/cm for 24 hr on a 0.4% agarose gel in Tris phosphate buffer containing 0.5 μg of ethidium bromide per ml. (*C*) Demonstration that fragments in the *Eco*RI* partial digest bear cohesive ends. Track 1, 8- to 10-kb *Eco*RI* fragments (0.5 μg) incubated for 16 hr under ligation conditions but without ligase. Track 2, the same fragments incubated with *E. coli* ligase.

When the *Eco*RI* technique was applied to mouse embryo DNA, a gel electrophoretic analysis (Fig. 3*B*) indicated that high molecular weight methylated DNA (track 1) was converted on partial digestion with *Eco*RI* to fragments with a broad distribution of sizes (track 2). A preparative electrophoretic fractionation allowed us to select 14- to 24-kb fragments (track 3). That the majority of *Eco*RI* fragments have two cohesive ends was verified by the results shown in Fig. 3*C*, which indicate that >60% of a set of 8- to 10-kb *Eco*RI* fragments (purified from the same partial digest as the larger fragments used for cloning) were converted to trimers or higher polymers by *E. coli* DNA ligase, which cannot ligate blunt ends (37).

During cloning, inadvertent ligation of two mouse *Eco*RI* fragments could produce a spurious clone containing two unrelated segments of mouse DNA. To minimize this risk, we selected fragments >14 kb in length, to ensure that the vast majority of any such dimers would exceed the 22-kb cloning capacity of Charon 4A (18), and determined the relative numbers of contaminating fragments <14 kb in length by quantitative densitometry of the gel shown in Fig. 3*B*, track 3. Assuming the chance of ligation of any intact end to be equal, we calculate that <0.2% of the clones could contain such unrelated mouse DNA segments.

By packaging the recombinant DNA into phage *in vitro*, we generated 1.0×10^6 clones of mouse DNA. Because the mouse genome is approximately 2×10^6 kb in length (38) and the average length of the cloned segments so far measured is 16 kb (see below), the clone library contains 8 genome equivalents. Assuming randomness, the probability that it contains any given mouse DNA segment is >99% (14). We segregated the library into 15 pools; this simplified subsequent screening because we could be sure that clones derived from separate pools represent independent cloning events. Because the individual phage generated by packaging were amplified by $\approx 10^6$ during plating, these pools represent a permanent library that can be repeatedly screened (14).

Isolation of Clones Containing V_{H76} Genes. To find clones with sequences homologous to that in pH76μ17, we scored $\approx 3 \times 10^4$ clones from each of nine pools by plaque hybridization (26). We detected 60 positive clones distributed among eight pools. So far we have purified 10 clones, derived from six pools, and characterized their DNA by digestion with *Eco*RI followed by Southern hybridization with the pH76μ17 probe; examples are given in Fig. 4. Nine of the 10 clones yielded distinctive *Eco*RI digestion patterns, indicating that each was of independent clonal origin. Because at least three pools contain more than one independent clone, there probably are additional independent clones among those not yet characterized. The *Eco*RI fragment patterns also established that the structure of the clones is consistent with their mode of construction. For example, Fig. 4 shows that a segment of mouse DNA remains joined to the left arm of the vector in clone ChMe-M48, to the right arm of ChMe-M51, but to neither in ChMe-M11. Moreover, the size of the mouse DNA inserts ranged from 14 to 19 kb and each insert contained internal *Eco*RI sites.

The hybridization pattern of each of the nine clones with the V_{H76} probe (fragment A in Fig. 1) was the same as with the entire V+C segment of pH76μ17, whereas C region fragments failed to hybridize. Hence, all these clones contain V_{H76} genes. Moreover, hybridization to *Bam*HI and to *Bam*HI plus *Eco*RI digests (not shown) revealed that the V_{H76} genes in at least six of the clones are surrounded by different sequences of mouse DNA and hence represent distinct V_{H76} genes.

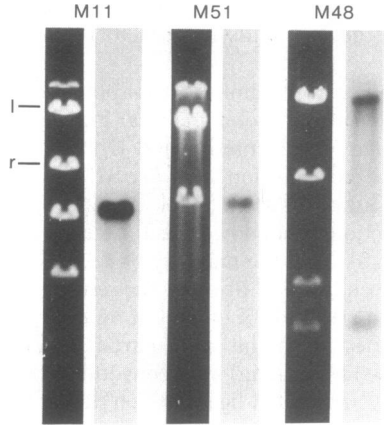

FIG. 4. Detection of V_{H76} regions within three cloned segments of mouse embryo DNA. After digestion with *Eco*RI, DNA (0.8 μg) from ChMe clones M11, M51, and M48 was fractionated by electrophoresis on 0.5% agarose, transferred to cellulose nitrate filters (29, 31), and then hybridized to the ^{32}P-labeled cDNA insert of plasmid pH76μ17 (0.5 × 10^6 cpm/ml) as in the legend to Fig. 2. For each clone, the left track shows the ethidium bromide-stained gel while the right track shows the autoradiograph after hybridization. The filters were washed for 5 hr in 0.30 M NaCl/0.030 M sodium citrate, pH 7, at 65°C followed by 0.5 hr in 30 mM NaCl/3.0 mM sodium citrate, pH 7, at 65°C (32).

Two Clones Each Bear Two V_H Genes. ChMe-M48 (Fig. 4) and ChMe-M31 (not shown) each possessed two *Eco*RI fragments with homology to our V region probe, suggesting that there might be separate V regions within each clone. To locate these regions, we constructed restriction maps of DNA from each clone by partial digestion with *Eco*RI, as well as digestion with three other enzymes, alone and in pairwise combinations.

ChMe-M48 contains two V regions, designated $V_{H76\text{-}1}$ and $V_{H76\text{-}2}$, separated by 15 kb of mouse DNA (Fig. 5*A*). These regions might represent two distinct V genes or, less likely, a single V gene containing a 15-kb intervening sequence. To distinguish between these possibilities, we examined the homology of each region to five segments spanning the entire V region of the pH76μ17 probe (B–F in Fig. 1). If $V_{H76\text{-}1}$ and $V_{H76\text{-}2}$ represented different portions of a single V gene, then

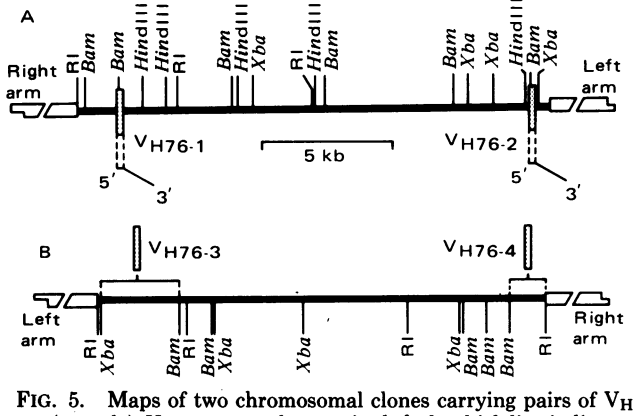

FIG. 5. Maps of two chromosomal clones carrying pairs of V_H genes (to scale). V_H genes are shown stippled; the thick line indicates the mouse DNA segment; only a portion of the Charon 4A vector arms is shown. (*A*) ChMe-M48. HPC 76 μ mRNA is shown below $V_{H76\text{-}1}$ and $V_{H76\text{-}2}$ on the same scale to indicate their orientation. Two additional *Bam*HI and two *Hin*dIII restriction sites occur at undetermined positions within the region 9–15 kb from the leftmost *Eco*RI site. (*B*) ChMe-M31. $V_{H76\text{-}3}$ and $V_{H76\text{-}4}$ genes lie within the regions indicated by brackets. Their orientation is not known. An additional *Xba* site occurs between the second and third *Xba* sites from the left.

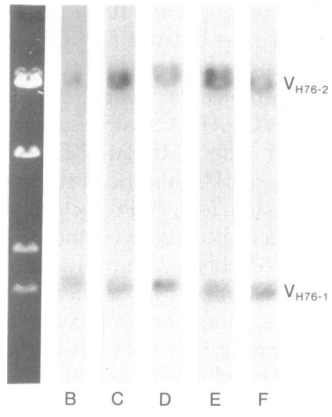

FIG. 6. Two separate V_{H76} genes within clone ChMe-M48. *Eco*RI digests (0.8 μg) of ChMe-M48 DNA were fractionated by electrophoresis on 0.5% agarose, transferred to cellulose nitrate filters (29, 31), and then hybridized as indicated to ^{32}P-labeled fragments B, C, D, E, and F from the V_{H76} sequence in pH76μ17 (see Fig. 1). The leftmost track shows the ethidium bromide-stained *Eco*RI fragments; tracks marked B–F show autoradiographs. The fragments bearing genes $V_{H76\text{-}1}$ and $V_{H76\text{-}2}$ are indicated. Hybridization and washing conditions were as in Fig. 2, but with 1.5 M NaCl/0.15 M sodium citrate, pH 7, at 55°C.

some segments should hybridize only to $V_{H76\text{-}1}$ and some only to $V_{H76\text{-}2}$. In fact, Southern hybridization showed that each probe segment hybridized well to both *Eco*RI fragments containing $V_{H76\text{-}1}$ and $V_{H76\text{-}2}$ (Fig. 6), clearly demonstrating that each region represents a separate V gene. Confirmatory results were obtained with an *Eco*RI/*Xba* I digest and with an *Eco*RI/*Bam*HI digest.

Because there is a *Bam*HI site within both $V_{H76\text{-}1}$ and $V_{H76\text{-}2}$, we were able to establish the orientation of each gene by determining which *Bam*HI fragments of ChMe-M48 hybridized with the 5′ V segment of pH76μ17 (B in Fig. 1) and the 3′ V segments (C–F). We found that both V genes had the same orientation (Fig. 5*A*).

The map of ChMe-M31 shows that it also contains two V regions, $V_{H76\text{-}3}$ and $V_{H76\text{-}4}$, separated by 14 ± 2 kb (Fig. 5*B*). Because the maps indicate that the sequences surrounding these two genes differ from those surrounding $V_{H76\text{-}1}$ and $V_{H76\text{-}2}$, each of the four must be a different gene. Hence, we have cloned two different segments of the mouse genome, each of which carries a pair of related V_H genes separated by a similar distance. In addition, we have recently isolated an independent clone (ChMe-M98) that contains a segment of the mouse genome indistinguishable from that in ChMe-M48, except having the opposite orientation with respect to the vector. This result rules out the possibility that $V_{H76\text{-}1}$ and $V_{H76\text{-}2}$ are two distant genes that were inadvertently ligated together during cloning.

DISCUSSION

Multiple Related V_H Genes Contribute to Immunoglobulin Diversity. Our results provide several lines of evidence that the germline contains multiple related V_H genes. First, the restriction analysis of mouse embryo DNA suggests that it contains at least eight distinct V_{H76} genes (Fig. 2). Second, scoring 2.7 × 10^5 clones (about two genome equivalents) gave a minimum of 9 independent V_{H76} clones, and this clonal frequency suggests that there are at least five V_{H76} genes per haploid genome. Third, DNA from some clones gave stronger signals than others by Southern hybridization with the V_H probe (Fig. 4), presumably due to different degrees of homology. Finally and most definitively, restriction analysis established that the nine independent V_{H76} clones so far isolated include

at least eight distinct V_{H76} genes. Undoubtedly there are additional V_{H76} genes. Moreover, another cloned V_H region (S107) hybridizes with a different set of V_H genes, which exhibits very little if any overlap with the V_{H76} set (our unpublished results). If each of the 5–10 subgroups already evident from amino acid sequences of V_H regions (10, 12) are encoded by a comparable number of genes, then there must be a minimum of 40–80 germline V_H genes. Thus, there is now direct evidence at the nucleic acid level that multiple related V_H genes in the germline contribute to immunoglobulin diversity.

Are V_H Genes Arranged in a Regular Repeating Unit? Certain multigene families, such as ribosomal RNA and histone genes, are organized in a tandem array having a basic repeating unit (reviewed in ref. 12), and it has been postulated (11, 12) that V genes are organized similarly. We propose that the two pairs of V_{H76} genes separated by similar distances define such a repeating unit. The other V_{H76} clones studied appear to contain only single V genes, as expected if the size of the repeating unit is comparable to the average size of the inserts (16 kb). That closely related V regions are adjacent in the genome, rather than interspersed with unrelated V genes, is a prediction of models that invoke homologous but unequal crossing-over to explain expansion and contraction of the V gene pool and the generation of V region diversity (e.g., refs. 6 and 11). It is therefore highly significant that we have found two pairs of *related* V_H genes in proximity.

It is evident from our restriction analyses of embryo DNA and of the clones that the sequences surrounding V_H genes are not highly conserved; *Eco*RI and *Bam*HI fragments of variable size bear V_{H76} genes (Figs. 2 and 4). Indeed, this heterogeneity extends even to the two pairs of adjacent genes, because the restriction sites surrounding each gene differ (Fig. 5). This situation is in striking contrast with the virtually exact preservation of restriction sites in the spacer DNA around the genes for ribosomal RNAs and for histones (reviewed in ref. 12). Nevertheless, we have demonstrated that homology of spacer sequences can be detected because a sequence from within the spacer region of ChMe-M48 (the 4.8-kb *Eco*RI fragment) hybridized to sequences within other clones including ChMe-M31 (our unpublished results). The observed degree of homology varied greatly, however, indicating that spacer sequences are not highly conserved. Seidman *et al.* (6) have proposed that extensive sequence homology surrounding V genes provides a large target for intergenic recombination, which could provide one mechanism for generation of V region diversity. Whether the limited degree of homology we find between sequences surrounding V_{H76} sequences would facilitate such recombination is unclear.

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