Studies of Ig gene structure and organization during the past decade have illuminated the central mechanisms of antibody diversification: the somatic rearrangement and reassortment of multiple gene segments, junctional flexibility, and point mutation. The inherited set of Ig gene segments provides a diversified genetic basis upon which these dynamic processes operate during ontogeny. Thus, the composition of these germline genes imposes a major influence on the development of the antibody repertoire.

The present study examines the germline content and organization of the mouse heavy chain variable region genes ($V_H$ genes). We set out to analyze the locus in sufficient detail and resolution to provide the basis for determining the extent of inherited $V_H$ gene diversity, the evolution of the germline repertoire, and any functional consequences of the physical arrangement of the $V_H$ gene segments.

The mouse $Igh$ locus consists of at least 100-200 $V_H$ genes (1-3). $V_H$ gene families, defined by nucleotide sequence relationships, comprise distinct sets of highly related $V_H$ genes that can be identified by hybridization using prototypic $V_H$ gene probes. This classification of $V_H$ gene families (1, 4) has provided a useful framework for the study of germline $V_H$ gene content, polymorphism, and utilization (1-7). Whereas the general organization of the $Igh$ locus is 5'-$V_H$-$D_J$-$C_H$-3' (8, 9), previous studies of $V_H$ gene family organization have resulted in partial, relatively low resolution maps lacking consistency between reports (10-12).

"Deletion mapping" takes advantage of the fact that $V_H$ gene rearrangements result in the deletion of DNA originally separating the rearranged $V_H$ gene segment and the $D_J$-$C_H$ region (13). We have constructed a panel of 32 pre-B cell lines, most of which have rearranged $V_H$ genes on both chromosomes. Since these cell lines were derived from F1 mice heterozygous at the $Igh$ locus, $V_H$ gene deletions can be identified using RFLPs. $V_H$ gene analyses of 51 independently rearranged chromosomes are consistent with a single $V_H$ gene map order of nine $V_H$ gene families. The genomic stability of these cell lines and consistent deletion profiles of all 51 rearranged loci provide a high resolution $V_H$ gene map that has compelling experimental support.
Materials and Methods

Mice. BALB/c.Ann.xid mice were obtained from Dr. Carl Hansen (National Institutes of Health, Bethesda, MD). BALB/cJ and C57BL/10J mice were obtained from The Jackson Laboratories, Bar Harbor, ME. C.AL-20 mice were provided by the late Dr. Frances Owen (Tufts Medical School).

Cell Lines. "CXXB" cell lines were generated using bone marrow cells from 8-wk-old (BALB/c x C57BL/10J)F1 mice. Cells were transformed in vitro using Abelson Murine Leukemia Virus (A-MuLV)1 and were initially cloned in soft agar as described by Rosenberg and Baltimore (14). Some lines were subsequently subcloned by limiting dilution in 96-well plates. Cells were grown in RPMI 1640 (Gibco Laboratories, Grand Island, NY) supplemented to contain 10% FCS (HyClone Laboratories, Logan, UT), 25 mM Heps, pH 7.2 (Sigma Chemical Co., St. Louis, MO), 5 x 10^{-3} M 2-ME, and penicillin/streptomycin (Gibco Laboratories). The CXAL 1.40 cell line was transformed as above from bone marrow cells of (BALB/c x C.AL-20)F1 mice. Although we have not analyzed these Abelson virus-transformed cell lines by immunofluorescence, based on previous studies of A-MuLV-transformed adult bone marrow cells, we assume that they are of the pre-B cell phenotype, i.e., cytoplasmic μ+ and surface IgM-.

The plasmacytoma PC3609 was induced in an NZB mouse by Dr. Martin Weigert, Fox Chase Cancer Center, Philadelphia, PA (15) and provided to us as a solid tumor. Before use in our studies, we adapted PC3609 to tissue culture using standard protocols. C3a is an LPS-dependent transformed B cell line of BALB/c origin (16).

Southern Blots. High molecular weight genomic DNA was prepared as described (1). DNA preparations were digested to completion with 3-5 U of restriction endonuclease per microgram. DNA for 5-12 h at 37°C using buffers recommended by the manufacturer (Bethesda Research Laboratories, Gaithersburg, MD). Digested DNA samples (10 μg/lane) were fractionated through 0.7% agarose gels (20 cm x 24 cm, 300 ml) at 45 V for 16-20 h in TAE buffer (40 mM Tris, 20 mM sodium acetate, 2 mM EDTA, pH 7.8). Fractionated DNA was transferred to nylon membranes (Nytran; Schleicher & Schuell, Inc., Keene, NH) by either electroblotting (25 V, 12-16 h in 0.25 x TAE) or by capillary transfer using 20 x SSC (1 x SSC = 0.15 M NaCl, 0.015 M sodium citrate). Blots were baked at 80°C for 2 h and hybridized with 32P-labeled DNA probes (17) as described (1). After hybridization, filters were washed twice at 68°C in 3 x SSC, 0.2% SDS for 15 min and once in 0.2 x SSC and 0.1% SDS for 30 min. Filters were exposed to KODAK XAR-5 film at -80°C using an intensifying screen (Lighting-Plus; DuPont Co., Wilmington, DE).

Probes. The hybridization probes used in this study were agarose gel-purified DNA fragments: VJ7183, 1.1-kbp Eco RI–HaeIII fragment from pVHSAPC-15 (1); VQ52, 250-bp Hha I fragment from pVQ52 (1); VJ107, 445-bp Pst I fragment from pV1 (18); VX24, 500-bp Eco RI fragment from pJ7 (19); VGAM3.8, 4.0-kbp Eco RI fragment (CBA germline) cloned in λgtWES; VJ36–60, 700-bp Hind III–Eco RI fragment from pRN5.15 (20); VJ606, 600-bp Bam HI–Eco RI fragment from pBV14J606 (1); VJ3609, 1.5-kbp Eco RI–Bam HI fragment (BALB/c germline); VJ558, 580-bp Eco RI fragment from pVAI (21); Jn, 1.9-kbp Bam HI–Eco RI fragment from pJ11 (22); β2 microglobulin, 1.0-kbp Hind III–Eco RI fragment (23).

Cloning and DNA Sequencing. The two rearranged Jn hybridizing fragments from cell line CXAL 1.40 were cloned in λgtWES and recombinant phage were identified using the pln1 probe. VJn containing fragments were subcloned in the plasmid pUC-18. The VJn558-bearing fragments (see Results) were subcloned into M13 phage and sequenced using standard dideoxynucleotide sequencing procedures (24).

Densitometry. Films of Southern blots hybridized with the VnS107 probe were analyzed using a densitometer (model 620; Bio-Rad Laboratories, Richmond, CA). The copy number of the monomorphic germline V1 gene was determined by comparing the ratio of the densities of V1 bands with polymorphic VnS107 family Eco RI fragments in BALB/c, C57BL/10, (BALB x C57BL)F1, and CXXB cell line DNA samples.

1 Abbreviation used in this paper: A-MuLV, Abelson murine leukemia virus.
Results

Pre-B cell lines were generated by in vitro transformation of adult bone marrow cells from F1 mice carrying the Igh<sup>a</sup> and Igh<sup>b</sup> parental haplotypes. These (BALB/c Ann.xid x C57BL/10)F1 cell lines are designated with the prefix "CXXB."

DNA from ~100 independent CXXB cell lines were initially screened by Southern blot hybridization with probes for the two V<sub>u</sub> gene families previously mapped most proximal to the D segments, V<sub>u</sub>Q52 and V<sub>u</sub>7183 (10). 29 CXXB cell lines, most of which have deleted the V<sub>u</sub>Q52 or V<sub>u</sub>7183 families from one or both chromosomes, were analyzed for the presence or absence of nine V<sub>u</sub> gene families.

Southern blots hybridized with probes for J<sub>u</sub> and the nine V<sub>u</sub> families are presented here for 17 CXXB cell lines (Figs. 1 and 2). Southern blot data from all 29 CXXB cell lines analyzed are presented in summary form (Figs. 3 and 4).

CXXB Cell Lines are Independent, Clonal, and Stable. Fig. 1 is a Southern blot of CXXB cell line DNA samples hybridized with the J<sub>u</sub> probe. No two lanes show identical patterns of rearranged Igh loci, demonstrating the independence of these transformed cell lines. 15 of the cell lines have two rearranged J<sub>u</sub> hybridizing Eco RI fragments, while two cell lines, F52 and F2, each have a single rearranged J<sub>u</sub> hybridizing fragment. None of the cell lines have retained an unrearranged, germ-line Igh locus (Fig. 1). Moreover, there is no evidence for further Igh locus rearrangement during culture; that is, no additional faintly hybridizing J<sub>u</sub> fragments were observed during in vitro propagation of these lines. We conclude that the CXXB cell lines analyzed here are clonal cell populations with stably rearranged heavy chain loci.

Construction of a V<sub>u</sub> Gene Map. Nine Southern blots, each with DNA from 17 CXXB cell lines and liver DNA from strains of the parental Igh haplotypes (BALB/c and C57BL/10) were analyzed. DNA was digested with Eco RI and electrophoresed through a 0.7% agarose gel, transferred to a Nytran membrane, and hybridized with probes for V<sub>u</sub> families (p1<sub>1</sub> or p2<sub>1</sub>).
**Figure 2.** V<sub>n</sub> gene analysis of 17 CXXB pre-B cell lines. DNA from indicated cell lines or liver (BALB/c and C57BL/10) was digested with restriction endonucleases, electrophoresed through 0.7% agarose gels, and transferred to Nytran membranes. Blots were hybridized with the indicated V<sub>n</sub> gene probe. All Southern blots are of EcoRI-digested DNA except V<sub>n</sub>X24 (Pst I) and V<sub>n</sub>36-60 (Hind III). These data are summarized in Fig. 3.

and C57BL/10), were hybridized with V<sub>n</sub> gene probes. As shown in Fig. 2, some cell lines have deleted two or more V<sub>n</sub> gene families from both chromosomes, for example, nine lines (F102, F52, F2, M6, M2, M4, F23, M38, and M9) have deleted all members of the V<sub>n</sub>7183 and V<sub>n</sub>Q52 gene families. Other cell lines have deleted V<sub>n</sub>7183 and V<sub>n</sub>Q52 on one chromosome only, for example, F100 has V<sub>n</sub>7183 and V<sub>n</sub>Q52 patterns identical to the Igh<sup>a</sup> (BALB) parental strain but has deleted all V<sub>n</sub>7183 and V<sub>n</sub>Q52 hybridizing fragments that are Igh<sup>b</sup> (C57BL) specific. Similarly, CXXB F<sub>1</sub> has deleted its Igh<sup>a</sup> V<sub>n</sub>7183 and V<sub>n</sub>Q52 families but retained V<sub>n</sub>7183 and V<sub>n</sub>Q52 patterns identical to those of the germline Igh<sup>a</sup> haplotype.
In many instances, a particular V<sub>n</sub> family is partially deleted from one (or both) alleles. For example, examination of the V<sub>n</sub>S107 pattern of CXXB M9 (Fig. 2) reveals the deletion of all but two germline Eco RI fragments.

The Southern blot data shown in Fig. 2 are summarized in Fig. 3; closed boxes indicate that the genes of that particular family are all in germline configuration, while open boxes indicate the deletion of the entire family. Partial deletion of a family is denoted by a half-filled box. The V<sub>n</sub> gene haplotype of each allele, Igh<sup>a</sup> and Igh<sup>b</sup>, is indicated for each cell line.

The analysis of deletions in the 29 CXXB cell lines (47 V<sub>n</sub> rearranged chromosomes) has yielded a relative chromosomal order of V<sub>n</sub> genes that is consistent with all observed patterns of V<sub>n</sub> gene deletion and retention. These deletion profiles are summarized in Figs. 3 and 4.

**V<sub>n</sub>Q52 and V<sub>n</sub>7183 Family Members are Interspersed.** Analyses of Igh-recombinant mouse strains have shown that the V<sub>n</sub>Q52 and V<sub>n</sub>7183 families are the most D-proximal V<sub>n</sub> gene families (10) and interspersion of these two families has been reported for NIH/Swiss (25) and NSF/N (26) strains. The CXXB cell lines show that V<sub>n</sub>Q52 and V<sub>n</sub>7183 family members are extensively interspersed in both Igh<sup>a</sup> and Igh<sup>b</sup> haplotypes. Partial deletion of both families was observed for nine cell lines; five having deletions on the Igh<sup>a</sup> allele (M21, F32, F45, M8, M52) and four having deletions on the Igh<sup>b</sup> allele (F29, M46, F10, M30). Most of these nine chromosomes have deleted one or a few germline restriction fragments from each family, indicating interspersion of the most 3' (D-proximal) V<sub>n</sub>Q52 and V<sub>n</sub>7183 genes. Notably, CXXB M21 has rearranged a V<sub>n</sub>7183 gene resulting in the deletion of most V<sub>n</sub>7183

![Figure 3](image-url)
and VₙQ52 germline fragments (Figs. 1 and 2). Since CXXB M21 has retained a single germline VₙQ52 fragment and two germline Vₙ7183 fragments, we conclude that the most 5' members of these two families are also interspersed. Therefore, members of these two families are extensively interspersed and together occupy the same physical subregion of the Igh locus.

Vₛ₁₀₇ Family Members are Dispersed. The Vₛ₁₀₇ family consists of four genes in the BALB/c genome (18). One member, V₁ (18), is the Vₙ gene encoding in the T15 idiootype bearing antiphosphorylcholine antibodies that predominate in the BALB/c primary response (27). The two other functional members of the BALB/c Vₛ₁₀₇ family are V₁₈ (5.7-kbp Eco RI) and V₁₃ (2.8 kbp Eco RI). The V₃ gene (3.4-kbp Eco RI) has several defects (28), rendering it nonfunctional.

C57BL/10 mice also have three functional Vₛ₁₀₇ genes and a pseudogene. The V₁ allele of the Ighᵇ haplotype is also present on a 7.8-kbp Eco RI fragment (29). The Eco RI Southern blot patterns of BALB/c and C57BL/10 have three monomorphic fragments corresponding to the BALB/c V₁, V₃, and V₁₃ genes as well as one polymorphic fragment (V₁₈ in BALB/c). With a slight increase in the hybridization stringency for Vₛ₁₀₇-probed Southern blots, the C57BL/10 V₁₃-like fragment, which comigrates with the BALB/c V₁₃ gene, is not detected. Therefore, Vₛ₁₀₇ gene analysis was performed using the increased hybridization stringency to allow a haplotype specific determination of the retention or deletion of the BALB/c V₁₃ gene (Fig. 2).

The Vₛ₁₀₇ family of BALB/c maps as two distinct pairs (V₃/V₁ and V₁₃/V₁₁), the V₃/V₁ pair mapping more proximal to the D segments and being the nearest V genes identified 5' of the VₙQ52/Vₙ7183 cluster. For example, both CXXB M₄ and M₁₀₁ have rearranged the 3' member of the two-gene Vᵢ₇X₂₄ family, and have
deleted V1 and V3 but not V11 and V13. CXXB M9 has rearranged a Vn,36-60 gene and also deleted V1/V3, but has retained V11/V13. CXXB F23 has retained two Vn,S107 genes, a germline V13 gene, and a rearranged gene that is presumably V11. These results are consistent with a map order of 5'-V13-V11-3'.

Within the Ighb haplotype the Vn,S107 family is also dispersed, as shown by the rearrangement of a VGAM 3.8 member (CXXB M2), which resulted in the deletion of only two of the three Ighb Vn,S107 fragments (Figs. 2, 3).

The Vn,X24 Family Maps 5' of Vn,S107 V3-V1. The Vn,X24 family consists of two genes in both BALB/c and C57BL/10 mice (19). Two independent cell lines, CXXB M4 and CXXB M101, have each rearranged a Vn,X24 gene on the BALB/c chromosome. In both cases, these rearranged Igh loci have deleted Vn,S107 V3/V1 and the entire Vn,Q52/Vn,7183 cluster without any detectable alteration in the germline patterns of the other seven Vn gene families. That both Vn,X24 members are located between Vn,S107 V13/V11 and V3/V1 is shown by the CXXB F23 and C3a cell lines (Figs. 2-4), which have each rearranged the Vn,S107 V11 gene and deleted both members of the Vn,X24 family. Furthermore, CXXB M9 has rearranged one of the two Vn,36-60 genes, which also map between V13/V11 and V3/V1 on the BALB/c chromosome, and has deleted both Vn,X42 genes. Therefore, the two Vn,X24 genes show no evidence of being dispersed.

The Vn,X24 genes also map between Vn,S107 members in the Ighb haplotype since CXXB M2 has deleted two Vn,S107 fragments (including V1), as well as both Vn,X24 genes, but has retained the 5.4-kbp Vn,S107 fragment on the C57BL/10 chromosome.

The Vn,36-60 Family Members Map to Three Locations. The Vn,36-60 family (20) consists of six and seven Hind III fragments for the Igh° and Ighb haplotypes, respectively. Two of the Igh° fragments and one Ighb fragment map between Vn,S107 V13/V11 and Vn,X24, whereas the majority of the Vn,36-60 family resides 5' of the Vn,S107 V13/V11 cluster. For example, the CXXB F23 (Figs. 3 and 5) and C3a (Fig. 4) cell lines have each rearranged the Vn,S107 V11 gene (Igh° allele) and have deleted the two Vn,36-60 3' members (the smaller Hind III fragment in the doublet at 12-kbp and the smaller Hind III fragment in the doublet at 7.5 kbp). The Vn,36-60 genes retained in CXXB 23 and C3a make up the 5' Vn,36-60 cluster, whereas the two fragments deleted by the Vn,S107 V11 rearrangement make up the 3' Vn,36-60 cluster of the BALB/c chromosome. Rearrangement of one of these 3' Vn,36-60 genes (CXXB M9, Figs. 2 and 3) resulted in the deletion of Vn,S107 V3/V1, Vn,X24, Vn,Q52, and Vn,7183 families but the Vn,S107 V13/V11 cluster and 5' Vn,36-60 clusters were retained.

The Igh° haplotype exhibits similar dispersion of Vn,36-60 members since the cell line CXXB M2 has rearranged a VGAM3.8 gene and deleted a single Hind III fragment (9.5 kbp) from the germline Vn,36-60 pattern of C57BL/10 (Figs. 2 and 3). As expected from the above results, rearrangement of a member of the 5' cluster of Vn,36-60 genes (CXXB M38, M6, and F45, Figs. 2 and 3) deletes the entire Vn,S107 family.

The Vn gene predominately expressed in the antiarsonate response of BALB/c mice, Vn,1210.7, is a Vn,36-60 family member and is located on a 2.4-kbp Hind III fragment in both the BALB/c and C57BL/10 genomes (20). However, the Vn,1210.7 gene resides on Eco RI fragments that are polymorphic between these two strains.
Southern blots of Eco RI-digested DNA (data not shown) show that the $V_n 1210.7$ gene is not deleted from chromosomes that have rearranged other members of the $V_n 36-60$ 5' cluster (CXXB M6, M38, F45), but is deleted from alleles that rearranged $V_n J 606$ genes (CXXB F10, F1). The $V_n 1210.7$ gene, therefore, is one of the most 5' members of $V_n 36-60$. For example, CXXB M6 has rearranged a $V_n 36-60$ gene and retained only two germline members of the family, one of which is $V_n 1210.7$. CXXB F24 has a single rearranged $V_n 36-60$ fragment and no germline $V_n 36-60$ fragments. Based on the intensity of hybridization (the $V_n 36-60$ probe used has 100% sequence identity with $V_n 1210.7$) and the data presented above, it is likely that the rearranged gene in CXXB F24 is $V_n 1210.7$. That the most 5' $V_n 36-60$ member is the germline $V_n 1210.7$ gene is consistent with the size of the $V_n 36-60$ hybridizing rearranged Hind III fragment in CXXB F24 (2.7 kbp) which is the predicted size of a $V_n 1210.7$ gene rearranged to $J_n 4$ (20).

As shown below, the most 5' member of the $V_n 36-60$ family is separated from the remainder of 5' $V_n 36-60$ cluster by at least one VGAM3.8 gene. Therefore, members of the $V_n 36-60$ family are located in at least three noncontiguous clusters.

**VGAM3.8 Genes Map to Two Distinct Regions.** The VGAM3.8 family of BALB/c consists of five Eco RI fragments (2). The germline 4.0-kbp fragment does not map in the same region as the remainder of the family as evidenced by deletions ($Igh^a$ allele) in cell lines CXXB F23, C3a, and M38. CXXB F23 has rearranged $V_n S 107 V 11$ ($Igh^a$ allele), deleting all VGAM3.8 members except the fragment at 4.0 kbp (Figs. 2, 5). The BALB/c-derived cell line C3a (16) has also rearranged $V_n S 107 V 11$ (Picarella, D., and N. Rosenberg, personal communication) and has similarly deleted all VGAM3.8 members except the 4.0-kbp fragment (Fig. 4). CXXB M38 has rearranged a 5' $V_n 36-60$ member and also retains only the 4.0-kbp VGAM3.8 fragment (Figs. 2, 3). Similarly, CXXB M6 has rearranged a 5' $V_n 36-60$ gene on the $Igh^b$ allele and deleted all VGAM3.8 members except the 4.0-kbp germline Eco RI frag-
ment (Figs. 2, 3). Therefore, in both BALB/c and C57BL/10 strains, the 4.0-kbp Eco RI VGAM3.8 fragment maps to a distinct 5′ subregion of the Igh locus relative to the remainder of that family.

The 5′ VGAM3.8 gene(s) of BALB/c and C57BL/10 mice (4.0-kbp Eco RI fragment) can be distinguished by polymorphic Bgl II restriction sites (data not shown). Southern blot analysis of Bgl II–digested DNA maps the 5′ VGAM3.8 fragment between the V_H606 (CXXB F10,F1) and 5′ V_H36-60 clusters (CXXB M6, M38, F45). CXXB F24 has rearranged the most 5′ member of the V_H36-60 family and has deleted all members of VGAM3.8. Therefore, the VGAM3.8 family has a single dispersed fragment that maps between the two most 5′ members of V_H36-60.

Most of the VGAM3.8 family, however, maps between V_S107 V13/V11 and the 3′ V_H36-60 cluster. CXXB M2 has rearranged a VGAM3.8 gene, deleting all germ-line VGAM3.8 fragments except the single VGAM3.8 Eco RI fragment at 4.0 kbp. Both CXXB M2 and M34 retain the germline V_S107 V11 fragment but delete the 3′ members of V_H36-60. CXXB M9 has rearranged a 3′ member of V_H36-60 (deleting both germline 3′ V_H36-60 fragments on the BALB/c chromosome), but has retained the entire VGAM3.8 family. Taken together, these results demonstrate that the 3′ VGAM3.8 cluster maps between V_S107 V11 and the two 3′ V_H36-60 fragments.

V_J606 Maps 5′ of V_H36-60. CXXB F10 and F1 have both rearranged a member of the V_J606 family (Igh^a allele), in each instance deleting two V_J606 members (10 and 5 kbp). In both cell lines, the entire V_H36-60 family is deleted, including the BALB/c V_H1210.7 gene–bearing fragment (Eco RI blot, data not shown). Rearrangement of the most 5′ V_H36-60 gene in CXXB F24 did not delete V_H606 fragments but resulted in the deletion of the entire VGAM3.8, V_S107, V_HX24, V_Q52, and V_H7183 families (Fig. 4). Therefore, the V_H606 cluster maps 5′ of all V_H36-60 members.

V_H3609 Maps 5′ of V_H606. Two cell lines of the CXXB panel have deleted the BALB/c V_H606 family and retained the BALB/c V_H3609 family (M6 and M2). Seven cell lines have C57BL/10 chromosomes with an intact V_H3609 family but no V_H606 members (CXXB M21, F100, M4, F23, M9, M8, F9.6). CXXB F2 has deleted all but four V_H3609 fragments and CXXB F52 has retained two V_H3609 fragments. Since the nondeleted fragments are monomorphic, it is not possible to assign them to either the BALB/c or C57BL/10 chromosome. Therefore, as shown in Figs. 3 and 4, the V_H3609 family of CXXB F2 and F52 is presented as a single, half-filled box to indicate the lack of assignment to a particular allele.

The Most 5′ V_H Gene Segments are Members of the V_H3558 Family. The great complexity of the V_H3558 family has impeded the construction of a fine-resolution map of these genes. CXXB F102 has no detectable V_H gene sequences except for five V_H3558 Eco RI fragments, indicating that these genes are the most 5′ V_H sequences we have identified. CXXB F52 and F2, each of which has deleted most germ-line V_H3609 genes, have retained 13 and 16 V_H3558 Eco RI fragments, respectively. Several other cell lines (CXXB F29, M2, M38) have clearly deleted some V_H3558 members. However, the large number of V_H3558 fragments (~35) makes it difficult to detect a partial loss of this family. Indeed, it is possible that most V_H3558 hybridizing Southern blot bands represent a number of unique, comigrating fragments that may not map in the same region of the Igh locus.
Evidence that a V\textsubscript{H}J558 Family Member Maps 3' of V\textsubscript{H}X24. A-MuLV-transformed lines were generated from (BALB/c × C.AL-20)\textsubscript{F1} mice (Igh\textsuperscript{a} × Igh\textsuperscript{a}). One of these cell lines, CXAL 1.40, has deleted V\textsubscript{H}S107 (V3-V1), V\textsubscript{H}Q52, and V\textsubscript{H}S7183 from the BALB/c chromosome (Fig. 4). We inferred that CXAL 1.40 has rearranged a V\textsubscript{H} sequence that is located between V\textsubscript{H}X24 and V\textsubscript{H}S107 (V3-V1) in the BALB/c germline. To determine the nature of this rearranged sequence, we cloned both J\textsubscript{H} rearrangements. One cloned fragment hybridized with a V\textsubscript{H}J606 probe and, based on the deletion profile of CXAL 1.40 (Fig. 4), most likely contains the VDJ rearrangement of the C.AL-20 (Igh\textsuperscript{a}) chromosome. DNA sequencing of the other V\textsubscript{H}J\textsubscript{H} rearrangement, however, revealed a V\textsubscript{H} gene with identity to the germline V\textsubscript{H} gene H4a-3, which belongs to the V\textsubscript{H}J558 family. The H4a-3 gene is one of a small number of BALB/c germline genes recognized, under high stringency, by a cDNA V\textsubscript{H} probe derived from the anti-GAT mAb, G8 Ca 1.7 (30). Although not yet formally proven, based on the deletion profile of CXAL 1.40 (Fig. 4) and the sequence identity with a BALB/c germline V\textsubscript{H} gene, we propose that the cell line rearranged a V\textsubscript{H}J558 gene on the BALB/c chromosome. Furthermore, using Igh-recombinant strains, at least one V\textsubscript{H} gene with high homology to our CXAL 1.40 sequence has been mapped to a region that includes the V\textsubscript{H}S107 V11 gene and part of the V\textsubscript{H}S107 V11 to V3 interval (Tutter, A., P. Brodeur, M. Shlomchik, and R. Riblet, submitted for publication). We provisionally conclude, therefore, that at least one V\textsubscript{H}J558 member is located between V\textsubscript{H}X24 and V\textsubscript{H}S107 (V3-V1) in the Igh\textsuperscript{a} haplotype.

We have examined the CXAL 1.40 cell line for the presence of V\textsubscript{H}ARS (V\textsubscript{H}36-65), the V\textsubscript{H}J558 member (1) expressed in the predominant idiotype response (CRI) to p-phenyl-arsonate in Igh\textsuperscript{a} strains (31). As previously reported, the V\textsubscript{H}36-65 probe, V1d-130 (32), hybridizes to the V\textsubscript{H}ARS-bearing Eco RI fragment (6.2 kbp) of the Igh\textsuperscript{a} haplotype but does not hybridize to BALB/c (Igh\textsuperscript{a}) genomic DNA. In agreement with the data of Rathbun et al. (12), we have demonstrated that the V\textsubscript{H}ARS gene is retained by CXAL 1.40 (Fig. 6), indicating that this gene is 5' of the rearranged V\textsubscript{H}J606 gene on the Igh\textsuperscript{a} chromosome. The Southern blot shown in Fig. 6 was also hybridized with a β2-microglobulin probe (23). The comparable intensities of the 1.8-kbp β2 fragment verify that each lane contained approximately the same amount of DNA.

The Relative Chromosomal Position of Nine V\textsubscript{H} Gene Families can be Inferred from Deletional Analysis. The data described above allow the positioning of 13 “clusters” of V\textsubscript{H} genes. Fig. 7 shows the relative order of these clusters along the chromosome and indicates the interspersion (V\textsubscript{H}Q52, V\textsubscript{H}S7183) and dispersion (V\textsubscript{H}S107, V\textsubscript{H}36-60, VGAM3.8) of the family clusters.

Discussion

We have constructed a panel of pre-B cell lines to elucidate the organization of the Igh locus by deletion mapping. We have determined the positions of nine V\textsubscript{H} gene families relative to the D-J\textsubscript{H}-C\textsubscript{H} region on chromosome 12 of BALB/c and C57BL/10 mouse strains. The members of a given V\textsubscript{H} gene family are generally clustered within a limited region of the locus, although three families consist of two clusters separated by members of one or more different families. The inferred map order is 5'-V\textsubscript{H}J558-V\textsubscript{H}3609-V\textsubscript{H}J606-V\textsubscript{H}36-60-VGAM3.8-V\textsubscript{H}36-60-V\textsubscript{H}S107(V13-V11)-VGAM3.8-V\textsubscript{H}36-60-V\textsubscript{H}X24-V\textsubscript{H}S107(V3-V1)-V\textsubscript{H}Q52-V\textsubscript{H}S7183-D-J\textsubscript{H}-C\textsubscript{H}-3': As sug-
FIGURE 6. The \textit{V_{\mu}ARS} (\textit{V_{\mu}36-65}) gene is 5' of the \textit{V_{\mu}J606} family. DNAs from the CXAL 1.40 cell line and livers of \textit{AL/N}, BALB/c, and (BALB x \textit{AL})F_{1} mice were digested with EcoRI, transferred to Nytran membrane, and hybridized with a mixture of \textit{Vld130} (\textit{V_{\mu}ARS}) probe (31) and \textit{\beta_{2}}-microglobulin probe (23).

gested by previous studies (11, 12, 25, 26), the two most \textit{D}-proximal \textit{V_{\mu}} families are completely interspersed and the two families most distal to the \textit{D} region, \textit{V_{\mu}J558} and \textit{V_{\mu}3609}, may be partially interspersed.

We initially reasoned that a valid \textit{V_{\mu}} gene map based on deletion analyses would require that deletions on all chromosomes of the same haplotype be consistent with a single map order. Such a map should be based on many independent deletions, yielding multiple consistent orderings. Our panel consists of 51 independently rearranged \textit{Igh-V} loci, all of which have deletion profiles consistent with a single \textit{V_{\mu}} map (Figs. 3, 4). We believe, therefore, that the size and consistency of the database presented here provide compelling support for the order of \textit{V_{\mu}} gene families shown in Fig. 7.

Recent studies using deletion mapping have been reported by Rathbun et al. (12) and Blankenstein and Krawinkel (11). Both studies examined a limited set of "haploid" cell lines. Rathbun and colleagues examined five cell lines and suggested a map order of \textit{V_{\mu}3609}-\textit{V_{\mu}J558}-\textit{V_{\mu}J606}-\textit{VGAM3.8}-\textit{Vhs107}-\textit{V_{\mu}36-60}-\textit{(V_{\mu}X24}, \textit{V_{\mu}Q52}, \textit{V_{\mu}7183}). Blankenstein and Krawinkel examined two haploid hybridomas, one \textit{Igh^{a}} and one \textit{Igh^{b}}, and a single recombination to infer a relative map order that differs significantly from that of Rathbun et al. (12) and the map derived from the present study. We do not know the causes of the discrepancies in map orders inferred from deletion analyses of hybridoma cell lines and that inferred from our data. It is possible that the inherent instability and complexity of hybridoma genomes (aneuploidy, translocations) and the possible anomalies associated with haploid loci generated in tissue culture lines could explain the differences. In contrast, the large number
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FIGURE 7. Relative chromosomal position of nine Vn gene families. The "complexity" of each family cluster is indicated by the lengths of the solid bars (VnX24 = 2). Actual physical distances are unknown.

of independent data points (51 deletions), the inherent stability of A-MuLV-transformed lines (33), and the internal consistency of our data strongly support the Vn gene map inferred from our study.

Vn gene deletion analyses of rearranged chromosomes of both Igkα and Igkβ haplotypes were consistent with a single Vn gene family organization. More limited data for the Igkα, Igkβ, and Igkγ haplotypes are also consistent with the Vn gene chromosomal positions shown in Fig. 7 (Fig. 4 and unpublished results).

Differences in Vn family utilization between BALB/c and C57BL strains have been reported (5, 7), although such differences have not been consistently observed in all systems (6). Based on our finding that Vn gene organizations of Igkα and Igkβ haplotypes are indistinguishable, it is unlikely that differences in family utilization are due to significant differences in the relative map positions of the Vn gene families. Indeed, Wu and Paige (34) and Jeong et al. (7) have presented evidence that such differences are controlled by other (non-Igh) loci.

The suggestion that mouse Vn genes belonging to the same family are clustered was first made by Kemp et al. (35) after isolating distinct genomic DNA clones, each bearing pairs of highly related Vn sequences (VnS107 and VnJ606). Bothwell et al. (36) and Givol et al. (37) reported similar findings for members of the VnJ558 family. Previous analyses of B cell lines for Vn gene deletions (11, 12, 38, 39) were consistent with a clustered organization of Vn gene family members. Vn gene analysis of Igh-recombinant mouse strains constructed by Riblet (40) also indicated a generally clustered organization of Vn families (10).

The clustered nature of mouse Vn gene families contrasts with the organization of the human Vn locus, which appears to have a more interspersed distribution of family members (41, 42). Although the data are currently more limited, the mouse Vκ genes also appear to have generally clustered family members (43, 44) while the human Vκ genes are highly interspersed (45). Data from wild-mouse populations and other mammals are not yet available.

The high degree of complexity and relative map position of the VnJ558 family impose certain limits on the present analysis. Because this set of Vn genes consists of at least 60 (1) and perhaps 500 (46), it is difficult to resolve very many polymorphic fragments on Southern blots. Furthermore, the fact that VnJ558 members map most distal to the D region makes it difficult to ascertain whether any VnJ558 genes...
are dispersed within the more D-proximal families. However, based on our finding that the cell line CXXB M18 has deleted all members of \( V_HJ606 \), \( V_HA36-60 \), \( V_HS107 \), \( V_HX24 \), \( V_HQ52 \), and \( V_H7183 \) but has retained an apparently complete \( Ig^h \) \( V_HJ558 \) pattern, we suggest that most \( V_HJ558 \) members are 5' of \( V_HJ606 \). Nevertheless, the apparent location of a \( V_HJ558 \)-like gene between \( V_HX24 \) and \( V_HS107 \) (V3-V1) on the BALB/c chromosome in the CXAL 1.40 cell line is supported by the finding that a gene of the H10/Sm7 (30, 47) set of \( V_HJ558 \)-related sequences maps to a distinct region 3' of the major \( V_HJ558 \) cluster (Tutter, A., P. H. Brodeur, M. Shlomchik, and R. Riblet, submitted for publication). The finding of \( V_HJ558 \) members in this 3' region has important implications. First, it reveals that \( V_HJ558 \)-related genes are dispersed over a wide range in the \( Ig^h-V \) locus. Second, it underscores the importance of using hybridization probes derived from distinct members of the \( V_HJ558 \) family, under high stringency conditions, to operationally define \( V_HJ558 \) family subsets and to apply deletion mapping analysis to map such subsets. With these means, a significant resolution of this complex set of genes may be attained. Third, the location of the H10/Sm7-like genes in the region between \( V_HX24 \) and \( V_HS107 \) (V3-V1) has implications for the origins of \( V_H \) family dispersion and for the evolutionary history of the \( Ig^h-V \) locus as discussed below.

There are three major groups of mouse \( V_H \) genes (I, II, III) based on the original classification of Kabat et al. (48) as recently modified by Tutter and Riblet (49). Accordingly, members of \( V_HQ52 \), \( V_H36-60 \), and \( V_H3609 \) belong to group I and are more closely related, by sequence, to each other than to other \( V_H \) gene families. As shown in Fig. 7, these three families are located in distinct regions of the \( Ig^h \) locus and are not adjacent. Similarly, family clusters of group II (VGAM3.8, \( V_HJ558 \)) and those of the group III families (\( V_H7183 \), \( V_HS107 \), \( V_HX24 \), \( V_HJ606 \)) are generally separated by members of one or two different groups (Fig. 8). This organization suggests a possible evolutionary pathway in which divergence of the ancestral groups II, I, and III (in that order) was followed by two independent duplications of the locus resulting in four repeats of the II-I-III unit. \( V_H \) families may have then evolved by more recent divergence including, perhaps, expansions and contractions. There is, in fact, evidence for both a large duplication within the human IgVx locus (50) and for smaller, incremental changes within mouse \( V_H \) gene families (51).

Several distinct mechanisms have been invoked to account for the evolution of multigene families (52). The interspersion of \( V_HQ52 \) and \( V_H7183 \) family members, typical of the human \( V_H \) (41) and \( V_x \) loci as well as both mouse and human T cell receptor \( V_H \) genes (53, 54), may be the product of illegitimate recombination be-

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**FIGURE 8.** Relative chromosomal position of the three major \( V_H \) groups (I, II, III) based on nucleotide sequence similarities (49): Group I, \( V_HQ52 \), \( V_H3609 \); Group II, VGAM3.8, \( V_n36-60 \), \( V_nJ558 \); Group III, \( V_n7183 \), \( V_nS107 \), \( V_nX24 \), \( V_nJ606 \). Solid rectangles indicate the relative chromosomal position of \( V_H \) gene clusters. The length of each rectangle indicate the approximate number of \( V_H \) gene-bearing restriction fragments within a given cluster. The position of the D-proximal Group II cluster is provisional (see Results), and is, accordingly, illustrated by an open rectangle.
between sequences (repetitive or nonrepetitive) conserved throughout a particular locus. Alternatively, the creation, expansion, and conservation of VH gene clusters might occur via homologous, unequal recombination and/or gene conversion events. It is possible that the only selective pressure on V region genes is the maintenance of a minimal V gene pool and the IgH-V locus may be permissive to a wide variety of evolutionary changes in size, composition, or organization. On the other hand, certain, as yet unrecognized, features of V gene organization may be important in the development of the antigen specific receptor repertoire, for example, the developmental read-out of particular V genes (55, 56) or antigen specificities (57, 58).

Despite the apparent evolution of a highly diversified immune system, various studies have demonstrated a biased usage of VH gene segments most proximal to the Ds segments (VH7183 family) in pre-B cells. Yancopoulos et al. (55) examined 9 A-MuLV-transformed BALB/c fetal liver derived pre-B cell lines, and documented 12 VDJH rearrangements in these cell lines, of which 11 involved the VH7183 gene family. Similar results were obtained by Perlmutter et al. (56), who found that 7 of 9 fetal liver hybridomas express the VH7183 family. We have recently generated fetal liver A-MuLV pre-B cell lines to examine the VH gene usage during ontogeny in CBA/Tufts mice. In contrast to initial reports, we and others (59-61) have observed the preferential utilization of both VH7183 and VHQ52 family members in pre-B cells (62). Taken together, the findings by ourselves and others (25, 26, 59) that VH7183 and VHQ52 family members are interspersed in a variety of haplotypes, and that both families are preferentially utilized in pre-B cells, strongly support the suggestion of Yancopoulos et al. (55) of a position-dependent rearrangement of VH gene segments in early ontogeny.

That the number and position of functional genes within a given family are unknown complicates attempts to relate VH gene family representation in the adult repertoire to gene organization. Thus, the relative contributions of family complexity (1) and chromosomal position as determinants of VH gene representation in the adult primary B cell repertoire are not clear. However, independent analyses have suggested that the most D-distal VH gene families (VHJ558 and VH3609) are under-represented relative to their apparent complexities. For example, the VHJ558 family is utilized only about six times more frequently than the three functional genes of the VH107 family in BALB/c adult splenic B cell populations (7, 63, Sheehan, K. M., and P. H. Brodeur, manuscript in preparation). If all functional VH segments have an equal probability of rearrangement and expression (as with a simple complexity driven model), the calculated number of functional VHJ558 family genes would be approximately eighteen (3 x 6). An estimate of 18 functional VHJ558 genes requires either a very large proportion of dysfunctional genes in this family, for which size estimates range from a minimum of 60 (1) to >500 members (46), or other factors that influence VH gene utilization. The uncertainty of the actual number of functional VHJ558 genes and the demonstration of non-Igh locus influence on VH gene usage (34), limits the impact of the data cited above in supporting a strict position-dependent model of VH gene expression. Therefore, additional analyses will be required to ascertain the full extent of and the basis for the shift from preferential rearrangement of D-proximal VH genes early in ontogeny (55, 63), toward a more probabilistic pattern of VH gene utilization that occurs in adult lymphoid tissues (7, 63).
Summary

We have constructed a panel of Abelson murine leukemia virus-transformed pre-B cells to study the organization of the mouse V<sub>H</sub> gene families. Based on the analyses of V<sub>H</sub> gene deletions on 51 chromosomes with V<sub>H</sub> gene rearrangements, we have inferred a map order of the Igh locus that holds for both the Igh<sup>°</sup> and Igh<sup>b</sup> haplotypes.

We show that members of each V<sub>H</sub> gene family are generally clustered, although three family clusters (V<sub>H</sub>S107, V<sub>H</sub>36-60, VGAM3.8) are dispersed in two or three subregions of the locus. Members of two V<sub>H</sub> gene families, V<sub>H</sub>Q52 and V<sub>H</sub>7183, are extensively interspersed and map within the same subregion. An examination of the distribution of V<sub>H</sub> group members (V.II, I, and III) within the locus suggests that two major duplications may, in part, explain the dispersed pattern of V<sub>H</sub> family clusters. The relationship of V<sub>H</sub> organization and functional expression is discussed in terms of position-dependent and complexity-driven models.

The authors wish to dedicate this paper with affection and respect to the memory of Dr. Frances L. Owen, our colleague, teacher, and friend, in recognition of her outstanding contribution to immunology during her deeply committed but unfortunately short life.

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