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Immunology

Genomic organization of the mouse T-cell receptor β-chain gene family

(deletion mapping/field-inversion gel electrophoresis)

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ABSTRACT **We have combined three different methods, deletion mapping of T-cell lines, field-inversion gel electrophoresis, and the restriction mapping of a cosmid clone, to construct a physical map of the murine T-cell receptor β-chain gene family. We have mapped 19 variable (V_β) gene segments and the two clusters of diversity (D_β) and joining (J_β) gene segments and constant (C_β) genes. These members of the β-chain gene family span ≈450 kilobases of DNA, excluding one potential gap in the DNA fragment alignments.**

The antigen-binding T-cell receptors are encoded in mice by two families of genes—the α-chain gene located on murine chromosome 14 and the β-chain genes on chromosome 6 (1–5). The β chains are encoded by a constant gene (C_β) and variable (V_β), diversity (D_β), and joining (J_β) gene segments, which rearrange and are joined together at the DNA level during T-cell differentiation to generate an assembled V_β gene (6–10). The β-chain gene family of murine T-cell receptors contains just 20–30 V_β gene segments by statistical estimates (11, 12), 19 of which have been identified from cDNA or genomic clones (13, 14). This family also encodes two closely linked D_β–J_β–C_β clusters, each containing 1 D_β and 7 J_β gene segments as well as a single C_β gene (6–10). One V_β gene segment, *V_β14*, is located 10 kilobases (kb) downstream from the *C_β2* gene (15). The relative order and locations of the 18 other V_β gene segments are unknown, although it is generally thought that they are encoded within hundreds of kilobases of DNA 5′ to the D_β–J_β–C_β gene clusters.

We have employed three distinct, but complementary, approaches for mapping the β-chain gene family. First, deletional mapping has permitted us to exploit the deletional rearrangement mechanisms of most T cells. On a chromosome that has experienced a V_β–D_β–J_β deletional rearrangement, the V_β gene segments located between the rearranging V_β and D_β gene segments are lost. The identity of the rearranged and deleted V_β gene segments in a clonal T-cell line can be determined by Southern blot analysis of the T-cell genomic DNA with various V_β-specific DNA probes, if both chromosome 6 homologues have undergone V_β–D_β–J_β deletional rearrangement. The examination of many T-cell lines with different rearranged V_β gene segments allows the generation of a deletion map of the β-gene family. Second, restriction enzyme mapping of germ-line genomic DNA was carried out by the newly developed field-inversion gel electrophoresis technique that has the capacity to separate DNA fragments ranging in size from 20 to 1600 kb (16). These two approaches, together with a third, the analysis of a cosmid clone, has allowed us to (*i*) determine a physical map of the murine T-cell receptor β-chain gene locus, (*ii*) position 19 V_β gene segments within this physical map, and (*iii*) link the V_β gene segments to the D_β–J_β–C_β clusters.

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MATERIALS AND METHODS

DNA Preparation. Genomic DNA used for field-inversion gel electrophoresis (17) and for conventional gel electrophoresis (18) was prepared as described.

DNA Probes. Sixteen murine V_β subfamilies have been described (11, 13, 14), where a subfamily is defined as one or more cross-hybridizing *V* gene segments that do not cross-hybridize with the members of other subfamilies (≈75% or more homology). DNA fragments corresponding to *V_β1*, *-2*, *-3*, *-4*, *-5.1*, *-8.1*, *-8.2*, *-8.3*, and *-14* gene segments were isolated from cDNA and genomic clones described previously (11, 15, 19) and subcloned into the plasmid pUC18. The *V_β10* subclone was obtained from Joan Kobori and Nilabh Shastri (California Institute of Technology), the *V_β5.2*, *-6*, and *-15* subclones were from Mark Davis (Stanford), the *V_β7* and *-16* subclones were from Edward Palmer (National Jewish Hospital), and the *V_β9*, *-11*, and *-12* subclones were from Paul Singer (Scripps Institute).

Southern Blot Analysis. Field inversion gels were transferred to Zeta-probe nylon membrane (Bio-Rad) and hybridized with oligonucleotide-primed ^{32}P-radiolabeled DNA probes as described (18). Conventional gels were either transferred to Zeta-probe and hybridized as described (18) or transferred to nitrocellulose membrane (Schleicher & Schuell) and hybridized to ^{32}P-labeled, nick-translated probe as described (11).

Cosmid Clones. A cosmid library constructed from liver DNA of a B10.D2dm1 mouse was screened with ^{32}P-labeled V_β DNA probes and positive clones were purified and mapped as described (20).

RESULTS

V_β Gene Segment Rearrangements and Deletions in T-Cell Clones. We have analyzed 16 separate cloned T-cell lines for V_β gene segment rearrangements and deletions using DNA probes that detect 14 different V_β gene segments. The V_β gene segment rearrangements or deletions were distinguished from unrearranged or germ-line V_β profiles by comparing the DNA blots of each T-cell line with C57BL/6J or C57L/J liver DNA. In 6 T-cell lines V_β segment deletions were observed (Table 1). The remaining 10 T-cell lines did not exhibit detectable V_β deletions and, therefore, their analyses were uninformative.

The logic of the deletional analysis is illustrated for T-cell line A10. The A10 cell line contains a single rearranged *V_β1* gene segment and one rearranged and one germ-line *V_β2* gene segment. The simplest interpretation of these results is that the *V_β2* gene segment is upstream to the *V_β1* gene segment and that on one chromosome 6 the *V_β2* gene segment has rearranged and deleted the downstream *V_β1* gene segment,

Abbreviations: V_β, D_β, J_β, and C_β, variable, diversity, and joining gene segments and constant genes encoding the β chain of the T-cell receptor.

Table 1. Deletional analysis of V_β gene segments in six T-cell lines

T-cell line	V_β probe																
	$V_\beta 2$	$V_\beta 4$	$V_\beta 10$	$V_\beta 16$	$V_\beta 1$	$V_\beta 5.2$*	$V_\beta 8.3$*	$V_\beta 5.1$*	$V_\beta 8.2$*	$V_\beta 8.1$*	$V_\beta 9$	$V_\beta 11$	$V_\beta 12$	$V_\beta 6$	$V_\beta 3$	$V_\beta 7$	$V_\beta 15$
A10	R, G	G	G	G	R	–	–	–	–	–	–	–	–	–	–	–	–
BW5147	G	G	G	G	R, G	R	–	–	–	–	–	–	–	–	–	–	–
VL3/1	G	G	G	G	G	G	R	–	–	–	–	–	–	–	–	–	–
SL17	G	G	G	ND	G	ND	R	ND	–	–	–	–	–	ND	–	ND	ND
RL17	G	G	G	G	G	G	G	R	–	–	–	–	–	–	–	–	–
L691	G	R, G	G	G	G	–†	–†	–†	–†	–†	–†	–†	–†	R	–	–	–

T-cell lines with two V_β rearrangements presumably undergo one rearrangement on each chromosome 6 homologue; T-cell lines with one V_β rearrangement could have just a single rearrangement on one chromosome 6 or two V_β rearrangements, one with an upstream V_β gene segment for which we are lacking a probe. –, Deleted V_β gene fragment; R, rearranged V_β gene fragment; G, germ-line V_β gene fragment. ND, not determined.

*The $V_\beta 5.1$ gene segment was distinguished from the $V_\beta 5.2$ gene segment and the $V_\beta 8.3$ gene segment was distinguished from the $V_\beta 8.1$ and $V_\beta 8.2$ gene segments by hybridization with specific probes and washing the Southern blots under conditions of increasing stringency.

†The L691 cell line is derived from a C57L/J mouse that carries a germ-line deletion for the $V_\beta 5$, $V_\beta 8$, $V_\beta 9$, $V_\beta 11$, $V_\beta 12$, and $V_\beta 13$ subfamilies.

whereas on the second chromosome 6 the $V_\beta 1$ gene segment has rearranged leaving the upstream $V_\beta 2$ gene segment in its germ-line configuration. Consequently, if the $V_\beta 1$ gene segment rearrangement occurred by deletion, those V_β gene segments downstream from the $V_\beta 1$ gene segment should be absent, whereas those upstream from the $V_\beta 1$ gene segment should be present. The $V_\beta 4$, *-10*, and *-16* gene segments are present in the germ-line configuration in the A10 DNA and, accordingly, appear to be upstream of the $V_\beta 1$ gene segment (Table 1). In contrast, the V_β gene segments *3, 5.1, 5.2, 6, 7, 8.1, 8.2, 8.3, 9, 11, 12*, and *15* are absent in A10 cells, indicating they are located downstream to the $V_\beta 1$ gene segment (Table 1).

Southern blot analysis of the other five T-cell clones yielded patterns of rearrangement and deletion that are consistent with the results obtained from the A10 cell line (Table 1). The $V_\beta 5$ and $V_\beta 8$ subfamilies have their individual V_β gene segments interspersed, as revealed by the deletional analysis of DNA from the T lymphomas VL3/1, SL17, and RL17 (Table 1 and Fig. 1). The VL3/1 line contains the $V_\beta 5.2$ gene segment in the germ-line configuration and a single rearranged $V_\beta 8.3$ gene segment. The gene segments $V_\beta 8.1$, *-8.2*, and *-5.1* are deleted in this cell line. These results indicate that the $V_\beta 8.3$ gene segment is located upstream from the $V_\beta 5.1$, *-8.1*, and *-8.2* gene segments. In the T-cell line RL17 there is a single rearranged $V_\beta 5.1$ gene segment and no $V_\beta 8.1$ or *-8.2* gene segments (Table 1). Therefore, the $V_\beta 5.1$ gene segment lies upstream from the $V_\beta 8.1$ and *-8.2* gene segments. We have assigned the $V_\beta 8.2$ gene segment to a position upstream from the $V_\beta 8.1$ gene segment based upon the observation that a genomic DNA clone isolated from a cytotoxic T-cell line containing a rearranged $V_\beta 8.2$ gene segment also includes an upstream germ-line $V_\beta 8.3$ gene segment but no $V_\beta 8.1$ gene segment (21).

The $V_\beta 9$, *-11*, and *-12* gene segments are deleted in all of the T-cell lines suggesting that these gene segments are located downstream to the $V_\beta 5.1$ gene segment. We have assigned the $V_\beta 9$, *-11*, and *-12* gene segments to a position upstream from the $V_\beta 6$ gene segment based upon the observation that in four inbred strains of mice these gene segments as well as the $V_\beta 5$ and $V_\beta 8$ subfamilies are deleted from the germ line (14). If this genetic event is a single deletion, then the $V_\beta 9$, *-11*, and *-12* gene segments should fall between the $V_\beta 1$ and $V_\beta 6$ gene segments. Field-inversion gel analysis confirms this hypothesis (see below).

The $V_\beta 3$, *-7*, and *-15* gene segments are deleted in all of the T-cell lines, indicating that they are nearest to the D_β–J_β–C_β cluster. The $V_\beta 6$ gene segment is the next most frequently deleted V_β gene segment in these T-cell lines, indicating that it is upstream from the $V_\beta 3$, *-7*, and *-15* gene segments but downstream from the other 13 V_β gene segments included in the analysis. We were unable to determine the relative order of the $V_\beta 3$, *-7*, and *-15* gene segments using the deletion mapping method. Likewise, it was not possible to order the $V_\beta 2$, *-4*, *-10*, and *-16* gene segments and the $V_\beta 8.1$, *-8.2*, *-9*, *-11*, and *-12* gene segments with the available group of T-cell lines. These data taken together have allowed us to construct a partial physical map of the relative order of the 17 V_β gene segments analyzed (Fig. 1).

Restriction Enzyme Mapping by Field-Inversion Gel Electrophoresis. DNA from fibroblast L cells was digested with one of several infrequently cutting restriction enzymes, electrophoresed, and subjected to Southern blot analysis using DNA probes specific for each of the different V_β gene segments (Fig. 2). The logic of mapping by field-inversion gel electrophoresis rests with the supposition that if V_β gene segments are located on similar-sized fragments in multiple restriction enzyme digests, they are probably closely linked. The alternative supposition that the V_β gene segments are located on independent, identical-sized fragments becomes more unlikely as comigration with increasing numbers of different enzyme digests is observed.

A summary of the field-inversion data is given in Fig. 3 with a combined restriction enzyme and physical map of the β-chain gene family. Three clusters of V_β gene segments can be defined by comigration on two or more restriction enzyme fragments generated by distinct enzymes. Cluster 1 contains the $V_\beta 1$, *-2*, *-4*, *-10*, and *-16* gene segments. Four of these five V_β gene segments ($V_\beta 1$, *-4*, *-10*, and *-16*) reside on a DNA fragment of the same size when digested with the restriction enzymes *Xho* I, *Cla* I, *Sal* I, and *Sfi* I (Figs. 2 *a* and *b* and 3). The $V_\beta 2$ gene segment resides on the same 60-kb *Sfi* I fragment as the other four V_β gene segments (Fig. 2*b*). These results indicate that $V_\beta 2$ is linked to $V_\beta 1$, *-4*, *-10*, and *-16*. The observation that $V_\beta 2$ is separated from the other four gene segments in *Xho* I, *Cla* I, and *Sal* I digests, together with the deletion analysis, which places $V_\beta 2$ upstream from $V_\beta 1$ (Fig. 1), demonstrates that $V_\beta 2$ must be located upstream from $V_\beta 4$, *-10* and *-16*.

$$[V_\beta 2, V_\beta 4, V_\beta 10, V_\beta 16]\text{-}V_\beta 1\text{-}V_\beta 5.2\text{-}V_\beta 8.3\text{-}V_\beta 5.1\text{-}[V_\beta 8.2, V_\beta 8.1, V_\beta 9, V_\beta 11, V_\beta 12]\text{-}V_\beta 6\text{-}[V_\beta 3, V_\beta 7, V_\beta 15]\text{-}D_\beta\text{-}J_\beta\text{-}C_\beta\text{-}V_\beta 14$$

FIG. 1. Relative order of V_β gene segments determined by deletion analysis of T-cell clones. The nomenclature for the different V_β gene segments has been described (11, 13). The relative order of V_β gene segments within the brackets could not be determined from these analyses. The location of the $V_\beta 14$ gene segment had been determined (15).

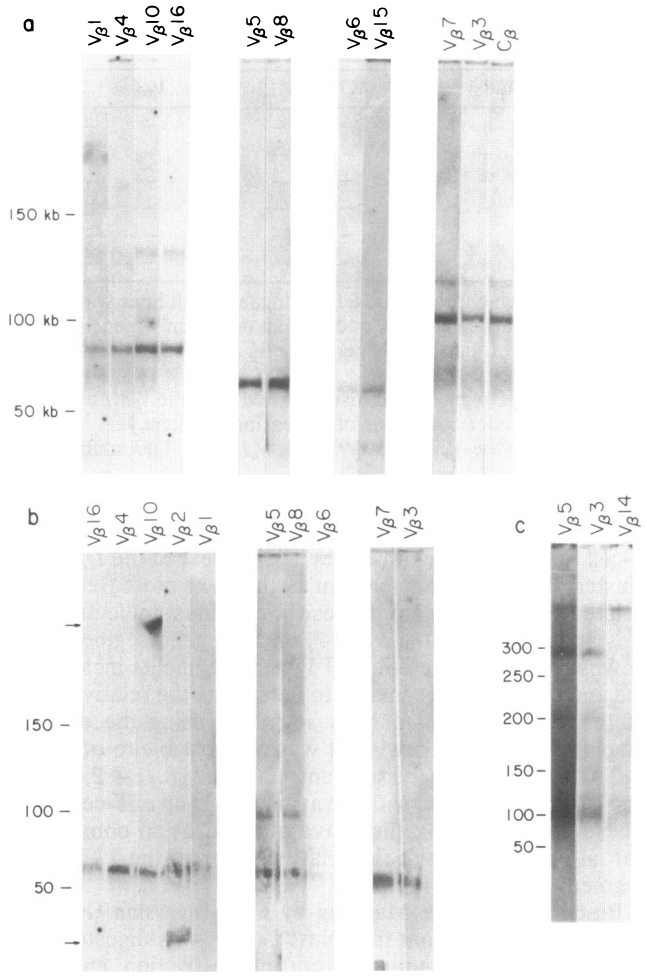

FIG. 2. Southern blot analyses of V_β gene segments on DNA fragments separated by field-inversion gel electrophoresis. The three panels contain DNAs digested with *Xho* I (*a*), *Sfi* I (*b*), and *Sal* I (*c*). Each lane contains 10 μg of L-cell DNA. Ligated λ DNA was used as size standards and switch times were selected to give optimal separation for the indicated size ranges. The arrows at the left of the lanes in *b* indicate artifacts in the Southern blots.

DNA probes specific for all of the members of the $V_\beta5$, $V_\beta8$, $V_\beta9$, $V_\beta11$, and $V_\beta12$ subfamilies hybridize to a single genomic DNA fragment when tested with the restriction enzymes *Xho* I, *Cla* I, *Sfi* I, *Sal* I, and *Pvu* I (Figs. 2 *a* and *b* and 3). These data are consistent with the evidence for interspersion of $V_\beta5$ and $V_\beta8$ subfamily members (Fig. 1) and indicate that these eight V_β gene segments are within 55 kb of each other (Fig. 3). In addition, the $V_\beta5.1$, -5.2, -6, -8.1, -8.2, -8.3, -9, -11, and -12 gene segments appear to reside on the same 55-kb *Sfi* I fragment (Fig. 2*b*). The $V_\beta6$ and $V_\beta15$ gene segments appear to be linked on an *Xho* I fragment (Fig. 2*a*). Based upon these observations, together with the conclusions from the deletion analysis, we assign the $V_\beta5.1$, -5.2, -6, -8.1, -8.2, -8.3, -9, -11, -12, and -15 gene segments to a second cluster.

We have assigned the $V_\beta3$ and $V_\beta7$ gene segments to a third cluster. Southern blot analyses indicate that both gene segments hybridize to the same 50-kb *Sfi* I and 100-kb *Xho* I genomic DNA fragments (Fig. 2 *a* and *b*). The demonstration of linkage between the $V_\beta3$ and $V_\beta7$ gene segments in conjunction with the linkage of the $V_\beta6$ and $V_\beta15$ gene segments indicates that the $V_\beta15$ gene segment is located upstream from the $V_\beta3$ and $V_\beta7$ gene segments, a conclusion that could not be drawn from the deletion analysis (Fig. 3).

To discern the relative order of the $V_\beta3$ and $V_\beta7$ gene segments, we used field-inversion gel electrophoresis to examine a T-cell hybridoma, BO4H.9.1, which possesses a rearranged $V_\beta3$ gene segment (J. Kobori and N. Shastri, unpublished observations). Field-inversion gel analysis of BO4H.9.1 DNA digested with either restriction enzyme *Xho* I or *Sfi* I and Southern blot hybridization with $V_\beta3$ and $V_\beta7$ probes revealed that the $V_\beta7$ gene segment is absent in the rearranged chromosome. This result indicates that the $V_\beta7$ gene segment is most probably located downstream from the $V_\beta3$ gene segment and is deleted on the chromosome that has undergone the $V_\beta3$ rearrangement.

Two larger fragments of DNA, each obtained from single restriction enzyme digests, allowed us to link V_β clusters *2* and *3* as well as V_β cluster *3* with the C_β genes (Fig. 3). The $V_\beta3$ and $V_\beta7$ gene segments appear to reside on the same 100-kb *Xho* I fragment as does the C_β gene and the $V_\beta14$ gene segment. Likewise, the V_β gene segments of clusters *2* and *3* appear to reside on a 290-kb *Sal* I fragment. These single observations must be confirmed by additional analyses.

Analysis of a Cosmid Clone Containing the $V_\beta1$, -4, -10, and -16 Gene Segments. One cosmid clone, D1A, contained the $V_\beta1$, -4, 10, and -16 gene segments. Restriction map analyses of clone D1A are shown in Fig. 4. All four V_β gene segments are encoded in a 15-kb DNA fragment with the 5′ to 3′ order: $V_\beta4$–$V_\beta16$–$V_\beta10$–$V_\beta1$.

DISCUSSION

Physical Map of the β-Chain Gene Family. We have constructed a partial restriction enzyme and physical map of the murine T-cell receptor β-chain gene family using deletional mapping of T-cell lines, field-inversion gel electrophoresis, and the analysis of a cosmid clone (Fig. 3). The physical map for the 17 V_β gene segments analyzed as well as the two D_β–J_β–C_β clusters and the previously mapped $V_\beta14$ gene segment (15) is complete apart from a single region between the $V_\beta1$ and $V_\beta5.2$ gene segments. The 45- and 290-kb *Sal* I fragments lying in this region could be adjacent or there may be one or more additional interposed *Sal* I fragments. Assuming that these fragments are adjacent, all 17 V_β gene segments upstream to the C_β genes are located on about 350 kb of DNA and the entire known β-chain gene family encompasses about 450 kb of DNA from the $V_\beta2$ to the $V_\beta14$ gene segments (Fig. 3).

In four inbred strains of mice the $V_\beta5$, -8, -9, -11, -12, and -13 subfamilies are deleted from the germ line (14). Our data indicate that eight of the nine V_β gene segments deleted in these mouse strains are mapped with a 55-kb fragment. This strongly suggested that this genetic event is a single deletion and the $V_\beta13$ gene segments must fall between the $V_\beta1$ and $V_\beta6$ gene segments. This is confirmed by our most recent finding that a genomic clone containing the $V_\beta8.1$ and $V_\beta8.2$ gene segments also includes the $V_\beta13$ gene segment.

Nearly 100 rearranged V_β genes have been analyzed to date and all use 1 of the 19 identified V_β gene segments. As indicated earlier, various statistical analyses suggest that there should be only 20–30 functional V_β gene segments. Therefore, perhaps most of the V_β gene segments of the mouse have already been identified. If so, the 450 or so kb of DNA that we have mapped, including any additional DNA in the putative gap between the $V_\beta1$ and $V_\beta5.2$ gene segments, should encode most of the murine β-chain gene family.

Rearrangement Mechanisms and V_β Gene Segment Organization. The murine V_β genes may assemble using any one of three mechanisms: (*i*) intrachromosomal looping out and deletion, (*ii*) interchromosomal sister chromatid exchange, and (*iii*) intrachromosomal inversion (see ref. 23 for a discussion). Studies on 44 T-cell lines suggest that the looping out and deletion mechanism is by far the most common (23). The sister chromatid exchange mechanism also leads to a deletion of intervening DNA on the chromosome that has the rearranged V_β gene and is, at the level of T-cell line analysis,

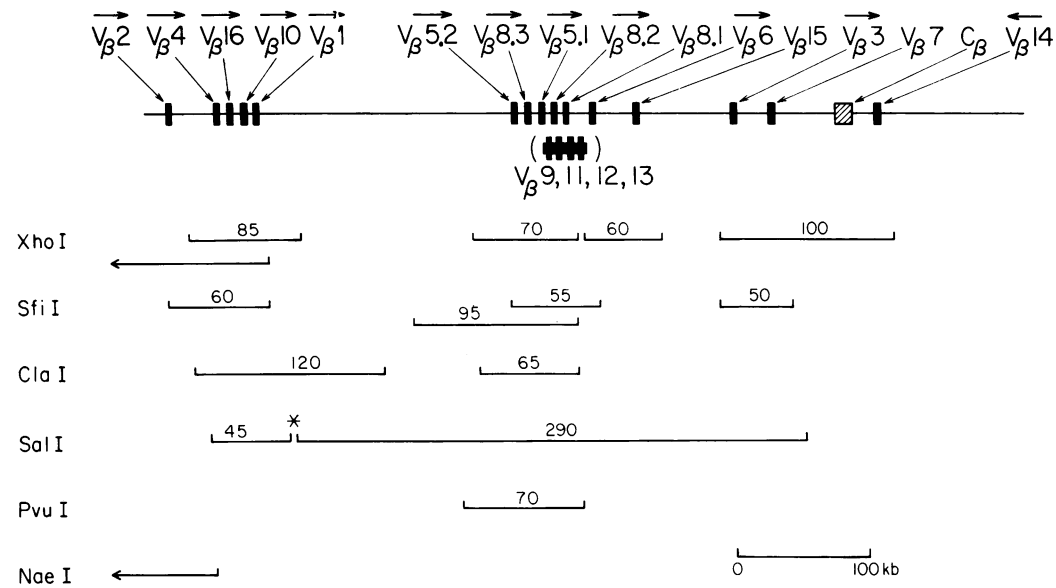

FIG. 3. Physical map of the murine β-chain gene family. This figure summarizes the data obtained from deletion analyses of T-cell lines, field-inversion gel analyses of germ-line DNA, and the characterization of the D1A cosmid. Vertical bars indicate V_β gene segments and horizontal arrows above each V_β gene segment indicate its transcriptional orientation. Overlapping fragments linking the $V_\beta 1$ and $V_\beta 5.2$ gene segments are lacking, as indicated by an asterisk between the *Sal* I fragments (see text). The position but not order of the $V_\beta 9$, *-11*, *-12*, and *-13* gene segments is indicated. The relative distances between V_β gene segments are not drawn to scale. The horizontal brackets below the line drawing and to the right of the restriction enzyme designations indicate restriction fragments of germ-line DNA that contain particular V_β gene segments. Numbers above horizontal brackets indicate the size of each particular DNA fragment in kilobase pairs (kb). Horizontal arrows immediately right of the *Xho* I and *Nae* I designations indicate partial enzyme digests. The vertical bar designated C_β on the line drawing represents the entire duplicated D_β–J_β–C_β cluster. The relative position and transcriptional orientation of the $V_\beta 14$ gene segment have been determined (15).

equivalent to the looping out and deletion mechanism. In contrast, the inversion mechanism never deletes intervening DNA; it only rearranges it. Any V_β gene that deletes other V_β genes during rearrangement and assembly must have arisen from mechanisms *i* or *ii*. These two mechanisms must operate with V_β gene segments in the same transcriptional orientation as the D_β–J_β–C_β clusters. Eleven of the V_β gene segments we analyzed are in the same transcriptional orientation as the C_β genes, indicating they must rearrange by deletion (Fig. 3). The remaining seven upstream V_β gene segments have not been analyzed in this manner. It is interesting to note that the single V_β gene segment known to be in an opposite transcriptional orientation ($V_\beta 14$) must rearrange by an inversional mechanism that rearranges but does not delete intervening DNA (15).

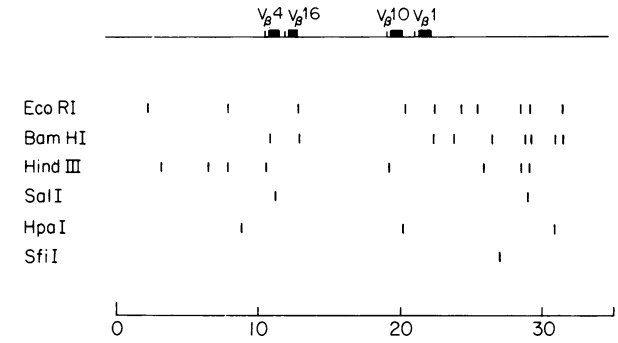

FIG. 4. Restriction map of cosmid D1A. Thin and thick boxes represent the first and second exons of the V_β gene segments, respectively. Vertical lines below the line drawing and to the right of the restriction enzyme designations represent the positions of restriction sites. Transcriptional orientations of the V_β gene segments were determined by restriction mapping with enzymes that cut asymmetrically within each V_β gene segment. Scale is in kilobase pairs. No sites for the restriction enzymes *Cla* I, *Not* I, *Pvu* I, and *Xho* I were detected. The discrepancy between the field-inversion gel and cosmid mapping-derived estimates for the size of the *Sal* I fragment containing the $V_\beta 4$, *-16*, *-10*, and *-1* gene segments may be due to partial digestion of the DNA used in the field-inversion gels. Such discrepancies have been observed (22).

Summary. We have determined a nearly complete map for all of the available murine β T-cell receptor probes—19 V_β, two D_β, and 14 J_β gene segments as well as two C_β genes. These elements extend over at least 450 kb of DNA on murine chromosome 6. We believe that this region includes most of the V_β gene segments routinely expressed in the mouse.

Note Added in Proof. We have recently linked an additional V_β gene segment, $V_\beta 17$ (provided by Paul Singer; ref. 24), to $V_\beta 3$ and $V_\beta 7$ by deletional mapping and field-inversion gel electrophoresis.

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