Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 1992–1996, April 1987
Immunology

Tandem linkage and unusual RNA splicing of the T-cell receptor β-chain variable-region genes

(alternative splicing/deletion mapping/gene duplication/T-cell-receptor genomic organization)

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Communicated by Herman N. Eisen, December 1, 1986

ABSTRACT **The variable-region (*V*) genes of the murine T-cell receptor β chain exist largely as single-element subfamilies. The $V_\beta 5$ and $V_\beta 8$ genes belong to the only two known three-member V_β subfamilies. We present studies on the linkage of these six genes and show that the genomic organization is that of alternating $V_\beta 5$ and $V_\beta 8$ genes. Our analysis suggests that these genes were tandemly duplicated, the unit of duplication being a pair of $V_\beta 5$ and $V_\beta 8$ genes. This tandem organization permits transcripts to initiate from the promoter of an unrearranged V_β located upstream of the rearranged V_β gene. These transcripts can generate functional β-chain gene messages by novel RNA splicing of the upstream leader exon to the V_β coding exon of the downstream rearranged gene. We extend the analysis of the T-cell receptor genomic organization to include 12 V_β genes and suggest that all V_β genes are closely linked on chromosome 6. In addition, we discuss the possible implications of the close linkage of the V_β genes on the development of the T-cell receptor β-chain gene repertoire.**

The variable regions of the murine T-cell receptor (TCR) genes are composed of discrete variable (*V*), diversity (*D*, in β chain), and joining (*J*) germ-line gene segments that rearrange during development to form functional transcription units (1–4). There exist relatively few TCR V_β genes (<30), most of which belong to single-element subfamilies (5, 6). The $V_\beta 5$ and $V_\beta 8$ subfamilies, each containing three V_β genes, are the only known three-member V_β gene subfamilies. In addition to the expansion by gene duplication, contraction in the size of the subfamily gene pool can also occur, as evidenced by the deletion of all $V_\beta 5$ and $V_\beta 8$ genes in the SJL mouse (7). However, the mechanisms by which a particular member of a multigene family undergoes gene expansion or contraction are not well understood.

As is the case in immunoglobulin heavy-chain variable (V_H) genes (8), the genomic organization of the V_β genes may influence the order of V_β gene rearrangement and the acquisition of the TCR β-chain gene repertoire during development. With the exception of $V_\beta 14$, which is located 3′ to, and has an inverted transcriptional orientation relative to, the D_β, J_β, and constant region (C_β) genes (9), the genomic organization of the 20 V_β genes so far identified is largely unknown. The majority of the V_β genes are presumed to lie 5′ of, and to have the same transcriptional orientation relative to, the D_β, J_β, and C_β genes.

Studies on the immunoglobulin genes have shown that, in addition to activating the promoter of the functionally rearranged *V* gene, the transcriptional enhancer located in the *J–C* intron can activate the promoter of an upstream, closely linked, germ-line *V* gene (10, 11). Recent studies have revealed the existence of nuclease hypersensitive sites in the J_β–C_β intron (12), suggesting the potential presence of transcriptional regulatory elements, such as an enhancer. It remains unclear whether the upstream germ-line V_β genes can be similarly activated and whether transcripts thus initiated can be functional.

To analyze the genomic organization of the two three-member V_β subfamilies, we constructed a complete genomic library from C57BL/6 liver DNA and isolated clones containing the six genes of the $V_\beta 5$ and $V_\beta 8$ subfamilies. Data presented here indicate that the two three-member subfamilies have probably expanded by the tandem duplication of a pair of $V_\beta 5$ and $V_\beta 8$ genes. The tandem organization of this complex has significant influence on the activation and expression of these closely linked V_β genes. In addition, we report here the analysis of the V_β genomic organization and present an overall map of the V_β family. Our results suggest that all V_β genes lie within a relatively short region on chromosome 6.

MATERIALS AND METHODS

Construction and Screening of Genomic and cDNA Libraries. Construction and screening of C57BL/6 and SJL genomic libraries and of a C57BL/6 spleen cDNA library were performed as described (13). The nucleotide sequence of relevant portions of cloned DNA was determined by either the method of Maxam and Gilbert (14) or the chain-termination method of Sanger *et al.* (15).

Hybridization of Genomic DNA Fragments. High molecular weight DNA from C57BL/6 liver and from a cytotoxic T cell clone F3 was digested to completion with *Eco*RI or *Pvu* II and fractionated in a 0.8% agarose gel. After electrophoresis, the gel was dried, and the DNA inside the gel was denatured and hybridized to radioactive DNA probes by the method of Purrello and Balazs (16). V_β-specific probes (200–350 bp) were labeled with [α-^{32}P]dCTP to a specific activity of $\geq 1 \times 10^9$ cpm/μg by using the large fragment of DNA polymerase I (Klenow fragment) and random oligonucleotide primers (17).

RESULTS

Tandem Duplication of the $V_\beta 5$ and $V_\beta 8$ Genes. Linkage of the rearranged $V_\beta 8.2$ gene and the unrearranged $V_\beta 8.3$ gene within 5 kilobases (kb) of DNA in a genomic phage clone (13) suggested to us that the three genes of the $V_\beta 8$ subfamily are tandemly duplicated and closely linked. To determine the germ-line organization of the $V_\beta 8$ genes, we screened a C57BL/6 liver genomic phage library with a $V_\beta 8$-specific probe. Four recombinant clones were obtained. Surprisingly,

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Abbreviations: TCR, T-cell antigen receptor; V, variable; D, diversity; J, joining; C, constant; H, heavy.

all four of the $V_\beta 8$-hybridizing clones also hybridized to a $V_\beta 5$ probe. These clones define a 25-kb segment of C57BL/6 genomic DNA (Fig. 1). Three $V_\beta 5$ and three $V_\beta 8$ genes were identified and linked within 18 kb of DNA. The order of these six genes (5′ to 3′) and the sizes of the *Eco*RI fragments on which they are located are: $V_\beta 5.2$ (4.6 kb), $V_\beta 8.3$ (0.85 kb), $V_\beta 5.1$ (4.2 kb), $V_\beta 8.2$ (3.2 kb), $V_\beta 5.3$ (1.5 kb), and $V_\beta 8.1$ (4.6 kb). The pattern of alternating $V_\beta 5$ and $V_\beta 8$ genes and the similarity of the distances between each $V_\beta 5$ and the $V_\beta 8$ gene immediately 3′ to it suggest that these genes have been tandemly duplicated, with the duplication unit being a pair of $V_\beta 5$ and $V_\beta 8$ genes.

An examination of the restriction map of the $V_\beta 5$–$V_\beta 8$ complex, however, did not clearly define either the length or the end points of the duplication unit. To characterize further the nature of the gene duplication events, we analyzed the restriction enzyme digests of the recombinant phage DNA by Southern blot analysis under low-stringency hybridization conditions. The only probes that hybridized to all three $V_\beta 5$–$V_\beta 8$ regions were those derived from either the V_β coding region or the 0.5-kb region 5′ of the $V_\beta 5$ and $V_\beta 8$ genes (data not shown). Thus, by the hybridization method, we are unable to define a duplicated segment of DNA much beyond the coding and its immediate 5′ regions. The duplication events probably occurred early in phylogeny, and the nucleotide sequences in the flanking regions have evolved significantly, so that cross-hybridization is no longer possible. This conclusion is supported by examination of DNA from a variety of wild mice by genomic Southern blot analysis, which showed that many subspecies of *Mus musculus* as well as species related to *Mus musculus* all have three copies each of the $V_\beta 5$ and $V_\beta 8$ genes (unpublished data). Thus, we conclude that the tandem duplication of the $V_\beta 5$ and $V_\beta 8$ genes occurred early in evolution, most likely prior to the speciation of the *Mus* genus.

Nucleotide Sequence Analysis. Analysis of the nucleotide sequence of the six $V_\beta 5$ and $V_\beta 8$ genes shows that each V_β coding exon is preceded by a leader exon of 76 bp ($V_\beta 5$) or 46 bp ($V_\beta 8$). All six genes share the same transcriptional orientation (Fig. 1). Of the three $V_\beta 5$ genes, $V_\beta 5.1$ and $V_\beta 5.2$ maintain open reading frames and are functional, whereas $V_\beta 5.3$ has a C-C dinucleotide insertion at codon 25 resulting in a premature termination of translation at codon 44 and is likely to be a pseudogene (Fig. 2). In contrast, all three $V_\beta 8$ genes maintain open reading frames and, thus, can be functional. As shown in Fig. 2, genes of the same V_β subfamily are highly homologous to each other, with 82% to 92% homology at the nucleotide level and 75% to 85% homology at the amino acid level. Thus, the sequence homology observed between murine V_β subfamily genes is similar to that seen between immunoglobulin V_H or TCR V_α subfamily genes (13, 18). The sequence diversity in these V_β genes is scattered throughout the V_β coding region and does not appear to cluster in identifiable hypervariable regions.

The nucleotide sequence of 1.5 kb of DNA between $V_\beta 8.3$ and $V_\beta 5.1$ was determined in order to identify DNA sequences that might have promoted the duplication of the $V_\beta 5$ and $V_\beta 8$ genes (data not shown). Three areas with repetitive DNA of low sequence complexity were found, including a stretch of simple C-T repeats interspersed with oligomers of cytosine, a region of T-G-C repeats, and an area with tandem repeats of a G-A-T-A-G-A hexamer. These sequences may have played a role in the development of the gene duplications we have observed. These sequences, however, differ from the putative recombination signals identified in human minisatellite regions (19) and in the murine E_β gene (20).

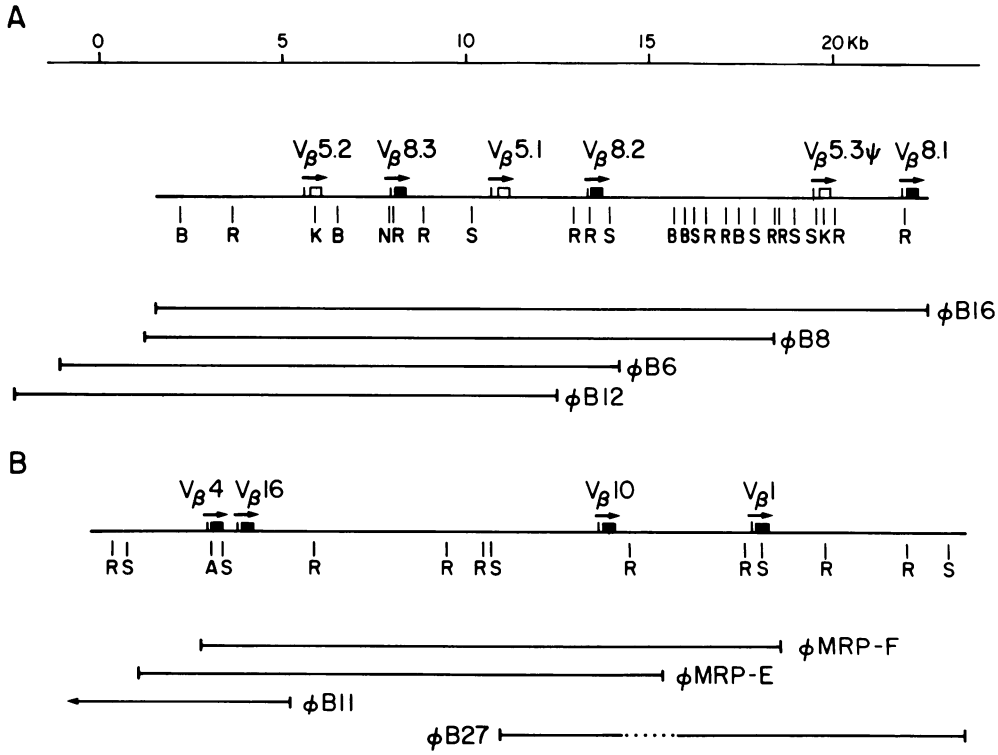

FIG. 1. Organization of the genomic phage clones containing the murine V_β genes. (*A*) Restriction enzyme map of the $V_\beta 5$–$V_\beta 8$ gene cluster. The leader and coding exons are indicated by vertical lines or boxes above the horizontal line. The four C57BL/6 genomic phage clones are shown below the map. The direction of transcription is shown by arrows. Restriction enzyme sites are indicated: A, *Sal* I; B, *Bam*HI; K, *Kpn* I; N, *Nru* I; R, *Eco*RI; and S, *Sac* I. (*B*) Restriction enzyme map of the $V_\beta 4$–$V_\beta 16$–$V_\beta 10$–$V_\beta 1$ gene cluster. φMRP-E and φMRP-F were isolated from an SJL genomic library; φB11 and φB27 were from a C57BL/6 genomic library. The dotted line in φB27 represents a 1.5-kb deletion, relative to the SJL genome, in C57BL/6 near the $V_\beta 10$ gene. This 1.5-kb deletion accounts for the restriction fragment length polymorphism observed in the $V_\beta 1$ and $V_\beta 10$ genes between these two strains of mice. Further analysis indicates that C57BL/6 and SJL are otherwise identical in this gene cluster (data not shown).

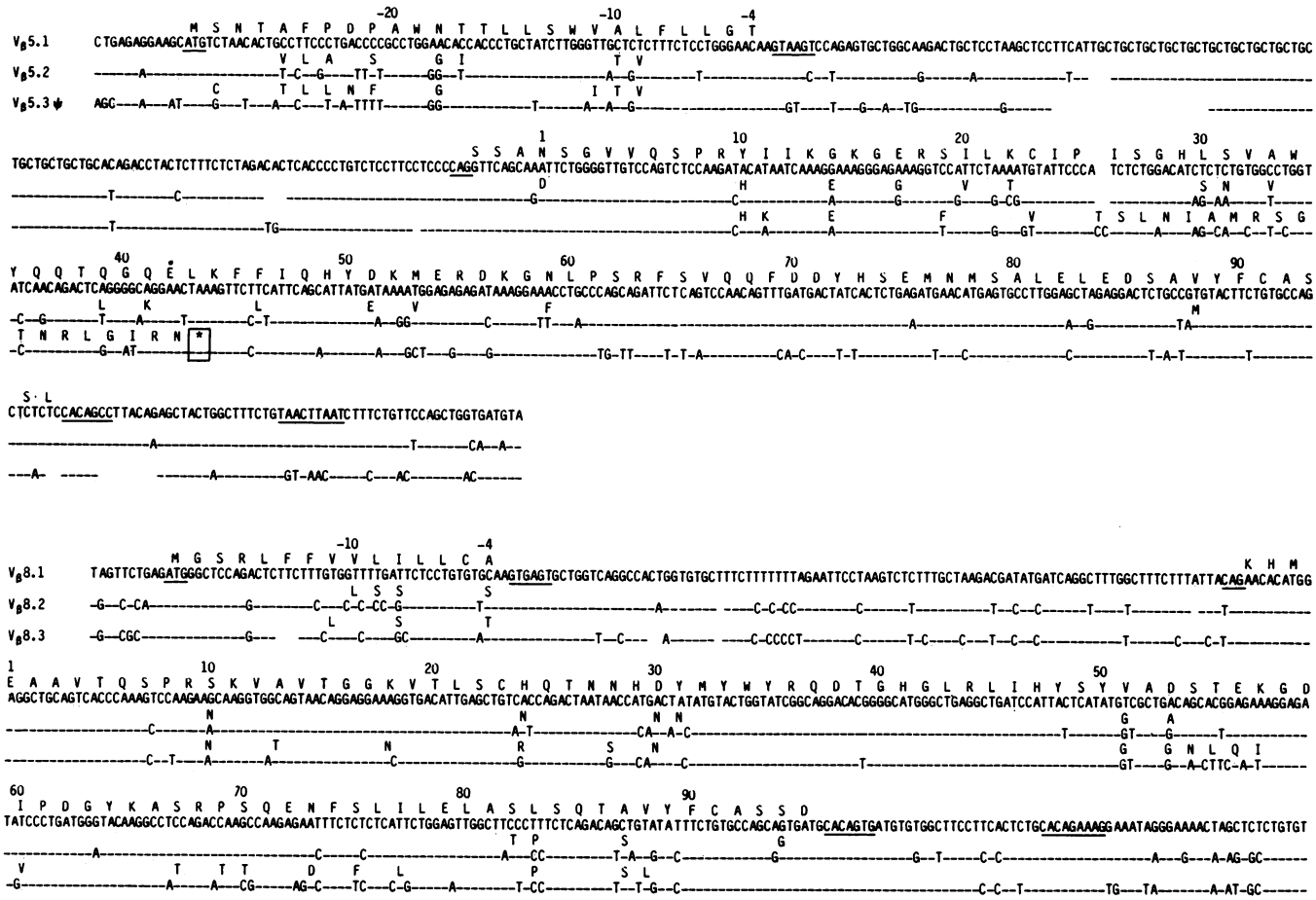

FIG. 2. Nucleotide and amino acid sequences of the $V_\beta 5$ and $V_\beta 8$ subfamily genes. Gaps are introduced to maximize sequence homology. Dashes represent nucleotide sequence identity to the top line; amino acid residues different from that of the top line are indicated. Consensus RNA splice sites, nonamer and heptamer recombination sequences, and putative initiation codons are underlined. The termination codon within the $V_\beta 5.3$ gene at codon 44 is boxed and indicated by an asterisk.

Alternative Splicing of $V_\beta 5$–$V_\beta 8$ Transcripts. When the nucleotide sequences of the germ-line $V_\beta 8$ genes were compared to our previously isolated $V_\beta 8$-containing cDNA clones, a striking discrepancy was noted. From the V_β–D_β junction to the −3 codon, the V_β sequence of a C57BL/6 spleen cDNA, pSA17, is identical to the germ-line $V_\beta 8.2$ gene (Fig. 3). However, the nucleotide sequence of the 5′-most 90 bp of pSA17 does not correspond to the leader exon of the $V_\beta 8.2$ gene. Surprisingly, this 90-bp sequence is identical to the leader exon of the $V_\beta 5.1$ gene, the V_β gene immediately 5′ to the $V_\beta 8.2$ gene. Therefore, transcripts can initiate from the promoter of an upstream germ-line V_β gene and splice correctly to a downstream rearranged V_β coding exon, forming functional TCR β-chain messages.

To investigate whether this novel alternative splicing between $V_\beta 5.1$ and $V_\beta 8.2$ is obligatory for the expression of the rearranged $V_\beta 8.2$ genes, we screened a C57BL/6 spleen cDNA library with a $V_\beta 8$-specific probe. Of the 15 independent $V_\beta 8/C_\beta$-containing clones analyzed, 10 contain the $V_\beta 8.2$ gene. Surprisingly, all 10 contain the $V_\beta 5.1$ leader exon spliced properly to the $V_\beta 8.2$ coding exon (Fig. 3). An examination of the published $V_\beta 8$ sequences revealed that the $V_\beta 8.2$ gene from a C57BL/6 thymus cDNA library reported by Barth *et al.* (5) also contains a properly spliced $V_\beta 5.1$ leader exon. Therefore, the $V_\beta 5.1$ leader exon is found correctly spliced to the $V_\beta 8.2$ coding exon in all $V_\beta 8.2$-containing cDNA clones from the C57BL/6 mouse. However, the $V_\beta 8.2$ leader exon has been observed to splice properly to the $V_\beta 8.2$ gene (designated $V_\beta 4$ in ref. 6) in a cDNA clone from BALB/c thymus. In addition, the $V_\beta 5.1$ leader is spliced correctly to the $V_\beta 5.1$ coding exon in all $V_\beta 5.1$-containing cDNA clones examined.

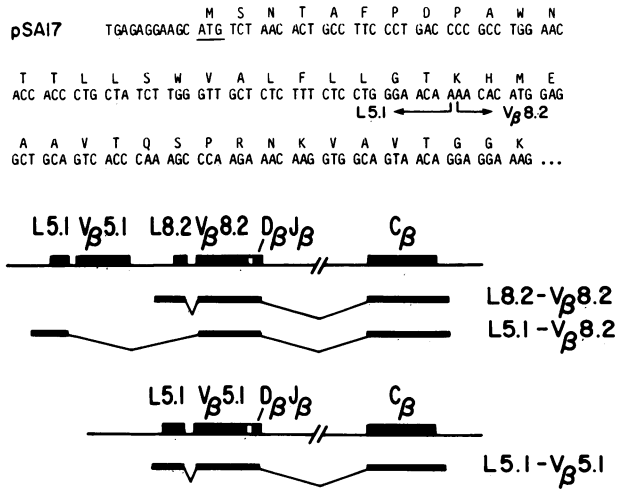

FIG. 3. Sequence of a C57BL/6 $V_\beta 8.2$ gene and the schematic representation of the RNA splicing of the $V_\beta 5$–$V_\beta 8$ transcripts. (*Upper*) Nucleotide and amino acid (single-letter code) sequences of pSA17, a C57BL/6 spleen cDNA clone containing the $V_\beta 5.1$ leader exon and $V_\beta 8.2$ coding exon. (*Middle*) TCR β-chain gene locus containing a rearranged $V_\beta 8.2$ gene. (*Lower*) TCR β-chain gene locus containing a rearranged $V_\beta 5.1$ gene. Exons found in mature RNA transcripts are represented by thick lines, and introns removed by RNA splicing are represented by thin lines. The exon–intron organization and the RNA splicing of the C_β gene are not shown. L, leader exon.

To ascertain whether such alternative RNA splicing between two V_β genes is a common phenomenon among the closely linked genes in the $V_\beta 5$–$V_\beta 8$ complex, we analyzed the remaining five clones containing both $V_\beta 8$ and C_β. One clone contains the $V_\beta 8.1$ gene and has the $V_\beta 8.1$ leader. Four clones contain the $V_\beta 8.3$ gene and have the natural $V_\beta 8.3$ leader exon. The β-chain gene of the C57BL/6 helper T-cell clone C5 (21) also contains the natural $V_\beta 8.1$ leader exon spliced to the $V_\beta 8.1$ coding exon. Furthermore, the natural $V_\beta 16$ leader exon was found in all 10 $V_\beta 16$-containing cDNA clones examined despite the presence of an unrearranged $V_\beta 4$ gene located less than 1 kb upstream (see below). Thus, the alternative RNA splicing of the leader exon of an upstream V_β to the coding exon of the downstream rearranged V_β gene is not a general phenomenon in the V_β gene family.

Genomic Organization of the V_β Gene Family. To determine if other V_β genes are closely linked, we screened a genomic library of C57BL/6 liver DNA with a mixture of 16 V_β probes. Positive clones were then hybridized with a panel of individual V_β probes. Two clones hybridized to more than one V_β-specific probe. Further analysis indicated that the $V_\beta 1$ and $V_\beta 10$ genes are linked within 5 kb of DNA in one clone and that the $V_\beta 4$ and $V_\beta 16$ genes are linked within 1 kb of DNA in the other clone (Fig. 1*B*). More recently, our analysis of genomic phage clones isolated from an SJL liver library revealed a 5′ $V_\beta 4$–$V_\beta 16$–$V_\beta 10$–$V_\beta 1$ 3′ organization within 16 kb of DNA, with all four genes sharing the same transcriptional orientation. To date, 12 of the 20 known V_β genes have been linked in three clusters. These three clusters of V_β genes may in turn be closely linked.

If most TCR V_β genes are located 5′ to the C_β gene and rearrange by chromosomal deletion, the V_β genes located 3′ to the rearranged V_β gene would be deleted in any given T cell. We have reported (13) that $V_\beta 8.2$ and $V_\beta 11$ are rearranged in a cytotoxic T-lymphocyte (CTL) clone, F3. If the $V_\beta 5$–$V_\beta 8$ cluster is located 5′ to the $V_\beta 12$–$V_\beta 11$ cluster, the germ-line $V_\beta 11$ gene would be absent, and single-copy germ-line $V_\beta 5.3$ and $V_\beta 8.1$ genes would be present in F3. Fig. 4 shows that this is indeed the case. Since none of the V_β genes of the $V_\beta 4$–$V_\beta 16$–$V_\beta 10$–$V_\beta 1$ cluster is deleted in F3, this cluster is probably located 5′ of the $V_\beta 5$–$V_\beta 8$ and $V_\beta 12$–$V_\beta 11$ clusters. We cannot rule out the possibility, however, that these four genes are located 3′ to the C_β and rearrange by chromosomal inversion. In contrast, deletion of the $V_\beta 3$ (2B4) and $V_\beta 6$ (LB2) genes in F3 suggests that these genes are located between the $V_\beta 12$–$V_\beta 11$ cluster and the C_β genes.

DISCUSSION

Data presented here indicate that a pair of $V_\beta 5$ and $V_\beta 8$ genes was tandemly duplicated to generate the two three-member V_β subfamilies. The duplication event occurred at least twice early in evolution, most likely before the speciation of the *Mus* genus. More recent studies have shown that the rat $V_\beta 5$ and $V_\beta 8$ genes also belong to multimember subfamilies among the otherwise single-member rat V_β subfamilies (unpublished data). Why the $V_\beta 5$ and $V_\beta 8$ genes were preferentially expanded in these species is not known. Divergence of the nucleotide sequence in the noncoding region since the last duplication event makes it difficult to identify the precise length and end points of the duplication unit and the sequence that might have mediated the gene duplication event.

Close linkage is often a distinctive feature of the organization of multigene families (22). Several *V* genes of immunoglobulin heavy or κ chain families have been physically linked on phage or cosmid clones (23–25). The murine TCR β-chain genes have been localized to chromosome 6 by somatic cell hybrid techniques (26, 27). Data presented here are consistent with the notion that all V_β genes lie within a relatively short distance. Assuming that the average distance between two adjacent V_β genes is 10 kb, we estimate that the 20 or so *V* genes of the murine TCR β chain are clustered within 200 kb of DNA on chromosome 6.

The TCR repertoire expressed in the developing fetal thymus may be important in thymic education, during which

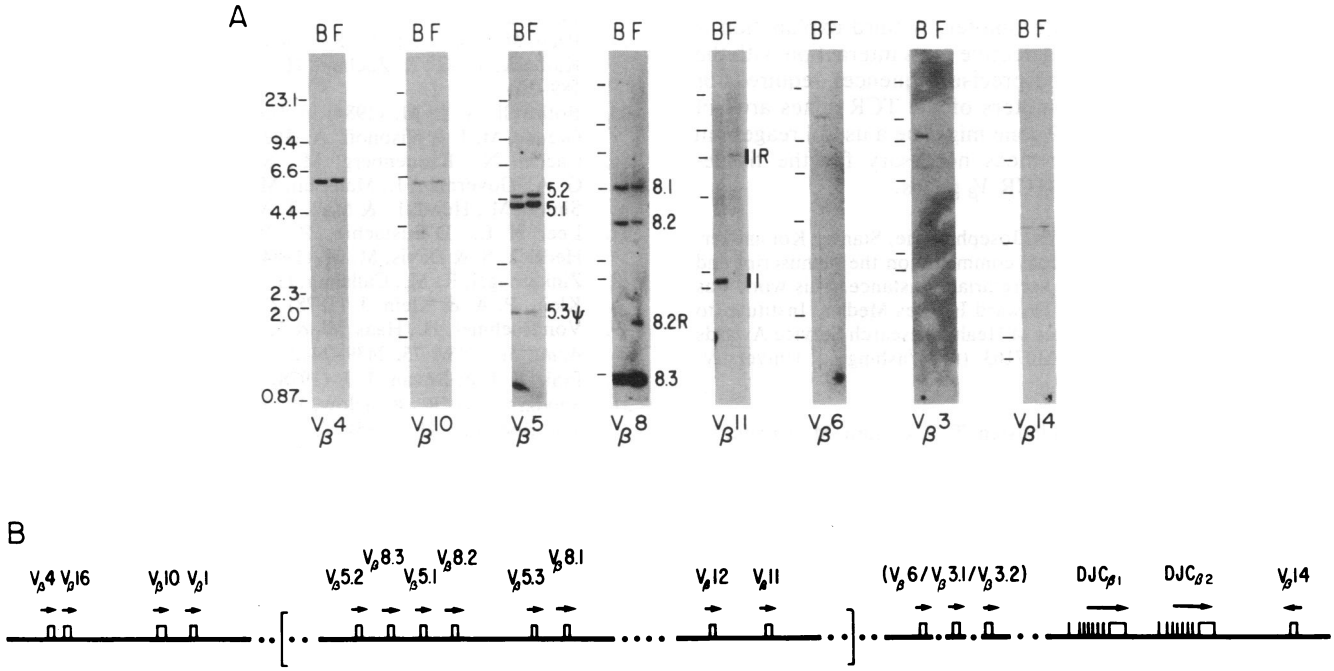

FIG. 4. Deletion mapping and genomic organization of genes of the murine V_β family. (*A*) Deletion mapping of the V_β genes. Genomic DNA from C57BL/6 liver (lanes B) and from the F3 CTL (lanes F) was digested with either *Pvu* II ($V_\beta 11$ lanes) or *Eco*RI (all other lanes), and the restricted fragments were hybridized with the indicated V_β probes. Rearranged V_β genes (labeled with an "R") are indicated. Size markers are indicated to the left in kilobases. (*B*) The overall map of the murine V_β gene family. Exons are indicated by vertical lines or boxes, and the transcriptional orientations are indicated by horizontal arrows. The dotted lines represent regions of DNA not yet molecularly linked. The V_β genes absent from the SJL mouse are enclosed by brackets.

precursor T cells are selected by the host thymus to acquire self-versus-nonself discrimination (28–30). The β-chain gene transcripts, assayed by a C_β probe, are detectable on day 15 of fetal development and are expressed at high levels on day 17, when the surface expression of the TCR first occurs (31, 32). Whether the β-chain gene transcripts expressed at the various developmental stages of the fetal thymus show preferential V_β gene usage remains unclear. Studies on the immunoglobulin H chain have revealed that the most J_H-proximal V_H genes are preferentially utilized in V_H–D_H–J_H rearrangements in pre-B-cell lines (8). By analogy to the V_H genes, we might expect that the V_β genomic organization influences the order of V_β gene rearrangement and, hence, the acquisition of the TCR β-chain gene repertoire. For example, the more J_β-proximal V_β genes, $V_\beta 3$ (2B4) and $V_\beta 6$ (LB2), may be preferentially rearranged and expressed early in the developing fetal thymus. Data presented here on the genomic organization of the V_β genes provide a clear basis for further investigation of this question.

Mature TCR β-chain gene messages are usually generated by RNA splicing of transcripts initiating from the promoters of the rearranged V_β genes. We show here that transcription from an upstream germ-line V_β can generate functional transcripts by alternative splicing of the leader exon of an upstream unrearranged V_β gene to the coding exon of the downstream rearranged V_β gene. This novel alternative splicing mechanism appears to dominate in the expression of the $V_\beta 8.2$ gene in the C57BL/6 mouse. As such, transcription from an upstream unrearranged V_β rescues the expression of the downstream productively rearranged V_β gene. Why alternative splicing of the leader exon is not observed in other closely linked V_β genes is not clear and awaits further study.

There are at least three explanations for why the $V_\beta 8.2$ gene does not use its own leader exon. One explanation is that the RNA splicing signals for the removal of the $V_\beta 8.2$ intron are defective. This possibility is unlikely in that at least the 5′ donor and 3′ acceptor sites in the $V_\beta 8.2$ intron are identical to those in the $V_\beta 8.3$ gene and agree with the consensus sequences. A second explanation is that the $V_\beta 5.1$ promoter is stronger than the $V_\beta 8.2$ promoter. A third explanation is that the $V_\beta 8.2$ promoter is defective in its interaction with the transcriptional factors. The precise sequences required for proper functioning of promoters of the TCR genes are not known. As such, the $V_\beta 8.2$ gene might be a useful reagent in identifying promoter sequences necessary for the tissue-specific expression of the TCR V_β genes.

We thank Drs. David Chaplin, Joseph Davie, Stanley Korsmeyer, and Mr. William Sha for critical comments on the manuscript and Madline Pearlman for expert secretarial assistance. This work was supported by funds from the Howard Hughes Medical Institute (to D.Y.L.) and National Institutes of Health Research Service Awards GM07067, GM07200, and AI07163 (to Washington University, H.S.C., S.J.A., and M.A.B.).

1. Chien, Y., Becker, D., Lindsten, T., Okamura, M., Cohen, D. & Davis, M. (1984) *Nature (London)* **312,** 31–35.
2. Saito, H., Kranz, D., Takagaki, Y., Hayday, A., Eisen, H. & Tonegawa, S. (1984) *Nature (London)* **312,** 36–40.
3. Siu, G., Kronenberg, M., Strauss, E., Haars, R., Mak, T. & Hood, L. (1984) *Nature (London)* **311,** 344–350.
4. Kronenberg, M., Siu, G., Hood, L. & Shastri, N. (1986) *Annu. Rev. Immunol.* **4,** 529–591.
5. Barth, R., Kim, B., Lan, N., Hunkapiller, T., Sobieck, N., Winoto, A., Gershenfeld, H., Okada, C., Hansburg, D., Weissman, I. & Hood, L. (1985) *Nature (London)* **316,** 517–523.
6. Behlke, M., Spinella, D., Chou, H., Sha, W., Hartl, D. & Loh, D. (1985) *Science* **229,** 566–570.
7. Behlke, M. A., Chou, H. S., Huppi, K. & Loh, D. Y. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 767–771.
8. Yancopoulos, G. D., Desiderio, S. V., Paskind, M., Kearney, J. F., Baltimore, D. & Alt, F. W. (1984) *Nature (London)* **311,** 727–733.
9. Malissen, M., McCoy, C., Blanc, D., Trucy, J., Devaux, C., Schmitt-Verhulst, A., Fitch, F., Hood, L. & Malissen, B. (1986) *Nature (London)* **319,** 28–33.
10. Wang, X. & Calame, K. (1985) *Cell* **43,** 659–665.
11. Atchison, M. L. & Perry, R. P. (1986) *Cell* **46,** 253–262.
12. Bier, E., Hashimoto, Y., Greene, M. I. & Maxam, A. (1985) *Science* **229,** 528–534.
13. Chou, H. S., Behlke, M. A., Godambe, S. A., Russell, J. H., Brooks, C. G. & Loh, D. Y. (1986) *EMBO J.* **5,** 2149–2155.
14. Maxam, A. & Gilbert, W. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 560–564.
15. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5463–5467.
16. Purrello, M. & Balazs, I. (1983) *Anal. Biochem.* **128,** 393–397.
17. Fineberg, A. P. & Vogelstein, B. (1983) *Anal. Biochem.* **132,** 6–13.
18. Bothwell, A. L. M., Paskind, M., Reth, M., Imanishi-Kari, T., Rajewsky, K. & Baltimore, D. (1981) *Cell* **24,** 625–637.
19. Jeffreys, A. J., Wilson, V. & Thein, S. L. (1985) *Nature (London)* **314,** 67–73.
20. Steinmetz, M., Stephan, D. & Lindahl, K. F. (1986) *Cell* **44,** 895–904.
21. Patten, P., Yokota, T., Rothbard, J., Chien, Y., Arai, K. & Davis, M. M. (1984) *Nature (London)* **312,** 40–46.
22. Hood, L., Campbell, J. H. & Elgin, S. C. R. (1975) *Annu. Rev. Biochem.* **44,** 305–353.
23. Rechavi, G., Bienz, B., Ram, D., Ben-Neriah, Y., Cohen, J. B., Zakut, R. & Givol, D. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 4405–4409.
24. Pech, M., Jaenichen, H.-R., Pohlenz, H.-D., Neumaier, P. S., Klobeck, H.-G. & Zachau, H. G. (1984) *J. Mol. Biol.* **176,** 189–204.
25. Bothwell, A. L. M. (1984) in *The Biology of Idiotypes*, eds. Greene, M. I. & Nisonoff, A. (Plenum, New York), pp. 19–34.
26. Caccia, N., Kronenberg, M., Saxe, D., Haas, R., Bruns, G. A., Goverman, J., Malissen, M., Willard, H., Yoshikai, Y., Simon, M., Hood, L. & Mak, T. W. (1984) *Cell* **37,** 1091–1099.
27. Lee, N. E., D'Eustachio, P., Pravcheva, D., Ruddle, F., Hedrick, S. & Davis, M. M. (1984) *J. Exp. Med.* **160,** 905–913.
28. Zinkernagel, R. M., Callahan, G. N., Althage, A., Cooper, S., Klein, P. A. & Klein, J. (1978) *J. Exp. Med.* **147,** 882–896.
29. Von Boehmer, H., Haas, W. & Jerne, N. K. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 2439–2442.
30. Fink, P. J. & Bevan, J. J. (1978) *J. Exp. Med.* **148,** 766–773.
31. Snodgrass, H. R., Kisielow, P., Kiefer, M., Steinmetz, M. & Von Boehmer, H. (1985) *Nature (London)* **313,** 592–595.
32. Raulet, D. H., Garman, R. D., Saito, H. & Tonegawa, S. (1985) *Nature (London)* **314,** 103–107.