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# Organization of the T-cell antigen-receptor β-chain locus in mice

**(orthogonal pulsed-field gel electrophoresis/variable-region deletions/inversion/looping-out and excision)**

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ABSTRACT **We used pulsed-field gel electrophoresis to determine the organization of the β-chain gene of the T-cell receptor for antigen in normal and mutant inbred strains of mice. In normal mice, the variable (*V*)- and constant (*C*)-region elements of this locus span 700–800 kilobases of chromosomal DNA. All but one of the $V_\beta$ gene segments analyzed lie 5′ of the $J_\beta C_\beta$ locus ($J_\beta$ represents the joining region), with the closest being 280–360 kilobases away. The mutant mouse strain SJL has an internal $V_\beta$-region gene deletion that compacts the $V_\beta$ region by 100–200 kilobases. Taken together with other data, these results indicate that the β-chain locus can use either a looping-out/deletion or an inversion mechanism to appose $V_\beta$ to $DJ_\beta$ gene segments (*D* is the diversity region) and can accomplish the former (at least) over very large distances.**

The antigen receptors of lymphocytes are the primary means of recognizing entities as foreign or abnormal. In the case of B lymphocytes, cell-surface and secreted forms of immunoglobulin fulfill this function. In T lymphocytes, the T-cell receptors for antigen are responsible. Because of the myriad ligands that these receptors may encounter, the organization of antigen-receptor genes seems highly specialized and complex. Both immunoglobulin and T-cell receptor genes consist of arrays of distinct gene segments [variable (*V*), joining (*J*), and, in some cases, diversity (*D*)], with one member of each type of segment able to join one of the others to form a specific *VJ* or *VDJ* combination. The number of functional *V* elements within a given antigen-receptor locus ranges from one to several hundred; the number of *D* elements varies from 2 to 12 and the number of *J* elements similarly encompasses 2 to more than 50 in the T-cell receptor α-chain locus (reviewed in refs. 1 and 2). Thus, the number of possible *VJ* or *VDJ* exons that can be formed is often in the thousands [$V \times J$ ($\times D$)]. This number is many orders of magnitude higher when variability at the joining sites and the apparently random addition of nucleotides between segments are included in the calculation. In all cases, the resulting *VJ* or *VDJ* exon is adjacent to an array of exons encoding a constant (C) region of the molecule, allowing for the expression of "VJC" or "VDJC" mRNA and proteins. Thus, antigen-receptor loci are large, multipartite entities with a number of interchangeable coding elements that can produce any one of an often huge number of polypeptides from a given chromosome. Because of their unique design and mechanism of rearrangement-dependent expression, it is of interest to know how much chromosomal DNA is required to encode such a complex type of gene and what the spatial orientation of the coding segments can be with respect to each other. There are only a few cases in which a physical linkage between *V* and *J* gene segments has been demonstrated (3–5), and all concern *V* loci that are located immediately adjacent to the *J–C* loci. One such case is that of the chicken immunoglobulin λ light chain gene, where the single functional *V* gene is located only 1.7 kilobases (kb) 5′ to the *J* gene segment (3). Another example is $V_{\beta 14}$, which is located about 10 kb 3′ to the $J_\beta C_\beta$ coding regions and in an inverted orientation (4). Rearrangement of $V_{\beta 14}$ appears to occur by inversion. In addition, classical genetic analyses show the *Igh* locus to encompass 2 map units of chromosome 12 of the mouse (6), although one cannot translate this number into nucleotides with any certainty because of variability in the frequency of recombination between different loci (7). The recent introduction of pulsed-field gel electrophoretic analysis, however, makes concrete determinations of distances up to several thousand kilobases possible (8, 9). We have used this technology to determine the linkage between the *V* gene segments and the *C* regions of the T-cell receptor β-chain locus. We find that this locus spans at least 700 kb of DNA and that most of the *V* gene segments are 5′ with respect to the $J_\beta C_\beta$ coding elements. Because the rearrangement of $V_\beta$ genes in this 5′ region is associated with the deletion of intervening DNA, these results indicate that both looping-out/deletion and inversion mechanisms of rearrangement can be utilized by the same antigen-receptor locus. This is consistent with and lends further support to the suggestion of Alt and Baltimore (10) that these are two potential consequences of the same basic rearrangement process, with the outcome (deletion or inversion) depending only on the relative spatial orientation of the *V* and the *J* (or *DJ*) elements.

## MATERIALS AND METHODS

**Preparation of DNA.** Liver DNA for pulsed-field gel electrophoresis was prepared as described (11). In brief, livers from C57BL/6 and SJL mice were frozen in liquid nitrogen and crushed using a mortar and pestle. Nuclei were prepared by resuspending the powder in 5% citric acid and homogenizing in a Dounce homogenizer as described (12). After washing in 5% citric acid, the nuclei were pelleted through a 0.88 M sucrose cushion. Finally, the nuclei were washed and resuspended at $2 \times 10^7$ per ml in RSB (10 mM NaCl/10 mM Tris, pH 7.5/25 mM EDTA). To prepare DNA in agarose blocks, a modification of the procedure described by van der Ploeg *et al.* (13) was used. The nuclei were mixed with an equal volume of 1% low-melting-point agarose in RSB containing proteinase K at 60 μg/ml. The suspension was transferred to sample holders and allowed to solidify in 100-μl aliquots. Each agarose block was then incubated for 16–24 hr in RSB with 1% $NaDodSO_4$ at 50°C. After lysis, the blocks were washed in 200 volumes of 10 mM Tris, pH 7.5/10 mM EDTA/0.1 mM phenylmethylsulfonyl fluoride, followed by equilibration in 100 volumes of the appropriate restriction buffer, as described (14). Half of each block was then placed in 50 μl of restriction buffer with 30 units of enzyme and digested overnight.

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Abbreviations: V, variable; D, diversity; J, joining; C, constant.

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**Gel Electrophoresis and Southern Blots.** After digestion, the restriction buffer was removed and the agarose block was melted at 68°C and pipetted into the slots of a 1% agarose gel. The electrophoresis apparatus used was built according to the specifications published by Carle and Olsen (9). The gels were run in 0.5× TBE at 11°C. (1× TBE is 90 mM Tris/90 mM boric acid/2.5 mM EDTA.) The pulse duration used was 20 sec, which gives good separation up to 450 kb. The gels were generally run for 10 hr at 300 volts. Self-ligated phage λ DNA, prepared as described (9), was used as size markers. To facilitate transfer to nylon membranes (GeneScreen, New England Nuclear), the gels were irradiated at 254 nm for 2 min following ethidium bromide staining. DNA was denatured (in the gels) with 0.3 M NaOH/0.6 M NaCl, followed by neutralization in 25 mM $Na_2HPO_4/NaH_2PO_4$ at pH 6.5. After the nylon membrane was baked in a vacuum oven for 2 hr, the DNA was crosslinked to the membrane by irradiation at 254 nm for 90 sec (8). Hybridization was performed according to the manufacturer's instructions (GeneScreen). The $D_{\beta 1}$ probe was a 1.2-kb *Pst* I genomic fragment covering sequences 5′ to the $D_{\beta 1}$ region (15). The $V_\beta$-region probes used were $V_{\beta E1}$ ($V_{\beta 2}$), *Pst* I(v)–*Pst* I fragment (16); $V_{\beta 86T1}$ ($V_{\beta 1}$), *Eco*RI(v)–*Bam*HI (17); $V_{\beta C5}$ ($V_{\beta 8.1}$), *Pst* I–*Pvu* II (16); $V_{\beta SJL73}$ ($V_{\beta 15}$ or $V_{\beta FN1\text{-}18}$), *Eco*RI(v)–*Xmn* I (18, 19); $V_{\beta LB2}$ ($V_{\beta 6}$), *Bst*EII–*Xho* I (16); $V_{\beta 2B4}$ ($V_{\beta 3}$), *Eco*RI(v)–*Rsa* I (20); $V_{\beta 14}$ ($V_{\beta J6.19}$), *Eco*RI(v)–*Bgl* I (ref. 4; from E. Loh). The symbol (v) represents sites generated in cloning. Inserts were purified from *V*-region subclones by polyacrylamide gel electrophoresis and radiolabeled by the hexamer labeling technique (21). Membranes were washed twice for 30 min in 2× SSPE/0.1% $NaDodSO_4$ at 55°C and then once for 30 min in 0.2× SSPE at 55°C (1× SSPE is 180 mM NaCl/10 mM $Na_2HPO_4$/1 mM EDTA, pH 7.4) as per Maniatis *et al.* (22).

## RESULTS

**Methylation-Sensitive Enzymes Create Large Restriction Fragments.** A panel of six $V_\beta$-region gene families were hybridized to various digests of C57BL/6 liver DNA. The DNA was prepared in agarose blocks (see *Materials and Methods*) to minimize shearing of the DNA. To create large restriction fragments, we used restriction enzymes with 6-base-pair recognition sequence containing the dinucleotide "CG" (11, 23, 24). The relative infrequency of this dinucleotide pair in mammalian DNA (25, 26), as well as the fact that the deoxycytosine residue is a frequent target of methylation (reviewed in ref. 27), contributes to the generation of high molecular weight fragments. We found three such enzymes, *Nae* I, *Sal* I, and *Xma* I, to be useful for mapping the T-cell receptor β-chain locus. As can be seen from the Southern blots in Figs. 1 and 2, these enzymes not only create large fragments but also can often form "ladders" of partial digestion products, presumably due to variable methylation of the restriction sites. These "partials" are often useful because they permit linking of genes located at a great distance from each other, but they can make exact mapping difficult.

**$V_{\beta 2B4}$ Is Located on the 5′ Side of $C_\beta$.** Deletion mapping indicates that $V_{\beta 2B4}$ is the most proximal in our panel of $V_\beta$-region gene segments (N.E.L. and M.M.D., unpublished data). Fig. 1*A* shows a Southern blot of *Nae* I-digested C57BL/6 liver DNA probed with a $D_{\beta 1}$-region probe. Two fragments of 360 and 280 kb hybridized to this probe. When the same filter was probed with a $V_{\beta 14}$-region probe, a fragment of 75 kb was seen (Fig. 1*A*). Thus, the internal *Nae* I sites in the $C_\beta$ region (data not shown) must have been digested to completion, because no overlapping bands are seen with these two probes. When the same filter was probed with the $V_{\beta 2B4}$ probe, one major band was seen at 360 kb, overlapping with the upper band seen with the $D_{\beta 1}$ probe, as well as a faint band at about 100 kb, corresponding roughly to the difference between the 360- and 280-kb bands. Thus,

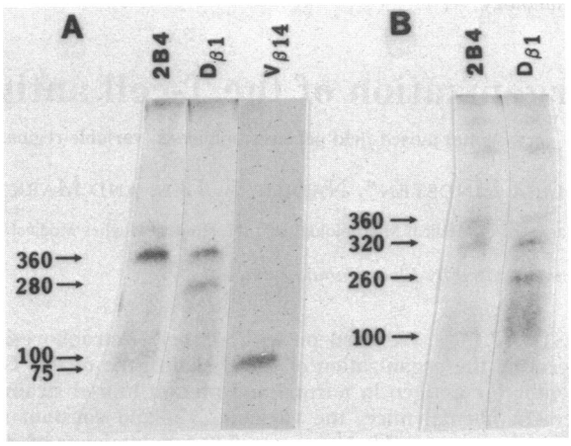


FIG. 1. Linkage of $V_{\beta 2B4}$ to the 5′ side of the $C_\beta$-region complex. C57BL/6 (*A*) and SJL (*B*) liver DNAs were digested with *Nae* I and separated by pulsed-field gel electrophoresis. The same filter strip was sequentially probed with $D_{\beta 1}$, $V_{\beta 2B4}$ ($V_{\beta 3}$), and $V_{\beta 14}$ probes. Fragment sizes are indicated in kb.

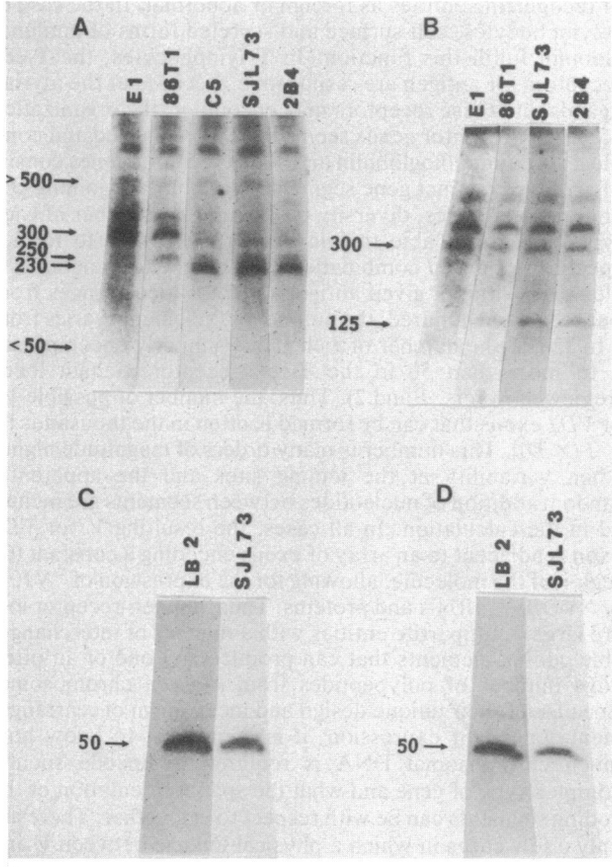


FIG. 2. Linkage of $V_\beta$-region genes. C57BL/6 (*A*) and SJL (*B*) liver DNAs were digested with *Sal* I and separated by pulsed-field gel electrophoresis. The same filter strip was sequentially hybridized with probes specific for $V_{\beta 2B4}$ ($V_{\beta 3}$), $V_{\beta SJL73}$ ($V_{\beta 15}$), $V_{\beta C5}$ ($V_{\beta 8.1}$), $V_{\beta 86T1}$ ($V_{\beta 1}$), and $V_{\beta E1}$ ($V_{\beta 2}$). C57BL/6 (*C*) and SJL (*D*) liver DNAs were digested with *Xma* I and separated by pulsed-field gel electrophoresis. The same filter strips were sequentially hybridized with probes specific for $V_{\beta LB2}$ ($V_{\beta 6}$) and $V_{\beta SJL73}$ ($V_{\beta 15}$).

$V_{\beta 2B4}$ appears to be located on the 5′ side of the $C_\beta$ region at a distance of more than 280 but less than 360 kb.

Further confirmation of this linkage was provided by a *Nae* I digest of SJL liver DNA (Fig. 1*B*). In addition to two high molecular weight partials, three major bands at 360, 320, and 260 kb hybridized with the $D_{\beta 1}$ probe. When the same filter was reprobed with the $V_{\beta 2B4}$ probe, four of these five fragments (not the 260-kb band) hybridized. The number of SJL bands that hybridize with both probes indicates that the single coincident band in C57BL/6 DNA represents true linkage between *V* and *C* regions. A band at about 100 kb was seen with the $V_{\beta 2B4}$ probe. In both C57BL/6 and SJL liver DNA, the intensity of the band at 100 kb was very weak. One possible explanation for this is that the presence of several *Nae* I sites accessible for digestion (i.e., unmethylated) on the same molecule is rare, thus diminishing the relative intensity of this band. As in C57BL/6 liver DNA, $V_{\beta 14}$ hybridizes to a 75-kb band (data not shown). Thus, both in C57BL/6 and in the mutant mouse strain SJL, the $V_{\beta 2B4}$ region is located on the 5′ side of the $C_\beta$ region at a distance of about 300 kb. Similar results were obtained with BALB/c and C57BL/Ka mice (data not shown).

**$V_{\beta 2B4}$ Is Linked to $V_{\beta SJL73}$, $V_{\beta LB2}$, $V_{\beta C5}$, and $V_{\beta 86T1}$.** Fig. 2*A* shows that $V_{\beta 2B4}$, $V_{\beta SJL73}$, and $V_{\beta C5}$ reside on the same 230-kb *Sal* I fragment in C57BL/6. Higher molecular weight partials of these fragments (particularly at 250 and 275 kb) also coincide. $V_{\beta 86T1}$ hybridizes to a fragment <50 kb long, as well as to fragments of 250, 300, and 375 kb. The 300-kb fragment appears to overlap with a $V_{\beta E1}$ probe, the most distal in our panel of $V_\beta$-region genes (N.E.L. and M.M.D., unpublished data). In addition, all the $V_\beta$-region genes share four high molecular weight partials of >500 kb (which cannot be accurately sized with a 20-sec pulse).

In the SJL mouse strain, $V_{\beta 2B4}$, $V_{\beta SJL73}$, and $V_{\beta 86T1}$ are located on the same 125-kb *Sal* I fragment as seen in Fig. 2*B*. Thus, the deletion in SJL encompasses about 125 kb as compared to C57BL/6. $V_{\beta E1}$ share four partials with $V_{\beta 86T1}$, the smallest being 300 kb. Clearly, $V_{\beta E1}$ must be linked to the other $V_\beta$-region genes. No band corresponding in size to the difference between the 300- and 125-kb fragments is seen. As suggested above, this could be due to the fact that *Sal* I sites accessible for digestion are rarely present in the same molecule. The maximum size of the $V_\beta$ region in SJL would be 300 kb.

In addition, Fig. 2 *C* and *D* show that $V_{\beta SJL73}$ and $V_{\beta LB2}$ reside on the same 50-kb *Xma* I fragment in both C57BL/6 and SJL mice. *Xma* I yields relatively short fragments and seems often to digest to completion, as overlapping partials are uncommon. This enzyme is useful, however, because by lining up the largest non-overlapping partials obtained with the different probes, the size of the $V_\beta$ region can be shown to be about 500 kb (Fig. 3). This confirms the size obtained with the *Sal* I digest. Note that the placement of the $V_\beta$ genes within the different fragments is arbitrary, particularly for $V_{\beta E1}$.

## DISCUSSION

The data presented here allow us to estimate precisely the portion of the genome taken up by one of the major antigen-

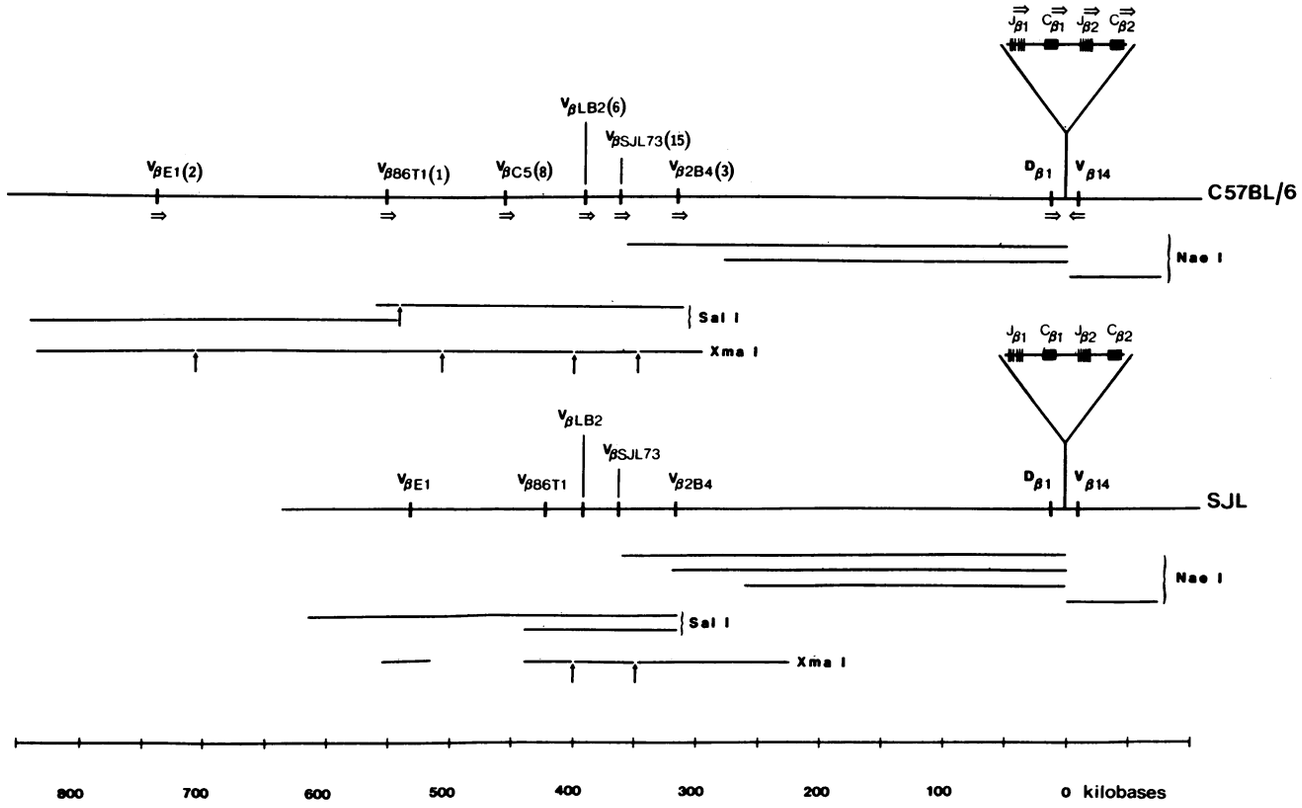


FIG. 3. The β-chain locus in the C57BL/6 and SJL strains of mice. Restriction endonuclease sites, deduced from digestions of liver DNAs, are indicated for *Nae* I, *Sal* I, and *Xma* I. The approximate location for each gene segment is shown. The specific $V_\beta$ gene segments are indicated with the numerical designations of Hood and coworkers (2) in parentheses. The relative positions of the fragments obtained with the different enzymes were not determined, except for the case of *Sal* I and *Xma* I in SJL. For *Xma* I the longest non-overlapping partials are shown, in order to estimate the size of the $V_\beta$ locus. The internal order between $V_{\beta SJL73}$ ($V_{\beta 15}$) and $V_{\beta LB2}$ ($V_{\beta 6}$) was determined by deletion mapping of AKR thymomas (N.E.L. and M.M.D., unpublished data). The arrows represent the direction of transcription. This is known from sequence analysis for the $DJC_\beta$ locus (reviewed in ref. 2) and for $V_{\beta 14}$ (ref. 4). For the 5′ *V* regions, the orientation was inferred by the deletion of intervening DNA accompanying rearrangement (ref. 28; N.E.L. and M.M.D., unpublished data).

receptor genes of lymphocytes. We have used probes representing nine distinct $V_\beta$ segments (including the three members of the C5 family). Chou *et al.* (28) demonstrated that $V_{\beta 86T1}$ is located on the same 16-kb fragment as $V_{\beta 10}$, $V_{\beta 4}$, and $V_{\beta 16}$ and that the $V_{\beta C5}$ ($V_{\beta 8}$) family is interspersed with the $V_{\beta TB21}$ ($V_{\beta 5}$) family on ≈16 kb of DNA. This means that at least 15 $V_\beta$ gene segments fall within the portion of the locus mapped here. The deletion in the SJL strain appears to be a single event, implying that the four other $V_\beta$ segments deleted in this strain (in addition to the $V_{\beta C5}$ and $V_{\beta TB21}$ families; ref. 18) are located between $V_{\beta 86T1}$ and $V_{\beta LB2}$. Thus, indications are that 19 of the 22 known $V_\beta$ elements, as well as the $C_\beta$ region, are contained on 700–800 kb, with the $V$ region comprising 400–500 kb. This is somewhat smaller than the human immunoglobulin κ light chain $V_\kappa$ locus, estimated by linking of cosmid clones to be longer than 900 kb (29). Note that all our measurements have utilized DNA prepared from liver-cell homogenates in order to avoid possible artifacts deriving from chromosomal rearrangements, insertions, and deletions that arise in established cell lines. Indeed, when we analyzed the organization of the β-chain genes in the B-cell tumor line Sp2/0 (30), we found that virtually every restriction fragment ran differently than in liver DNA from the original strain of origin (BALB/c; data not shown). Although some of these differences may be due to differential methylation between these DNAs, it seems unlikely that all of them are.

The data presented here also show that whereas the one previously described $V_\beta$ linkage ($V_{\beta 14}$) is 3′ to $J_\beta C_\beta$ and uses an inversion mechanism for rearrangement, at least 14 of the other known $V_\beta$ gene segments are 5′ of $J_\beta C_\beta$ and appear to use looping-out and deletion of the intervening DNA as a mechanism for rearrangement (ref. 28; N.E.L. and M.M.D., unpublished data). Also in favor of the looping-out/excision mechanism of rearrangement is the evidence of Yamagishi and Sakano and their colleagues (31, 32) that circular, supercoiled, extrachromosomal deletion products from both α- and β-chain genes can be found in T-cell populations known to be rearranging antigen-receptor genes. That both deletion and inversion can be accomplished by the same type of joining mechanism has been proposed to occur in the immunoglobulin κ gene, the only important parameter being the relative spatial orientations of $V$ and $J$ elements (10, 33). How rearrangement may work in T-cell receptor β-chain genes is shown in Fig. 4. In the principal case (Fig. 4*A*), a $V_\beta$ in the same orientation as, and located 5′ to, $J_\beta C_\beta$ rearranges to a $D$ region by looping out of the intervening DNA. This results in a $VDJ_\beta$ joint maintained in the chromosome and a heptamer–heptamer joined circle that is presumably lost later, although probably not instantaneously judging from the fact that significant numbers of such circular DNA molecules can be purified from neonatal thymocytes (31, 32). Alternatively (Fig. 4*B*), if the $V_\beta$ is located 3′ and in the reverse orientation, joining of $V$ to $DJ$ and of the respective heptamers to each other will constitute an inversion and need not result in the loss of any DNA. The enzymatic mechanism by which the different segments are joined together is not known but has similar general properties regardless of the net result (deletion or inversion), as pointed out previously (10, 33).

Another interesting point about these data is their implications for the question of the distribution of $V$ regions. Many $V_\beta$ gene segments are only 2–7 kb apart (28, 34), and the repertoire of functional $V$ regions appears to be encoded by only about 20–30 genes (2). Therefore, in principle, the $V$-region locus could all be fit into ≈200 kb vs. the 700-kb minimum size that we see here. One possibility is that there are large numbers of pseudogenes encoded in the β locus that have not yet been discovered. Another is that the distribution of $V_\beta$ gene segments is very heterogeneous. It is particularly difficult to understand why there would be such a large

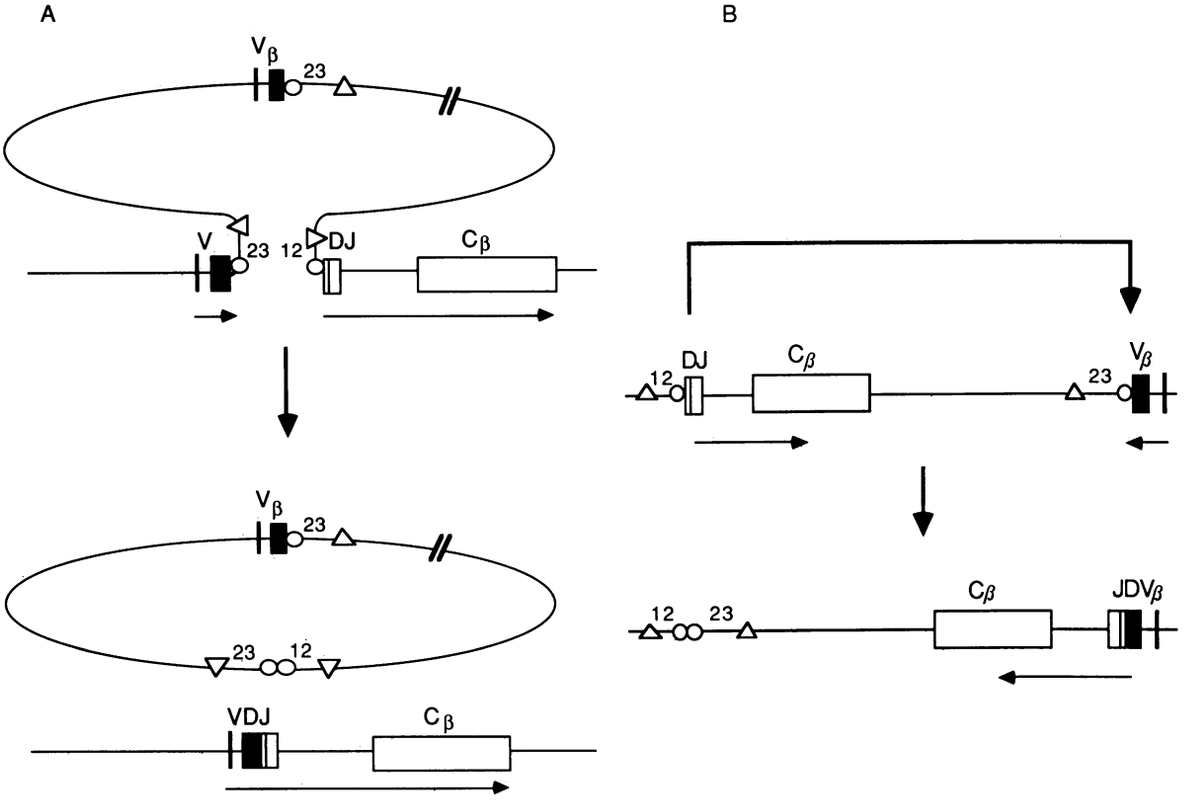


FIG. 4. Looping-out/deletion and inversion mechanisms for β-chain gene rearrangement. Circles and triangles represent the heptamer and nonamer components of the rearrangement recognition sequences, respectively. See text for discussion.

distance (≈300 kb) between the 5′ *V* regions and the $J_\beta C_\beta$ region, especially since *VJ* rearrangement can clearly occur over very short distances (e.g., as in the chicken immunoglobulin λ genes; ref. 3). Perhaps the topology of the gene segments in the chromosome favors rearrangement by the looping-out/deletion mechanism when $V_\beta$ genes are located beyond some minimal distance. No simple correlation between the chromosomal localization and the frequency of $V_\beta$ usage in adult mice is apparent. It has been observed that the immunoglobulin heavy chain $V_H$ gene segments located proximal to the *C* region are preferentially utilized in early B-cell differentiation (reviewed in ref. 35). In this regard, it is interesting that Pardoll *et al.* (36) have seen expression of $V_{\beta 2B4}$-containing RNA 2 days before that of $V_{\beta TB21.1}$ in fetal thymocytes.

Recently, two other groups have reported the use of pulsed-field electrophoresis and cosmid clones to map the mouse β-chain locus (37, 38). Although there is substantial agreement among the data presented, Lai *et al.* (37) found a much smaller distance between $V_{\beta 2B4}$ ($V_{\beta 3}$), the closest 5′ $V_\beta$ in our study, and the $C_\beta$ coding regions (≈80 kb versus the 280–360 kb that we report here). They also found the distance between $V_{\beta E1}$ ($V_{\beta 2}$) and $V_{\beta 86T1}$ ($V_{\beta 1}$) to be considerably smaller than our results indicate. The results of Chou *et al.* (38) are consistent with our data for the distance between $V_{\beta 2B4}$ and $C_\beta$. We believe that the discrepancies between our data and those of Lai *et al.* (37) are due to the fact that we used DNA from normal liver cells for our mapping studies, whereas they used L-cell DNA. This line has been propagated for many years (39) and may have undergone numerous deletions and rearrangements with respect to the germ line. Variations in the methylated state of the DNAs alone are not sufficient to explain the differences in our respective maps.

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