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Rearrangement by inversion of a T-cell receptor δ variable region gene located 3′ of the δ constant region gene

(inversional rearrangements/reciprocal joints/N regions/ordered variable region gene usage)

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ABSTRACT **We have located a T-cell receptor variable (V) δ gene segment immediately 3′ of the δ constant (C) region gene and 5′ to the known joining (J) α gene segments. This V_δ gene is in the opposite transcriptional polarity to C_δ and has rearranged to C_δ by inversion in a γ/δ-expressing hybridoma, DN7.3. This V_δ gene is commonly rearranged in adult but not fetal γ/δ-expressing thymocytes and has not been observed among α gene rearrangements reported to date. The reciprocal joining sequence isolated from this cell line contains N region nucleotides between the recombination signal sequences, in contrast to previously analyzed reciprocal joints. The results are discussed in the context of models accounting for ordered V gene usage during lymphocyte development.**

Murine thymocytes express clonally distinct T-cell receptor (TCR) heterodimers that consist of either α and β chains (expressed by the majority of T cells) or γ and δ chains (expressed by a subpopulation of thymocytes and peripheral T cells and dendritic epidermal cells of $CD4^-$, $CD8^-$, $CD3^+$, or "double-negative" phenotype) (1–7). Each of these chains is composed of segments of DNA [variable (V), diversity (D) (in some cases), and joining (J) region gene segments] that rearrange during thymocyte maturation and comprise the final gene. Recently, the partial organization of the δ gene locus has been described (8, 9). The δ gene locus encodes two D_δ, two J_δ, and one constant (C) δ gene segment(s), which are located 75 kilobases (kb) upstream of the C_α gene. While this arrangement suggests that TCR V region usage for α and δ chains may overlap, many V_δ segments described thus far have not been detected as part of rearranged α genes (9–11). In investigating the diversity of V region gene sequences expressed in γ/δ thymocytes, we have found that a V_δ gene segment [$V_\delta 7.3$¶ (11) or $V_\delta 5$ according to the nomenclature of Elliott *et al.* (10)] is located ≈2.5 kb downstream of the C_δ gene and ≈5 kb upstream of J_α gene segments. The $V_\delta 7.3$ gene segment is oriented in reverse transcriptional polarity relative to D_δ, J_δ, and C_δ gene segments and has rearranged to $D_\delta 1$–($J_\delta 1$)–C_δ by inversion in a T-cell hybridoma, DN7.3.

MATERIALS AND METHODS

Cells. DN hybridomas (DN7.1, DN12.1, DN7.3, DN2.3, and DN1.1) were isolated by fusion of highly enriched $CD4^-$, $CD8^-$, CD5 (Ly-1)^{dull} cells with BW5147(β^-) (a TCR β-chain loss variant of BW5147) as described (12, 13). Double-negative dull CD5 (Ly-1) cells were isolated by depletion of C57BL/6 thymocytes with anti-CD4, anti-CD8, and anti-CD5 antibodies and complement. $CD3^+$ γ/δ cells were prepared by culture of these cells with recombinant interleukin 1, rat Con A supernatant, and WEHI-3 supernatant for 3–4 days.

DNA Cloning. To isolate the 7.0-kb C_δ-containing *Eco*RI fragment, DNA from DN7.3 was digested with *Eco*RI and electrophoresed on 0.7% agarose gels. DNA ≈7 kb long was isolated from the gel and ligated to λgt10. Recombinant bacteriophage were screened with a 900-base-pair (bp) *Eco*RI C_δ fragment isolated from δ7.3cDNA-1. The isolation of δ7.3cDNA-1 has been described (11); δ7.3cDNA-2 (see Fig. 3*A*) was isolated from the same library. DNA sequences were determined by primer extension of oligonucleotides hybridized to denatured supercoiled plasmids using Sequenase (United States Biochemical, Cleveland) (14). Southern hybridizations were performed as described (15). DNA probes were labeled by random hexamer priming (16). The filters were washed with 0.2× SSC (1× SSC = 0.15 M NaCl/0.015 M sodium citrate) at 68°C for the final washes.

RESULTS AND DISCUSSION

We have previously characterized a small panel of TCR γ/δ-expressing T hybridomas derived by fusion of $CD4^-$, $CD8^-$, $CD3^+$, $CD5^{dull}$ adult thymocytes with BW5147 (12). cDNA clones corresponding to productively rearranged δ genes were isolated from three of the hybridomas, and the δ gene rearrangements in the panel were identified by Southern hybridization analyses of *Eco*RI-digested DNAs with V_δ and J_δ probes (11). Southern blots of *Eco*RI-digested hybridoma DNAs were also hybridized with a C_δ probe, which was not expected to reveal V_δ/J_δ rearrangements because *Eco*RI sites are located between the J_δ and C_δ gene segments. A 10-kb germ-line *Eco*RI fragment was present in all of the lines except BW5147, which has deleted the δ gene as reported (8) (Fig. 1 *Left;* data not shown). Surprisingly, however, the DN7.3 hybridoma harbored an additional rearranged 7.0-kb *Eco*RI fragment that hybridized with the C_δ probe. This fragment was also present in DNA isolated from a population of total $CD4^-$, $CD8^-$, $CD5^{dull}$ thymocytes, which are highly enriched for cells expressing CD3-associated γ/δ receptors after culture in a lymphokine cocktail (DNγ/δ^+Thy). A faint band at ≈8 kb was also observed in the total population (see below). The 7.0-kb *Eco*RI fragment was cloned from DN7.3 and a restriction map was constructed (Fig. 2). For comparison, a map was constructed for the germ-line C_δ fragment [isolated from the cosmid TA25.1 (16)] (Fig. 2). A recombination breakpoint was localized to a 550-bp *Eco*RV/*Sac* I restriction fragment (≈2.5 kb to the 3′ side of the end of the C_δ gene).

The observed recombination could have resulted from deletional, inversional, or interchromosomal recombination events. To distinguish among these possibilities, a restriction

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Abbreviations: TCR, T-cell receptor; V, variable; D, diversity; J, joining; C, constant; V_H, V region heavy chain.

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¶For clarity in indicating the derivation of our $V_\delta 5$ cDNA from the DN7.3 hybridoma, this V segment is denoted "$V_\delta 7.3$" in this report.

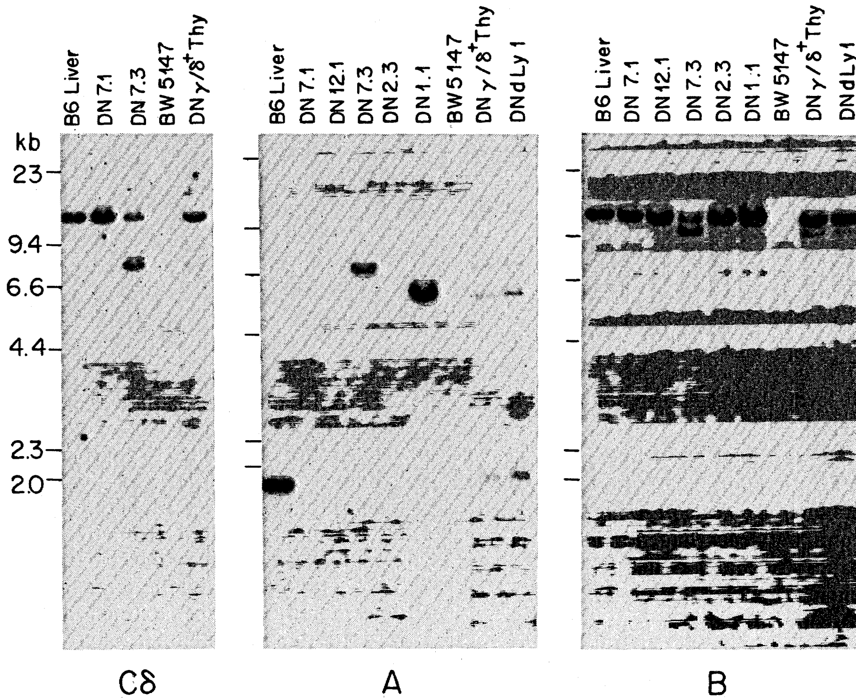

FIG. 1. Southern hybridization to DNA isolated from DN hybridomas (DN7.1, DN12.1, DN7.3, DN2.3, DN1.1), C57BL/6 liver (B6 liver), and populations of adult double-negative thymocytes (DNγ/δ^+Thy and DNdLyl). DNγ/δ^+Thy refers to a population of purified CD4$^-$, CD8$^-$, CD5 (Ly-1)^{dull} thymocytes, which have been cultured in lymphokine cocktail for 3 days (12). This treatment results in cells that are >80% CD3$^+$, γ/δ^+. DNdLyl refers to purified CD4$^-$, CD8$^-$, CD5(Ly-1)^{dull} thymocytes that have not been cultured; these cells are normally 5–10% CD3$^+$, γ/δ^+ (7). DNA probes used for hybridization are indicated. C_δ, a 900-bp *Eco*RI fragment from the 3′ half of a δ7.3 clone (11); probe A, a 600-bp *Hin*dIII fragment from 3′ of the recombination breakpoint in the 7.0-kb *Eco*RI fragment (see Fig. 2); probe B, a 700-bp *Hin*dIII fragment 3′ of the recombination breakpoint from germ-line DNA (see Fig. 2). DNA was digested with *Eco*RI, electrophoresed on 0.7% agarose gels, and transferred to nitrocellulose (15). *Hin*dIII-digested λ DNA size markers are indicated.

fragment immediately 3′ of the recombination breakpoint from germ-line DNA (Fig. 2, probe B) was used to analyze DNA from the γ/δ hybridomas (Fig. 1 *Right*). This analysis revealed the presence of a single band (≈10 kb) in all of the hybrids except for BW5147, where it was deleted, and DN7.3, where a second band (≈9.5 kb) was observed. In addition, the γ/δ population also contained the rearranged band. The presence of two fragments that hybridize with probe B suggested that the rearrangement in DN7.3 did not involve a simple deletion of sequences 3′ of the breakpoint but indicated that inversion or possibly chromosomal translocation of these sequences had occurred. We noticed that this hybridization pattern was identical to that obtained with a probe that consisted solely of the V region of a δ7.3 cDNA clone (11). This observation raised the possibility that the germ-line V_δ7.3 gene is in close proximity to probe B on the same germ-line DNA fragment.

Analysis of the nucleotide sequence 3′ of the recombination breakpoint in germ-line DNA (Fig. 3*A*) confirmed the presence of the δ7.3 V region ≈2.5 kb downstream of C_δ. In addition, the V_δ7.3 gene is situated in opposite transcriptional orientation to C_δ (and D_δ–J_δ gene segments). Consensus heptamer–nonamer recombination signals with a 23-bp spacer are present at the 3′ end of the V_δ7.3 gene; this would be predicted based on the observation that D_δ (and J_δ) gene segments are flanked on their 5′ end by recombination signals with 12 bp spacers (9). This genomic organization indicates that rearrangement of V_δ7.3 to (D_δ–J_δ) C_δ gene segments occurs by an inversional mechanism. Consistent with this interpretation, a J_δ1 probe also detected the 9.5-kb rearranged *Eco*RI fragment in DN7.3 (11). A distinct J_δ1 rearrangement of similar size is also present in DN7.3.

Based on the foregoing analysis, rearrangement of V_δ7.3 by inversion should result in the presence of the reciprocal

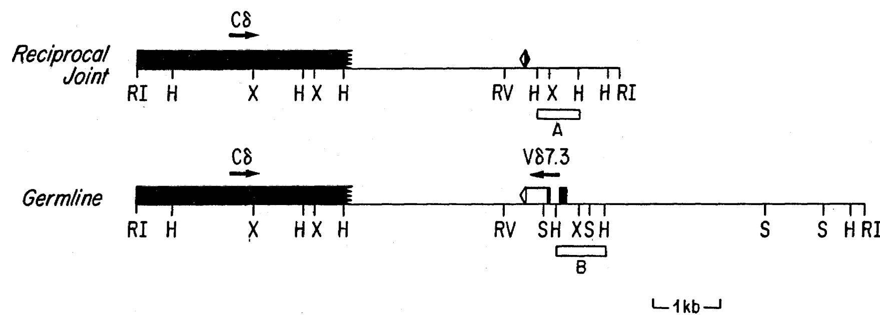

FIG. 2. Restriction maps of the 10-kb germ-line C_δ fragment and 7.0-kb *Eco*RI C_δ fragment from the DN7.3 hybridoma. The germ-line C_δ fragment was subcloned from cosmid TA25.1, previously isolated from a library prepared from B10.D2^{dm2} DNA (17) (a kind gift of L. Hood). Restriction sites for the following enzymes were determined: *Eco*RI, RI; *Eco*RV, RV; *Hin*dIII, H; *Sac* I, S; *Xba* I, X.

A

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TCTAGATGGTTTGAAAACCAACATTTCTGTAAACCACAACAGTTCCTTGGTAGAGGTATTCACCCCCACAATTCCAGTTA   germ-line

                                                    CCCTCCTGTCCCCTCCCTAGACAAAGCAATA   δ7.3cDNA-2
ATCCAGTCACATGACAGTCATGTGAGGGGCTGCCTGCCTCTCCTCCTTTCCCTCCTGTCCCCTCCCTAGACAAAGCAATA   germ-line

                                                               M  I  L  A  A  T
GGGATGGTTAAAGAGAGTCCAGCAGCCCTCCTCCACGCTGTGCCCCTGTACCGACTGGAAGGATGATTCTTGCCGCGACC   δ7.3cDNA-2
GGGATGGTTAAAGAGAGTCCAGCAGCCCTCCTCCACGCTGTGCCCCTGTACCGACTGGAAGGATGATTCTTGCCGCGACC   germ-line

 L  T  L  L  F  A
CTTACCCTTCTGTTTGCCT{donor---------------------------------------------------------   δ7.3cDNA-2
CTTACCCTTCTGTTTGCCTGTAAGAGGCGTATTTCCCGCTTGCTGCACCCTGCTTCCCTCTCTCTGACCTTCGGAAGCTTG   germ-line

                                                                            TGAG   δ7.3cDNA-1
--------------------------------------------------------------------------------
CTCTCTGTCTCTACACACTTAGGTTTCAGTCATTCTCTGCCCGTGACTTTCATTCCTATCGTCTGTTGAGTCCCTCTGAG   germ-line
                                                                    +1
                  M  Q  T  L  L  W  P  P  F  F  F  T  D  K  D  V  L  C ...
CTGGTCAGTGTCTGGGATGCAGACGCTACTATGGCCTCCTTTCTTCTTCACAGACAAGGATGTGCTGTGCATCACGCTGA   δ7.3cDNA-1
                                                      Y  K  D  V  L  C ...
---------------------------------------------acceptor}ACAAGGATGTGCTGTGCATCACGCTGA   δ7.3cDNA-2
CTGGTCAGTGTCTGGGATGCAGACGCTACTATGGCCTCCTTTCTTCTTCACAGACAAGGATGTGCTGTGCATCACGCTGA   germ-line

CCCAGAGCTCCACTGACCAGACAGTGGCAAGCGGCACTGAAGTAACACTGCTCTGCACGTACAATGCGGATTCTCCAAAC   δ7.3cDNA-1
CCCAGAGCTCCACTGACCAGACAGTGGCAAGCGGCACTGAAGTAACACTGCTCTGCACGTACAATGCGGATTCTCCAAAC   δ7.3cDNA-2
CCCAGAGCTCCACTGACCAGACAGTGGCAAGCGGCACTGAAGTAACACTGCTCTGCACGTACAATGCGGATTCTCCAAAC   germ-line

CCAGATTTATTTTGGTATCGCAAAAGGCCAGACAGATCCTTCCAGTTCATCCTTTATAGGGACGACACTAGTTCCCATGA   δ7.3cDNA-1
CCAGATTTATTTTGGTATCGCAAAAGGCCAGACAGATCCTTCCAGTTCATCCTTTATAGGGACGACACTAGTTCCCATGA   δ7.3cDNA-2
CCAGATTTATTTTGGTATCGCAAAAGGCCAGACAGATCCTTCCAGTTCATCCTTTATAGGGACGACACTAGTTCCCATGA   germ-line

TGCAGATTTTGTTCAAGGTCGATTTTCTGTGAAGCACAGCAAGGCCAACAGAACCTTCCATCTGGTGATCTCTCCAGTGA   δ7.3cDNA-1
TGCAGATTTTGTTCAAGGTCGATTTTCTGTGAAGCACAGCAAGGCCAACAGAACCTTCCATCTGGTGATCTCTCCAGTGA   δ7.3cDNA-2
TGCAGATTTTGTTCAAGGTCGATTTTCTGTGAAGCACAGCAAGGCCAACAGAACCTTCCATCTGGTGATCTCTCCAGTGA   germ-line

                                         Dδ1 N      Dδ2          Jδ1
GCCTTGAAGACAGCGCTACTTATTACTGTGCCTCGGGGTAT TAT AC CGGAGGGATACGAG CTACCGACAAA ...   δ7.3cDNA-1
GCCTTGAAGACAGCGCTACTTATTACTGTGCCTCGGGGTAT TAT AC CGGAGGGATACGAG CTACCGACAAA ...   δ7.3cDNA-2
GCCTTGAAGACAGCGCTACTTATTACTGTGCCTCGGGGTATCACTGTGGTGCAGGTGCCCAGGGAGCCTGTACCCAAACC   germ-line

CCTGGAGCACAGTTTAGGAGTGCCCTCCCCATCCCCCTTCCACAGAAAGTCACATGCCAGGTTGTCAGGCATCTGGCACT   germ-line
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B

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AAGCTTTTGGTTCTTAACTTACAAGTGTGACAGTGTTGTGCACCTGAGTTTTAGGACTCTAAACTGCAAC   recip.jt.

                                                        AAATGCCAAGGGAAGAA   Dδ1 germ-line
AGCTTTGGAGGAAGATTCTGGGCCAGTGGGTATGGCAGAGGGTGGTGATGGCAAAATGCCAAGGGAAGAA   recip.jt.
GTTCAAGGTCGATTTTCTGTGAAGCACAGCAAGGCCAACAGAACCTTCCATCTGGTGATCTCTCCAGTGA   Vδ7.3 germ-line

                       12                    Dδ1                   23
ACAAAGGGTGTTTTTGTACGGCTGTGTTTCACTGTGGTGGCATATCACACAGGTTGAAGTATATTAAACC   Dδ1 germ-line
ACAAAGGGTGTTTTTGTACGGCTGTGTTTCACTGTGCGCCCACTGTGGTGCAGGTGCCCAGGGAGCCTGT   recip.jt.
GCCTTGAAGACAGCGCTACTTATTACTGTGCCTCGGGGTATCACTGTGGTGCAGGTGCCCAGGGAGCCTGT   Vδ7.3 germ-line

TCTGTTCAGAAACACTCAGTGTGACTCCCTGTATAAGTCT                                 Dδ1 germ-line
ACCCAAACCCCTGGAGCACAGTTTAGGAGTGCCCTCCCCATCCCCCTTCCACAGAAAGTCACATGCCAGG   recip.jt.
ACCCAAACCCCTGGAGCACAGTTTAGGAGTGCCCTCCCCATCCCCCTTCCACAGAAAGTCACATGCCAGG   Vδ7.3 germ-line

TTGTCAGGCATCTGGCACTCCCAAGTGACTAGGAACGAAA                                 recip.jt.
TTGTCAGGCATCTGGCACTCCCAAGTGACTAGGAACGAAA                                 Vδ7.3 germ-line
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FIG. 3. (A) Sequence of the $V_\delta7.3$ gene (germ-line) and its comparison to two cDNAs derived from the DN7.3 hybridoma (δ7.3cDNA-1 and δ7.3cDNA-2). The sequence of the germ-line $V_\delta7.3$ derived from B10.D2[dm2] and cDNAs derived from C57BL/6 are identical (refs. 10 and 11; this study). The δ7.3cDNA-2 is indicated together with splice donor and acceptor sequences. Heptamer–nonamer recombination signal sequences are underlined. The sequence of the germ-line $V_\delta7.3$ region has been reversed relative to its orientation shown in Fig. 2 for comparison to $V_\delta7.3$ cDNA sequences. $D_\delta1$ and $D_\delta2$ sequences (in italics) and N region nucleotides (in boldface) present in the $V_\delta7.3$ cDNAs are also indicated. The single-letter amino acid code is used. (B) DNA sequence of the reciprocal joint (recip. jt.) from the C_δ-hybridizing 7.0-kb *Eco*RI fragment from DN7.3 and comparison to $D_\delta1$ germ-line sequence (9) ($D_\delta1$ germ-line) and germ-line $V_\delta7.3$ sequence ($V_\delta7.3$ germ-line). Putative N region nucleotides between the heptamer–nonamer sequences in the reciprocal joint are indicated in boldface. The DNA sequence of the reciprocal joint has been reversed relative to its orientation in Fig. 2 to compare it to $D_\delta1$ sequences, which are presented in the 5′–3′ orientation.

rearrangement products or "reciprocal joint" on the rearranged 7.0-kb *Eco*RI C_δ fragment. Reciprocal joints are the result of the head-to-head joining of two heptamer–nonamer recombination signal sequences and have been observed in immunoglobulin light chain and TCR gene rearrangement (18–21) (see below). In this case, the 3′ flank of $V_\delta7.3$ should be rearranged to the 5′ flank of a D_δ or J_δ gene sequence. Sequence analysis (Fig. 3B) of the 7.0-kb *Eco*RI fragment revealed the presence of a reciprocal joint at the recombination breakpoint consisting of sequences 3′ of germ-line $V_\delta7.3$

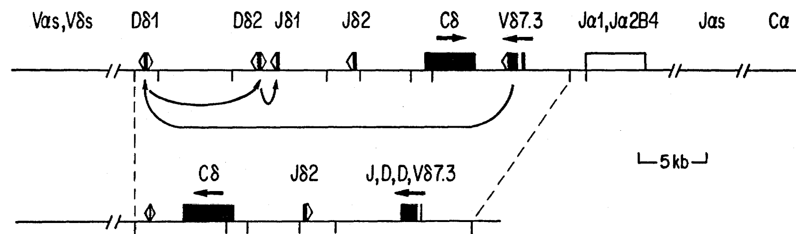

FIG. 4. Organization of the TCR δ- and α-chain locus and proposed inversional rearrangement of $V_δ7.3$ ($V_δ5$). The location of *Eco*RI sites (depicted as downward tics) was adapted from BALB/c and B10.D2^{dm2} (8, 9, 11, 17). C57BL/6 has been shown to exhibit polymorphism of *Eco*RI sites in the vicinity of $J_δ2$ (11, 22) (data not shown). Heptamer–nonamer recombination sequences are indicated as open triangles adjacent to D and J sequences. $V_δ7.3$ is followed by heptamer–nonamer sequences separated by 23 bp; rearrangement to $D_δ1$, which has a heptamer–nonamer sequence separated by 12 bp, is in accord with the 12/23 recombination rule (23). The open rectangle 3′ of $V_δ7.3$ indicates the approximate location of the nearest known $J_α$ sequences (8, 17). $V_δ7.3$ rearranges by inversion to $D_δ1$, which rearranges to $D_δ2$ and $J_δ1$ by deletion. This results in the presence of a reciprocal joint on the rearranged $C_δ$ fragment together with rearranged $J_δ1$ and $V_δ7.3$ fragments.

juxtaposed to a fragment whose sequence is identical to the sequence 5′ of $D_δ1$ as determined by Chien *et al.* (9). These results, together with the V–D–J junctional sequence derived from $V_δ7.3$ cDNAs (11) (Fig. 3*A*), indicate that the productive δ rearrangement in DN7.3 resulted from inversional rearrangement of $V_δ7.3$ to $D_δ1$, and deletional joining of $D_δ1$, $D_δ2$ and $J_δ1$ (Fig. 4).

To confirm and extend these conclusions, Southern hybridization using a 5′ $D_δ1$ probe (probe A) was performed (Fig. 1 *Center*). This probe detected a 1.9-kb germ-line *Eco*RI fragment in liver DNA, the 7.0-kb reciprocal joint fragment in DN7.3, and a 6.0-kb rearranged fragment in the DN1.1 cell line. The 6.0-kb fragment may correspond to an incomplete $D_δ1$–($D_δ2$)–$J_δ1$ rearrangement based on the finding that a 6.0-kb *Eco*RI fragment in DN1.1 hybridized with a $J_δ1$ probe (11). Sequences hybridizing with probe A are deleted in DN7.1, DN12.1, and DN2.3, indicating that both chromosomes in these cells have undergone deletional rearrangements of $V_δ$s to $D_δ$–$J_δ$ gene segments.

Each of the rearranged fragments observed in DN7.3 with probes A, B, and $C_δ$ are also detected in the double-negative thymocyte populations (DNγ/δ$^+$Thy and DNdLy1) (Fig. 1). These findings are consistent with the high frequency of $V_δ7.3$ rearrangement (see below). In addition, Southern hybridization with the $C_δ$ probe also detected a faint band at ≈8 kb in the γ/δ population; this band is consistent with the inversion of $V_δ7.3$ to a $D_δ2$–$J_δ1$ rearranged fragment. Presumably, the 9.5-kb *Eco*RI band detected in the population with a $V_δ7.3$ probe is a mixture of rearrangements of $V_δ7.3$ to either $D_δ1$–($D_δ2$)–$J_δ1$ or $D_δ2$–$J_δ1$.

Comparison of the sequence of the germ-line $V_δ7.3$ gene segment with the sequence of the reciprocal joint reveals that four additional nucleotides are present between the recombination signal sequences (Fig. 3*B*). These nucleotides cannot be accounted for by sequences flanking either the $V_δ7.3$ or the $D_δ1$ gene segments.‖ Consequently, these nucleotides may be the result of template-independent nucleotide additions, which are thought to be catalyzed by terminal deoxynucleotidyltransferase (24, 25). Most reciprocal joints reported to date are present as perfect head-to-head recombination signals. Additional sequences observed in some reciprocal joints (19, 20) may be accounted for by nucleotides immediately adjacent to the heptamer–nonamer signals contained in the DNA segments that are recombined. The reciprocal joint described here documents the presence of N region nucleotides in a reciprocal joint and suggests that during the recombination events the noncoding recombination signals are exposed to the action of terminal deoxynucleotidyltransferase. This finding may be related to the high levels of N region diversification observed in adult δ gene rearrangements (10). N region nucleotides have also been observed in reciprocal joints in recombination substrates that have undergone rearrangement after introduction into tissue culture cells (32).

Analysis of the region upstream of the $V_δ7.3$ gene indicates an unusual genetic organization for the $V_δ7.3$ signal sequences (Fig. 3*A*). Comparison of the germ-line sequence with a cDNA sequence isolated from DN7.3 (δ7.3cDNA-1) (11) and three cDNA sequences reported by Elliott *et al.* (10) indicates that the 5′ untranslated sequences and signal peptide sequences are contiguous with the V region gene sequence. Most V region gene segments for immunoglobulin and TCRs contain a small intron between the 5′ untranslated region–signal peptide exons and the V region exons. The absence of an intron between the signal sequence and the V region has also been observed in the pre-B-specific λ5 immunoglobulin light chain (26). Considering the frequency of isolation of this sequence, it is unlikely that these cDNAs represent unspliced primary δ7.3 transcripts. Interestingly, we have isolated a second cDNA clone from DN7.3 (δ7.3cDNA-2), which is distinct from those described above (Fig. 3*A*). This cDNA encodes a different 5′ untranslated region and signal peptide sequence, which is located 195 bp upstream of the V segment in germ-line DNA; the corresponding mRNA apparently results from splicing this sequence to a position corresponding to amino acid position −4 of the $V_δ7.3$cDNA-1 signal sequence. Infrequent splicing of a single transcript or usage of two distinct promoters may account for the presence of these two messages.

The results presented here document the rearrangement of a downstream $V_δ$ gene to the ($D_δ$–$J_δ$)–$C_δ$ gene, a situation highly reminiscent of that reported for the $V_β14$ and $C_β$ genes (19). Inversional rearrangement of human immunoglobulin $V_κ$ genes has also been documented (27, 28), as have reciprocal joints suggestive of inversion in the murine immunoglobulin κ locus (18, 24). Inversional rearrangements of δ genes may also occur in fetal thymocytes. Chien *et al.* (9) reported the isolation of DNA clones from fetal thymocytes that contain reciprocal joints of rearrangements of unidentified sequences to $D_δ2$ sequences. The possibility that these joints were present in the cells on extrachromosomal circles resulting from deletional rearrangements was not excluded, however.

Positional effects on rates of V gene rearrangement have been reported in the immunoglobulin heavy (H) chain locus, where those V_H segments most proximal to J_H rearrange most frequently in pre-B cells; thereafter, V gene usage is randomized, perhaps by selection (reviewed in ref. 29). In

‖Strain differences are unlikely to account for the observed N region nucleotides since the sequence of $D_δ1$ is identical in BALB/c and 129 and differs by one nucleotide in B10.A (9, 10) and $V_δ7.3$ is identical between B10.D2^{dm2} germ-line, C57BL/6 cDNA, and (BALB/c × 129)F_1 cDNAs (refs. 10 and 11; this study); the DN7.3 hybridoma is derived from the C57BL/6 strain.

contrast, $V_\delta 7.3$, which is the most proximal of any reported V gene to its corresponding C gene, is rearranged infrequently in γ/δ hybridomas derived from fetal thymocytes (A. Kruisbeek, P. Leblanc, S. Marusic-Galesic, A.J.K., and D.H.R., unpublished data) and among genomic δ rearrangements cloned from fetal thymocytes (8). Instead, $V_\delta 7.3$ rearrangements represented 16–30% of δ rearrangements in adult γ/δ-expressing thymocytes (11) and represented 11/21 C_δ-hybridizing cDNA clones isolated from adult double-negative thymocytes (10). Moreover, the predominance of $V_\delta 7.3$ rearrangements among adult thymic γ/δ cells may not be due to cellular selection, since $V_\delta 7.3$ rearrangements are also very abundant among $CD4^-$, $CD8^-$, $CD5^{dull}$ thymocytes, only 5–10% of which express surface CD3-associated γ/δ receptors (7) (Fig. 1, DNdLy1). V_δ rearrangements may be generally subject to different regulation than V_H genes; alternatively, rearrangement of $V_\delta 7.3$ may be subject to independent regulation owing to its location to the 3′ side of C_δ or its inverted orientation relative to C_δ. The data presented here also bear on models in which ordered V gene usage is accomplished by secondary rearrangements, which replace a rearranged V gene with another V gene (30, 31). If most of the $V_\delta 7.3$ rearrangements in adult γ/δ thymocytes resulted from V replacements, the reciprocal joints would represent a diverse collection corresponding to the sequence 3′ of $V_\delta 7.3$ juxtaposed to various other V_δ genes. Instead, the frequency of reciprocal joints in the population containing sequences 5′ of germ-line D_δ gene segments approaches that of $V_\delta 7.3$–D_δ rearrangements (as assessed by densitometry) (Fig. 1). In this instance, at least a considerable portion of the $V_\delta 7.3$–D_δ rearrangements apparently do not involve V replacement. Finally, it is worth noting that there are no documented examples of $V_\delta 7.3$–J_α rearrangements, despite the proximity of $V_\delta 7.3$ to several J_α gene segments and the apparent compatibility of their respective recombination signals. In this case, it is possible that selection accounts for distinguishing the V_α and V_δ repertoires.

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