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Thymocyte circular DNA excised from T cell receptor $\alpha-\delta$ gene complex

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We have characterized thymocyte circular DNA excised from the T cell receptor $\alpha-\delta$ gene complex. Some δ gene clones contained unusual recombinant structures derived from V−(D)−J joining: (i) a reciprocal joint of direct V to J_δ joining, skipping the D_δ segment; (ii) a V−D_δ coding joint lacking an adjacent $D_\delta-J_\delta$ coding joint; (iii) a V−D structure containing two D_δ segments. Many of the α gene clones contained both coding and reciprocal joints of V_α−to−J_α joining on the same structure. Most of these coding joints were out of phase; however, in one clone there was an in-phase V−J_α structure. Interestingly, some α gene clones contained the same V gene sequence as rearranged in the δ gene clone, indicating that the same V gene family, at least in part, could be utilized for both the α and δ gene systems.

Key words: thymocytes/circular DNA/T cell receptor/V−D−J joining

Introduction

T cell receptor (TCR) genes are activated by somatic DNA rearrangement, during the development of T lymphocytes (Davis, 1985; Kronenberg *et al.*, 1986). Like immunoglobulin (Ig) genes, TCR variable region genes are split into three DNA segments: variable (V), diversity (D) and joining (J). Furthermore, V−(D)−J joining in TCR genes follows the same 12/23-bp spacer rule (Early *et al.*, 1980; Sakano *et al.*, 1980, 1981) as established for Ig genes. The TCR gene system consists of four gene families: α, β, γ and δ. Among them, the α and δ genes are carried by the same chromosome (Chien *et al.*, 1987a), chromosome 14 (Dembić *et al.*, 1985), while other TCR genes, β and γ, are contained in separate chromosomes. Moreover, the δ gene J-C region has been identified in the middle of the α gene locus, i.e. between the V_α cluster and the J_α cluster (Chien *et al.*, 1987b), although it is not yet determined whether the same gene pool is shared between the α and δ gene families. Because of the unusual gene organization, it is of particular interest to study how these genes undergo the rearrangement during T cell differentiation. It is generally accepted that the δ gene is expressed with the γ gene (Bonyhadi *et al.*, 1987; Born *et al.*, 1987; Loh *et al.*, 1987), while the α gene is expressed with the β gene (Allison and Lanier, 1987). No other combinations have been reported for the TCR gene expression. During fetal development, γ/δ-producing cells appear much before α/β producers (Pardoll *et al.*, 1987). However, it is not clear at this moment whether or not these two types of T cells are of the same developmental lineage.

In this report, we describe the characterization of thymocyte circular DNA excised from the TCR$\alpha-\delta$ gene region. We have previously analyzed TCRβ gene sequences in circular DNA prepared from mouse thymocyte nuclei, and identified reciprocal recombinant structures of V−D−J joining (Okazaki *et al.*, 1987). Two pairs of recombination signal sequences (RSSs) (Akira *et al.*, 1987) were joined in a head-to-head fashion at the reciprocal joint. A reciprocal structure was also reported for the TCRα-chain gene (Fujimoto and Yamagishi, 1987). These observations provided direct evidence for the intramolecular DNA deletion mechanism for the TCR gene rearrangement. Molecular analysis of circular DNA also permits us to study other recombination events occurring in the TCR gene region before the excision of circular DNA. In the present study, we have identified five δ gene clones and >200 α gene clones using J_δ and J_α probes among 200 000 recombinant phages made from thymocyte circular DNA. We have characterized two δ gene clones and four α gene clones by restriction enzyme mapping and nucleotide sequence determination.

Results

Identification of TCR δ gene sequences in thymocyte circular DNA

Thymocyte circular DNA prepared from 3-week-old BALB/c mice was used for making the phage library (Okazaki *et al.*, 1987). The library was screened with the $J_{\delta1}$ probe, a 3.6-kb *Eco*RI−*Xba*I fragment of the germline $J_{\delta1}$ clone. Two probe-positive clones, TCDδ1 and TCDδ2 were characterized by restriction mapping (Figure 1). Since the sizes of cloned *Eco*RI fragments are different from the germline $J_\delta1$ clone, both circular DNA clones must have been rearranged, unless they carried distinct but cross-hybridizable J_δ sequences. Using the cloned *Eco*RI fragments, germline components for the rearranged sequences were also cloned from liver DNA of Balb/c mice. Two different germline components were isolated for each rearranged clone, in addition to the germline $J_\delta1$. Restriction maps of the three germline fragments are compared with those of the rearranged fragments (Figure 1).

Nucleotide sequence determination of the germline $J_\delta1$ clone revealed a possible D_δ segment ~0.9 kb upstream from the $J_\delta1$ coding region (Figures 1A-a, 3A). The D_δ segment was flanked by two pairs of recombination signals: one pair on the 5′ side is separated by a 12-bp spacer, and the other pair on the 3′ side by a 23-bp spacer. The coding sequence was in agreement with a putative D_δ region in the cDNA sequence reported by Chien *et al.* (1987a), and turned out to be the same D_δ segment, $D_\delta2$, as recently reported by Chien *et al.* (1987b).

The clone TCDδ1 contained two recombination sites; one is in the Dδ region, and the other is at the $J_\delta1$ segment (Figure 1A-d). In this clone, the 0.9-kb intervening sequence

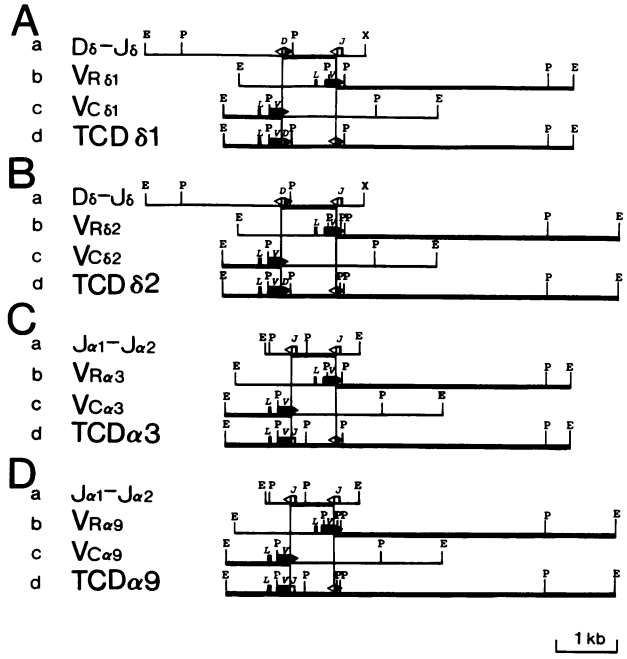

Fig. 1. Restriction enzyme cleavage maps for the circular DNA clones from mouse thymocytes. Two TCRδ gene clones, TCDδ1 (**A**) and TCRδ2 (**B**), and two TCR α gene clones, TCDα3 (**C**) and TCDα9 (**D**), were analyzed. The germline components, J and D (**a**) and V (**b** and **c**), are compared with the recombinant clones (**d**). In the figure, recombination sites are indicated by vertical lines. Exons for leader (L), variable (V), diversity (D) and joining (J) regions are shown. Recombination signal sequences (RSSs) are indicated by triangles (◁ for 12-bp RSSs and ▶ for 23-bp RSSs). In all the circular DNA clones, two recombination sites were identified; one is a coding joint (VD or VJ), and the other is a reciprocal joint (◁ ▶). Restriction enzyme cleavage sites are indicated for *Eco*RI (E), *Pst*I (P) and *Xba*I (X).

between D_δ and $J_\delta1$ was retained; however, the 5′-D and the 3′-J sequences appeared to be replaced with unknown DNA, judging from the restriction maps (Figure 1A-a and d). A similar structure was also found in another circular DNA clone, TCDδ2 (Figure 1B).

Reciprocal recombinants of V–J_δ joining skipped D_δ

Nucleotide sequence determination revealed that a 7-mer–7-mer structure was formed at the $J_\delta1$ site on the clones TCDδ1 and TCDδ2 (Figure 2A,B). The 5′ sequence of $J_\delta1$ was found next to the 7mer, but no $J_\delta1$ coding sequence was left at the joint. In the 3′ region of the $J_\delta1$ joint, another pair of recombination signals of unknown origin were identified. In order to characterize the 3′ sequence, we have cloned and sequenced the germline component attached to the reciprocal joint of $J_\delta1$ (Figure 1A-b). As shown in Figure 4A, a typical germline V sequence was identified in the clone $V_{R\delta}1$, a germline component for the clone TCDδ1. It contained recombination signals with a 23-bp spacer, whose sequences were exactly the same as those found in the reciprocal joint in the rearranged clone TCDδ1 (Figure 2A). A similar reciprocal structure of V–J joining was also found in the clone TCDδ2 (Figure 2B). These observations indicate that the clones TCDδ1 and TCDδ2 were excision products of V–J joining skipping D in the δ gene. Since the 12-bp RSSs and 23-bp RSSs are arranged in an alternating fashion in the TCRδ gene

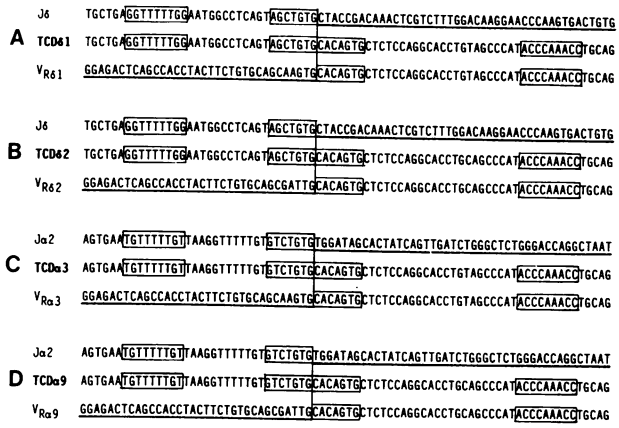

Fig. 2. Nucleotide sequences for reciprocal joint of TCRα and δ gene rearrangement in the circular DNA of thymocyte nuclei. Two δ gene clones, TCDδ1 (**A**) and TCDδ2 (**B**), and two α gene clones, TCDα3 (**C**) and TCDα9 (**D**) were analyzed. The recombinant sequences in the middle are compared with the corresponding germline sequences, J on the top and V at the bottom. Note that two pairs of recombination signals are joined in a head-to-head fashion at the reciprocal junction. The 7mer and 9mer sequences, sufficient recombination signals for V–(D)–J joining (Akira *et al.*, 1897), are in boxes. Coding regions in the germline sequences are underlined.

system, V can join directly to J, without violating the 12/23-bp spacer rule. Although the D-skipped V–J joining has been predicted (Davis, 1985; Okazaki *et al.*, 1987), the data presented here for the δ system are the first evidence for such a phenomenon.

V gene rearrangement to the germline D_δ segment

We have characterized another DNA rearrangement in the circular DNA clones TCDδ1 and TCDδ2. In addition to the reciprocal joint, both clones contained another recombination site 0.9 kb upstream from the reciprocal joint (Figure 1A-d and 1B-d). Nucleotide sequence determination revealed that the V sequence was joined to D_δ, but no J segment was attached to the V–D structure. Thus, the 3′ recombination signals of D_δ remained unused in the coding joint (Figure 3A). This observation is curious, since D–J joining has been considered a prerequisite to V–D joining in the IgH and TCRβ gene systems. It should be noted that a long stretch of inserted nucleotides was found at the coding joint, 13 bases in both TCDδ1 and TCDδ2 clones (Figure 3A). These insertions are rather long for the non-germline elements, and might be contributed by D–D joining (see Discussion).

TCR α gene sequences in thymocyte circular DNA

Using the J_α probe, a 1.5-kb *Eco*RI–*Eco*RI fragment containing $J_\alpha1$ and $J_\alpha2$ sequences (Hayday *et al.*, 1985) (Figure 1C), >200 probe-positive clones were isolated from the phage library of thymocyte circular DNA. Among them, four clones were randomly chosen and characterized further by restriction mapping and nucleotide sequencing. As described below, clones TCDα3 and TCDα9 contained two recombination sites, a reciprocal joint and a coding joint of V–J joining (Figure 1C-d, 1D-d). Two other clones, TCDα6 and TCDα10, contained only a coding joint.

In the clone TCDα3, DNA between $J_\alpha1$ (inclusive) and $J_\alpha2$ (exclusive) was retained; however, 5′-$J_\alpha1$ and 3′-$J_\alpha2$ sequences have been replaced with other DNA sequences

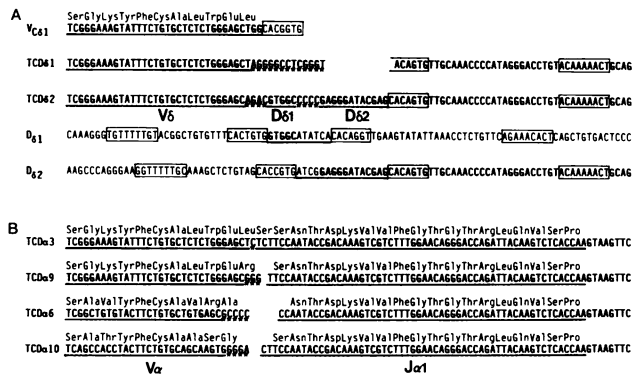

Fig. 3. Nucleotide sequences for coding joints of TCRα and δ gene rearrangement identified in thymocyte circular DNA. Two δ gene clones (**A**), TCDδ1 and TCDδ2, and four α gene clones (**B**), TCDα3, TCDα9, TCDα6 and TCDα10, were analyzed. To determine the recombination sites of δ gene clones, sequences of germline components, V, D and J sequences are compared (A). The $D_\delta 1$ sequence was taken from Chien *et al.* (1987b). Extra nucleotides inserted at the junctions of V−D, D−D, D−J and V−J are indicated by asterisks. Coding sequences of V, D and J segments are underlined. Recombination signal sequences are in boxes. Note that two D_δ sequences are linked in tandem at the V−D junction in the clone TCDδ2.

(Figure 1C-a and d). Two clones, $V_{R\alpha}3$ and $V_{C\alpha}3$, were isolated from mouse liver DNA as germline components for the rearranged sequences (Figure 1C-b and c). Nucleotide sequencing revealed that both germline clones contained a V_α sequence rearranged in the clone TCDα3. Nucleotide sequences around the two recombination sites are shown in Figures 2C and 3B; one is a reciprocal joint at $J_\alpha 2$, and the other is a coding joint at $J_\alpha 1$. The reciprocal joint contained a 7mer−7mer structure generated by the recombination between $J_\alpha 2$ and $V_{R\alpha}3$, a member of the $V_\alpha 2$ family TA19 (Arden *et al.*, 1985) (Figure 4A). No nucleotide insertion or deletion was found at the joint (Figure 2C). The coding joint of TCDα3 was formed between $J_\alpha 7$ and $V_{C\alpha}3$ (Figure 4B), a member of the $V_\alpha 7$ family (Arden *et al.*, 1985), and contained a one-nucleotide insertion (Figure 3B). It is worth noting that the coding joint in TCDα3 is in phase, and did not cause a frame-shift of the genetic code in the J coding region (see Discussion). In the entire V−J coding region, no stop codon was noted (Figure 4B) indicating that the V−J exon in the clone TCDα3 is functional.

Likewise in the clone TCDα9, two recombination sites were identified; one was a reciprocal joint and the other was a coding joint (Figure 1D). Two germline components, $V_{R\alpha}9$ ($V_\alpha 2$ family) and $V_{C\alpha}9$ ($V_\alpha 7$ family), were cloned and their nucleotide sequences were compared with the recombinant sequence of TCDα9 (Figures 2D and 3B). Like the clone TCDα3 described above, TCDα9 contained a 7mer−7mer structure derived from $J_\alpha 2$ and $V_{R\alpha}9$, representing the reciprocal product of V−J joining. Although TCDα9 also contained a V−J coding joint, the translational reading frame was out of phase, in contrast to TCDα3 (Figure 3B).

Two more circular DNA clones, TCDα6 and TCDα10, were analyzed by DNA sequencing (Figure 3B). Both clones contained a V−J coding joint at $J_\alpha 1$. Although the reciprocal structure was not identified in these clones, it is most likely that the reciprocal joint was separated by the *Eco*RI site downstream from $J_\alpha 2$. Coding joints were out-of-frame in both TCDα6 and TCDα10 (Figure 3B).

Comparison of V gene sequences between the α and δ gene clones

Since the D−J−C region of δ genes has been mapped between the V and J−C regions of TCRα genes (Chien *et al.*, 1987a), it is possible that the same V gene pool, at least in part, is shared between the α and δ gene systems. Figure 4B compares two V_α and two V_δ sequences identified in the coding joint of the circular DNA clones. The V region sequence of TCDδ1 was identical to the published V_α sequence of TA27, a member of $V_\alpha 7$ (Arden *et al.*, 1985), in the entire V coding region except at two positions. The other δ gene clone, TCDδ2, contained exactly the same V sequence as in the α gene clones TCDα3 and TCDα9. Furthermore, the 3′-flanking sequences of V genes identified in the reciprocal joint are also similar between the α and δ gene clones (Figure 4A). For the reciprocal joint, the δ gene clone TCDδ1 appears to share the same V gene with the α gene clone TCDα3, as does TCDδ2 with TCDα9.

Discussion

Circular DNA as an excision product of TCR gene rearrangement

In the present study, we have analyzed mouse thymocyte circular DNA excised from the TCR$\alpha-\delta$ gene complex. A phage library was prepared from circular DNA of thymocyte nuclei (Okazaki *et al.*, 1987) and screened with TCR J_α and J_δ region probes. Four α and two δ gene clones were characterized by DNA sequencing. In both types of clones, α and δ, a reciprocal recombinant of V−(D)−J joining was identified. At the reciprocal joint, two pairs of 7mer and 9mer sequences (Max *et al.*, 1979; Sakano *et al.*, 1979) were recombined in a head-to-head fashion; one pair was from V and the other from J. These observations indicated that in both α and δ genes, V and J segments were arranged in the same orientation on the germline chromosome and brought together by a DNA deletion mechanism.

Recombination pathways to generate the circular DNA molecule

Most circular DNA clones characterized in this study contained an additional recombinant structure upstream from the reciprocal joint (Figure 1). It was a coding joint: V−J in the α clones and V−D in the δ clones (Figure 3). Two successive joining events must have occurred to generate such structures. Two possible pathways are schematically shown in Figure 5 for the production of these molecules. In pathway A (Figure 5A), we assume that the coding joint was generated first on the chromosome and then excised in the circular DNA by the second V−J joining. The other pathway (Figure 5B) assumes that the first V−J joining excised unused V and J segments as well as the reciprocal joint into the circular molecule. Next, the secondary joining occurred on the circular DNA between the unused V and J segments forming the coding joint on the circle. This recombination generates two circular molecules from the substrate circular DNA (Figure 5B).

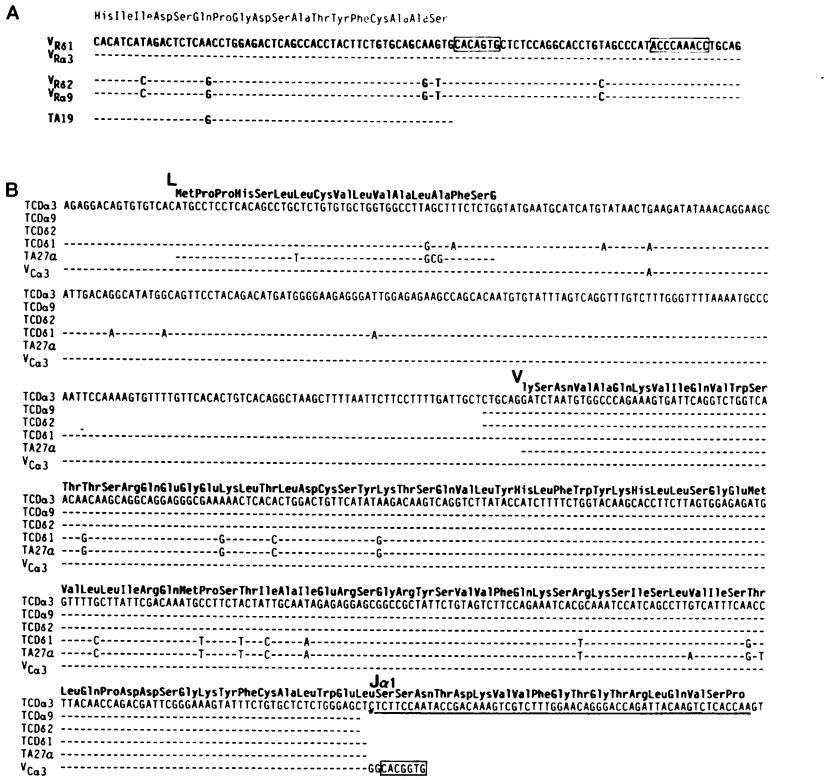

Fig. 4. Comparison of V gene sequences rearranged in the circular DNA clones. (**A**) Germline V sequences used in the reciprocal joint are compared. Nucleotide sequences in the vicinity of recombination sites are shown for the clones TCDδ1, TCDα3, TCDδ2 and TCDα9. The sequence of TA19, a cDNA clone of $V_\alpha 2$ family (Arden *et al.*, 1985) is also compared. Note that $V_{R\delta}1$ and $V_{R\alpha}3$ sequences are identical at least in the sequenced region. $V_{R\delta}2$ has the same sequence as $V_{R\alpha}9$. Predicted amino acid sequence for the germline clone $V_\alpha 2$ is shown. Recombination signals 3′ of the coding sequence are in boxes. (**B**) V gene sequences rearranged in the coding joint are compared for the entire V exons. Two α gene sequences (TCDα3 and TCDα9) and two δ gene sequences (TCDδ2 and TCDδ1) are compared. $V_{C\alpha}3$ is a germline sequence rearranged in the coding joint of TCDα3. These sequences appear to belong to the α gene family $V_\alpha 7$, the sequence identified in the cDNA clone, TA27 (Arden *et al.*, 1985). Predicted amino acid sequences are shown for leader (L), variable (V) and joining (J) regions of the in-phase recombinant identified in TCDα3.

Since V−(D)−J joining is not actively regulated to preserve the reading frame of genetic code, non-productive V−(D)−J structures must be produced quite frequently (Hagiya *et al.*, 1986). Although the V gene replacement (Kleinfield *et al.*, 1986; Reth *et al.*, 1986) could be one possible way to rescue the out-of-phase coding joint, successive trials of V−(D)−J joining could be another way to replace the non-productive recombinant with a productive one. For this reason, pathway A (Figure 5A) may be biologically significant, because the out-of-phase V−J joint can be removed from the chromosome by the second joining between the upstream V and downstream J segments to make an in-phase coding joint. In the TCRα gene system, >20 germline J segments have been identified (Hayday *et al.*, 1985; Winoto *et al.*, 1985). A greater number is estimated for the germline V_α segments (Arden *et al.*, 1985). The large number of pairs of V and J segments should not only help to diversify the V−J sequences, but also ensure that T cells eventually make an in-phase recombinant.

Identification of an in-phase V−J structure in the circular DNA clone TCDα3 is somewhat curious, since it has been assumed that further joining events in the same antigen receptor gene family cease as soon as a functional V-region structure is formed (Alt *et al.*, 1984; Hagiya *et al.*, 1986). There are three possible explanations for the in-phase V−J structure of TCDα3. First, TCDα3 could in fact be part of a non-functional gene, due to a mutation in some important sequences, e.g., transcriptional control elements. Secondly,

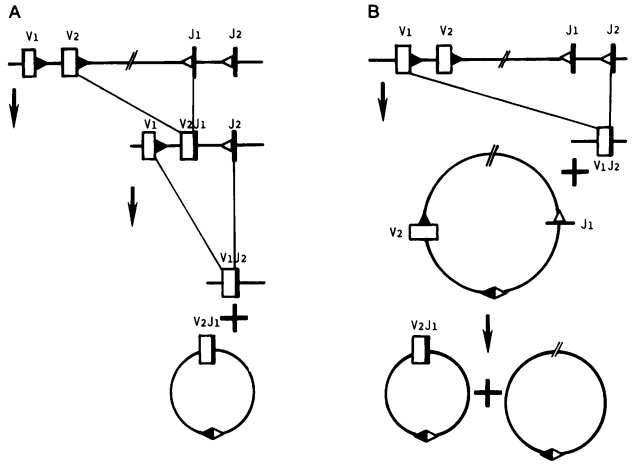

Fig. 5. Two possible pathways to generate double recombinant structures on the circular DNA molecules. Most clones characterized in this study contained two recombination sites; one is a coding joint and the other is a reciprocal joint. In pathway **A**, we assume two successive V−J joinings on the chromosomal DNA; the first joining is with the inside pair of substrates, V2 and J1, and the second joining is with the outside pair, V1 and J2. Pathway **B** assumes that the first joining occurs between the outside pair of substrates, V1 and J2, excising the unused pair, V2 and J1. The second joining would occur on the circular DNA between the V2 and J1 segments, generating two smaller circles. V and J segments are shown by boxes and bars respectively. Recombination signals are indicated by triangles (► and ◅).

TCDα3 could have been formed by pathway A (Figure 5A), with the in-phase joint forming first; the reciprocal joint is then rapidly formed, before the protein product of the in-phase structure can exert its putative signal effect. The third explanation is that TCDα3 could be formed via pathway B (Figure 5B). Here we are supposing that the coding joint was formed on the circular DNA after excision from the chromosome. It has been shown that the V−J type of recombination can occur on extrachromosomal plasmid DNA (Hesse *et al.*, 1987).

Unusual V−(D)−J joining in the TCRδ gene

In contrast to the Ig heavy-chain genes, V−J joinings skipping D may be possible in the TCRβ and δ gene systems. This is because the two different spacer lengths are arranged in an alternating fashion, i.e. V(23 bp)···(12 bp) D(23 bp) ···(12 bp)J. It is usually difficult to tell whether the D-skipped V−J recombination is taking place from the nucleotide sequence of the coding joint, since the D sequence is too short at the V−(D)−J junctions and is modified by nucleotide deletion and addition. The analysis of reciprocal joints on circular DNA permitted us to test the possibility of V−J joinings that skipped D segments. The reciprocal joint in clones TCDδ1 and TCDδ2 consisted of the 5′-J and 3′-V recombination signals (Figure 2A,B), thus providing direct evidence for the D-skipped V−J joining.

Another interesting structure found in the circular DNA was a coding joint of V−D without the J sequence. The δ gene clones, TCDδ1 and TCDδ2, contained such structures (Figure 3A,B). In these δ gene clones, the V segment is joined to the germline D segment without having D−J joining. In Ig genes, D−J joinings usually occur prior to V−D joinings (Alt *et al.*, 1984; Hagiya *et al.*, 1986). This sequential joining may indicate that the D−J structure is somehow prerequisite to the V−D recombination. The V−D recombinants identified in this study seem inconsistent with the sequential model for V−D−J joining. Similar chromosomal V−D sequences were also recently reported by Chien *et al.* (1987b). It may well be possible that the order and efficiencies of joinings are affected by distances separating the recombination substrates. Closer substrates tend to be used more frequently in the Ig heavy-chain V−D−J joining (Coleclough *et al.*, 1981; Yancopoulos and Alt, 1985). Although the linkage between V and D regions in the δ gene is yet to be established, the D_δ segments could be quite close to the 3′ proximal V segments in the V gene cluster.

As mentioned earlier, TCR δ segments have 12-bp RSSs and 23-bp RSSs configured in an alternating fashion. Because of this configuration, D−D joinings are also possible as well as D-skipped V−J joinings. In the V−D structures described above, we identified a long stretch of inserted nucleotides at the V−D junction, 13 bases both for TCDδ1 and TCDδ2 (Figure 3A,B). Since the inserted sequences are unusually long for the non-germline elements, D−D joining may be contributing to the inserted sequences. Recently, Chien *et al.* (1987b) identified the second D segment ($D_\delta1$) ~10 kb upstream from the D described in Figure 3A ($D_\delta2$). It is interesting that a part of the inserted sequence of the clone TCDδ2, five nucleotides GTGGC, is in agreement with the coding sequence of the $D_\delta1$ segment (Figure 3A). A similar example of $D_\delta1-D_\delta2$ joining was also described by Chien *et al.* (1987b). The 13-bp insertion in the clone TCDδ1 could not be accounted for by the $D_\delta1$ sequence. There could be an additional D segment(s), yet to be identified, that contributed to the insertion sequence of TCDδ1.

It has been reported that the TCRδ gene is located in the middle of the α gene cluster on chromosome 14 (Chien *et al.*, 1987a,b). The δ gene J−C region has been mapped only 20 kb upstream from the J_α region. It is intriguing to study whether the same V gene pool is utilized both for the α and δ gene systems. Our sequence data of V genes rearranged in the circular DNA supported the notion that some germline V segments could be used both in the α and in the δ gene rearrangements (Figure 4). For example, the same germline V segment was used in the reciprocal joints of the δ gene clone TCDδ1 and the α gene clone TCDα3. A similar situation was also found in the V genes used for the coding joint (Figure 4B). It is yet to be determined, however, whether the entire V gene pool is shared by both α and δ gene systems. Furthermore, it is not evident whether the V_α sequence was indeed utilized for the δ gene expression, because the sequence is found on the excision product from chromosomal DNA. More cDNA sequences of α and δ chains will clarify the issue. During the preparation of the manuscript, we learned from Dr Chien that the TA27 V_α sequence (Figure 4B) is found in functional δ genes, preferentially in fetal thymocyte hybridomas (Elliott *et al.*, 1988).

In the present paper as well as our previous report (Okazaki *et al.*, 1987), we have characterized all four kinds of TCR genes−α, β, γ and δ−in the thymocyte circular DNA. From 200 000 recombinant phages of thymocyte circular DNA from 3-week-old mice, we have identified >200 rearranged clones for α, 24 for β, five for δ and two for γ, using the respective J region probes. Based on the Northern blot analysis of T-lineage cells, it has been proposed that γ and δ gene sequences could be expressed even in the pre-thymic T cells (Chien *et al.*, 1987b; Loh *et al.*, 1987b). Both α and β genes undergo the rearrangement in the thymus, although the β gene rearranges before the α gene. Relative frequencies of getting circular DNA clones for four different TCR families are consistent with the order of the expression of their genes during the T cell development. It is of interest to compare the ratios of TCR gene clones in similar circular DNA libraries prepared from different stages of fetal development. Characterization of circular DNA will continue to be a powerful approach to study the developmental control of rearranging genes.

Materials and methods

Phage library of circular DNA

Circular DNA from thymocyte nuclei was prepared from 3-week-old BALB/c mice according to the method described by Okazaki *et al.* (1987). The phage library was prepared by the method of Hohn (1979) using λgtWES (Tiemeier *et al.*, 1976) as a vector.

DNA probes

The J_α probe was a 1.5-kb *Eco*RI−*Eco*RI fragment containing $J_\alpha1$ and $J_\alpha2$ (Hayday *et al.*, 1985). The J_δ probe was a 3.6-kb *Eco*RI−*Xba*I fragment containing the $D_\delta2$ and $J_\delta1$ sequences (Chien *et al.*, 1987a,b).

DNA sequencing

Nucleotide sequences were determined by the chain-termination method with dideoxynucleotides (Sanger, 1981), or by the chemical degradation method of Maxam and Gilbert (1977).

Other methods for DNA analysis

DNA clones were analyzed by the standard procedures as described by Maniatis *et al.* (1982). Enzymes used for DNA experiments were obtained

from Bethesda Research Laboratories, Boehringer Mannheim, New England Biolabs and United States Biochemicals.

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