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Organization of variable region segments of the human immunoglobulin heavy chain: duplication of the D_5 cluster within the locus and interchromosomal translocation of variable region segments

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We have studied the organization of variable region (V) genes of the human immunoglobulin heavy chain (H) by cosmid cloning. We isolated two independent immunoglobulin D_5 clusters ($D_{5\text{-a}}$ and $D_{5\text{-b}}$) from cosmid libraries of the human genome. Restriction maps of these two regions showed that downstream 15 kb portions of the 55 kb overlap were different although upstream 40 kb portions were almost identical. Four more D segments, (D_M, D_{XP}, D_A and D_K) were found around the D_5 segment in the conserved region of each cluster. Nucleotide sequences of the corresponding D segments from each cluster were almost identical and they encoded potentially functional D regions. Analysis using human – rodent somatic cell hybrids demonstrated that both clusters were located in the immunoglobulin heavy chain (H) locus on chromosome 14, suggesting that the $D_{5\text{-a}}$ and $D_{5\text{-b}}$ regions evolved by internal duplication within this locus. We also isolated a 60 kb DNA region carrying four V_H segments, designated as $V_{H\text{-F}}$ region, which was located on chromosome 16. Nucleotide sequences of the four V_H segments were determined. Two of them encoded potentially functional V_H segments, and the other two were pseudogenes. Some more V_H segments were found to be located outside chromosome 14, by Southern blot hybridization of human – rodent hybrid cell DNAs. These results provide further evidence that the human V_H locus has undergone recent reorganization.

Key words: cosmid clones/human – rodent somatic cell hybrids/immunoglobulin gene/intrachromosomal duplication/orphon V_H segments

Introduction

Both variable (V) and constant (C) region genes of the human immunoglobulin (Ig) heavy chain (H) are located at chromosome 14q32 (Croce *et al.*, 1979). The germ line V_H region genes consist of three groups of discontinuous DNA segments, i.e. V_H, D and J_H, which are brought together by VDJ recombination during lymphocyte differentiation (reviewed by Tonegawa, 1983; Honjo and Habu, 1985).

The human V_H segments are classified into six families. It is obvious that the numbers and sequences of the V_H, D and J_H segments provide the germ line basis of the Ig repertoire. In addition, recent results indicate that the organization in this locus such as the relative location and orientation of each segment seems to affect the relative frequency of its usage and thus the diversity of the V_H repertoire. Several investigators showed that V_H segments that are located proximal to the J_H segments are more frequently used especially during early stages of ontogeny (Schroeder *et al.*, 1987; Berman *et al.*, 1988, Humphries *et al.*, 1988).

We showed previously that a functional D segment, D_5, is not directly linked to the J_H segments but located within the V_H cluster (Matsuda *et al.*, 1988). V_H segments downstream of the D_5 segment are thus unable to recombine with the D_5 segment unless the V_H segments are inversely oriented like some of the human V_κ segments (Lorenz *et al.*, 1987). These results indicate that not only the numbers and sequences of the V_H, D and J_H segments but their organization on the chromosome play crucial roles in the V_H repertoire formation.

Earlier studies on the human V_H locus organization have revealed several unique features. In addition to the interspersed D_5 segments described above (Matsuda *et al.*, 1988), members of different V_H families were shown to be interspersed (Kodaira *et al.*, 1986). In contrast, the murine D segments are located immediately 5′ to the J_H segments (Ichihara *et al.*, 1989), and members of each V_H family tend to cluster in the murine V_H locus (Brodeur *et al.*, 1989). Different V_H locus organization between man and mouse suggests that the V_H locus has undergone recent reorganization after segregation of the two species. Comparison of the human and murine V_H locus organization may provide interesting insights into the dynamic reorganization of a multigene family during evolution.

In this study we will describe two examples of recent reorganization of the human V_H locus; (i) duplication of the D_5 cluster within the H chain locus on chromosome 14, and (ii) translocation of V_H segments to chromosome 16. Surprisingly, two out of four orphon V_H segments sequenced were apparently functional.

Results and discussion

Internal duplication of the human D_5 cluster

In our previous paper (Matsuda *et al.*, 1988), we discussed the possibility that there are more than two human D_5 segments in the human genome on the basis of studies using pulsed field gel electrophoresis. To test this possibility by physical mapping, we isolated several cosmid clones carrying the D_5 segment from human genomic libraries using the D_5 segment as a probe. Restriction maps of these clones using eight restriction endonucleases allowed us to classify them into two major groups, designated as the $D_{5\text{-a}}$ and $D_{5\text{-b}}$ regions as shown in Figure 1. The $D_{5\text{-a}}$ region encompasses 60 kb DNA consisting of five overlapping cosmid clones,

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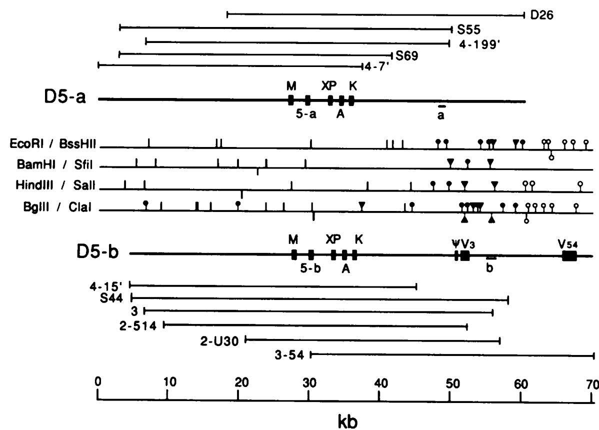

Fig. 1. Restriction maps of human D_5 clusters. The $D_{5\text{-a}}$ and $D_{5\text{-b}}$ regions are shown by thick lines with closed boxes indicating exons. Location of the region specific probes a and b are shown by underlining. The transcriptional orientation of D_M, D_5, D_{XP}, D_A, D_K and V_3 genes is from left to right. Nucleotide sequence of the V_{54} segment was not determined. Restriction sites common to the $D_{5\text{-a}}$ and $D_{5\text{-b}}$ regions are shown by vertical lines. Vertical lines with closed circles and triangles indicate restriction sites which exist only in the $D_{5\text{-a}}$ and $D_{5\text{-b}}$ regions, respectively. Vertical lines with open circles represent sites identified in the $D_{5\text{-b}}$ region only. Cosmid clone DNAs are shown by thin lines above and below each region.

and the $D_{5\text{-b}}$ region contains six overlapping cosmid clones encompassing 65 kb DNA. About 55 kb of the $D_{5\text{-a}}$ and $D_{5\text{-b}}$ regions overlap each other. The restriction maps of the upstream 40 kb DNA of the overlapped region were almost identical whereas those of the downstream 15 kb DNA were very different. Divergence between the two regions began ~47 kb 3′ from the 5′ end of the clone 4-7′. A continuous transition from the conserved region to the diverged region was found in at least three clones for each region. Furthermore, the probe derived from the diverged portion of each region did not hybridize to the counter portion of the other region yet both (probes a and b) were localized to the distal end of chromosome 14 as described below. Taking these results together, we can exclude the possibility that the 3′ divergence of these regions is due to a cloning artifact.

The downstream of the $D_{5\text{-b}}$ region contained two V_H segments, i.e. V_3 and V_{54}, which were absent from the $D_{5\text{-a}}$ region. The V_3 segment which belongs to the $V_{H\text{-III}}$ family is a pseudogene as described previously (Matsuda *et al.*, 1988), and the V_{54} segment was assigned to the $V_{H\text{-I}}$ family by Southern blot hybridization although the nucleotide sequence of the V_{54} segment is not yet determined.

Since each of the human D_1, D_2, D_3 and D_4 regions contains five additional D segments, namely D_M, D_A, D_{XP}, D_K and D_N (Ichihara *et al.*, 1988b), we tried to find D segments other than the $D_{5\text{-a}}$ and $D_{5\text{-b}}$ segments in the two D_5 regions by Southern hybridization using the D_{M2}, D_{A4}, D_{XP1}, D_{K1} and D_{N4} segments (Ichihara *et al.*, 1988b) as probes. Both $D_{5\text{-a}}$ and $D_{5\text{-b}}$ regions contained these D segments except for the D_N segment between 2.5 kb upstream and 5.5 kb downstream of the D_5 segments in the order 5′-D_{M5}−D_5−D_{XP5}−D_{A5}−D_{K5}−3′ (Figure 1). When the nucleotide sequences of the corresponding D segments in the two D clusters $D_{5\text{-a}}$ and $D_{5\text{-b}}$ were compared, their coding sequences were identical with each other, suggesting that duplication of these clusters took place rather recently (Figure 2). All 10 newly identified D segments have two open reading frames, indicating that they are potentially functional. However, the upstream heptamer of the recombination signal sequences of the D_{A5} (TGCTATG) and D_{K5} (GATTGTG for $D_{K5\text{-a}}$ and GACTGTG for $D_{K5\text{-b}}$) segments were slightly diverged from the consensus heptamer sequence, CAC$^{A}_{T}$GTG. Furthermore, the $D_{A5\text{-b}}$ segment contained a single base deletion in the upstream spacer. As a single base substitution of the third C or the fifth G nucleotide in the heptamer sequence drastically reduces the recombination activity (Akira *et al.*, 1987), the D_{A5} and $D_{K5\text{-a}}$ segments might not be used efficiently. Although a few mismatches were found in the flanking sequences of the $D_{5\text{-a}}$ and $D_{5\text{-b}}$ regions, nucleotide sequences of the corresponding D segments as well as restriction maps in the upstream 40 kb portions of the two D_5 regions are almost identical with each other, indicating that this duplication must have taken place very recently within the human V_H locus.

Duplication of the D_5 region is an intrachromosomal event

To test whether the D_5 region duplication took place between different chromosomes or within chromosome 14, chromosomal mapping of the $D_{5\text{-a}}$ and $D_{5\text{-b}}$ regions was carried out. A probe which is specific to each D_5 region was isolated from the diverged downstream portion (Figure 1). The 1.0 kb *Eco*RI fragment from the clone D26 (probe a) and the 0.55 kb *Pst*I−*Hin*dIII fragment (probe b) derived from the 4.8 kb *Hin*dIII fragment of clone 3 were isolated, and used as probes for Southern hybridization of *Hin*dIII digested DNA of a human−mouse somatic cell hybrid (Rag/G04) which contains a single human chromosome with the IgH locus (band 14q32) translocated to the short arm of chromosome X. High molecular weight DNAs from FLEB14-14 (human germ line control) and Rag cell (mouse control) were also included, and digested with *Hin*dIII. Probe a hybridized with a single 2.0 kb fragment in FLEB14-14 and Rag/G04 DNAs (Figure 3). A similar experiment using probe b detected the identical set of six bands containing the 4.8 kb fragment in FLEB14-14 and Rag/G04 DNAs. However, no bands were detected in Rag DNA with either probe. These results led us to conclude that both $D_{5\text{-a}}$ and $D_{5\text{-b}}$ regions are located within the Ig locus in chromosome 14 (14q32) as a result of intrachromosomal duplication.

Isolation and mapping of two distinct D_5 clusters in the V_H locus on chromosome 14 established that the human V_H locus was generated by multiple duplication events during the course of evolution. In addition, we isolated other D_5-containing cosmid clones which have different restriction maps and cannot be classified into either $D_{5\text{-a}}$ or $D_{5\text{-b}}$ regions (our unpublished observation). This might reflect the fact that there are more D_5 clusters in the human genome. Since all the murine D segments are located in the 80 kb region between the V_H and J_H clusters (Ichihara *et al.*, 1989), the human V_H locus must have evolved through extensive reorganization of large chromosomal regions after divergence from mouse.

Are the D_5 segments functional?

It is worth noting that at least seven out of the ten D segments in the D_5 region are apparently functional as they have more than one open reading frame in both orientations as well as the conserved recombination signal sequences.

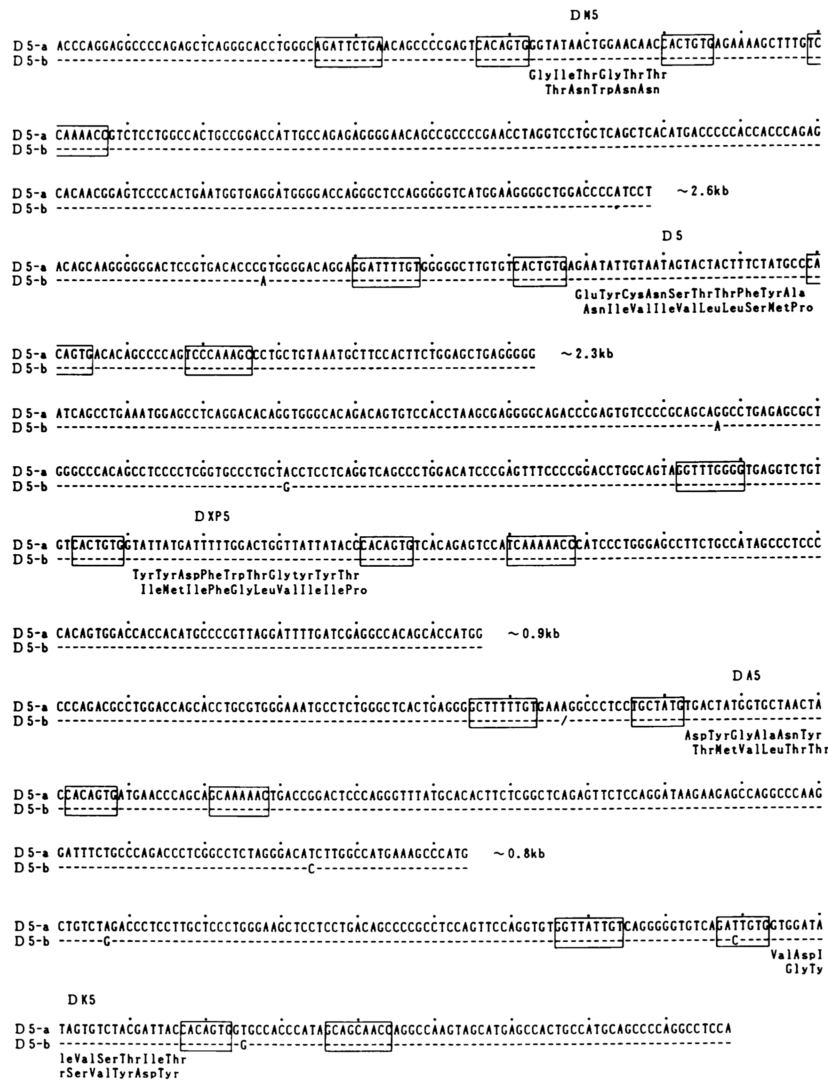

Fig. 2. Nucleotide sequences of D segments in $D_{5\text{-}a}$ and $D_{5\text{-}b}$ regions. D exons were identified by alignment with sequences published previously (Ichihara *et al.*, 1988a; Zong *et al.*, 1988). Open reading frames of each D segment are shown below. Dashes in $D_{5\text{-}b}$ indicate bases identical to those of $D_{5\text{-}a}$ and a slash in $D_{5\text{-}b}$ shows deletion. Intervals between sequenced regions are shown in kb. The recombination signal sequences are boxed.

Although the computer assisted homology search did not find Igs containing these ten D sequences, these D segments could have been used for functional V_HDJ_H formation, followed by modification due to N sequence insertion and somatic mutation. In rabbit, as in human, some unknown D segments might be dispersed among V_H segments because many somatic D segments do not have high homology with any of the D segments located at the immediate 5′ region flanking the J_H cluster (Becker *et al.*, 1989).

The D segments in upstream D_5 clusters could be used to rescue an allele with a non-functionally rearranged V_HDJ_H gene by subsequent rearrangement between more upstream D segments and more downstream J_H segments, resulting in excision of the non-functional V_HDJ_H gene from the chromosome. Secondary DJ_H rearrangement to preformed V_HDJ_H genes was reported in Abelson murine leukemia virus-transformed pre-B cell lines (Reth *et al.*, 1986; Maeda *et al.*, 1987). Such a rescue rearrangement would increase the probability of succeeding in functional rearrangement.

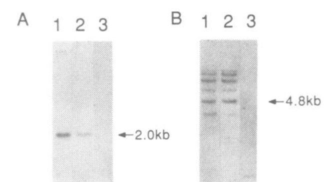

Fig. 3. Southern hybridization of the $D_{5\text{-}a}$ and $D_{5\text{-}b}$ specific probes to DNA from a mouse–human hybrid cell line. Southern blot filters of *Hin*dIII digested DNAs (2 μg each) of FLEB14-14, Rag/G04 and Rag cells were hybridized with probe a (**A**) or probe b (**B**) as indicated in Figure 1. Origins of DNAs are: **lane 1**, FLEB14-14; **lane 2**, Rag/G04; **lane 3**, Rag.

Orphon V_H segments were found on chromosome 16

A 60 kb DNA region which consists of three overlapping cosmid clones, namely 3-14, 3-27 and 3-65, was isolated from human genomic libraries using $V_{H\text{-}I}$ and $V_{H\text{-}III}$ probes. This cluster, designated as $V_{H\text{-}F}$, carries two each of the

V_{H-I} and the V_{H-III} hybridizing fragments as shown in Figure 4. V_H segments belonging to different families are interspersed in this cluster as previously reported (Kodaira et al., 1986).

The probe P27 (0.3 kb *Pst*I fragment) was used to test whether the V_{H-F} locus is on chromosome 14 (Figure 5A). Unexpectedly, the P27 probe did not hybridize with Rag/G04 DNA at all, indicating that the V_{H-F} cluster is located on a chromosome other than chromosome 14 (Figure 5A, lane 6). Chromosomal mapping analysis using the P27 probe and a panel of human–mouse somatic hybrid cells showed that the scores of discordance to the chromosomes 9, 15 and 16 (25%, 30% and 19%, respectively) were lowest whereas those to other chromosomes including chromosome 14 were >37% (data not shown). In order to obtain a conclusive answer, we examined four more hybrid DNAs, 2D5-A1, H/B2-1 ①, H/B1B1-43 ② and C/B CL-17 which allow us to distinguish chromosomes 9, 14, 15 and 16 (Table I). The 5.6 kb *Hind*III band was found only in the H/B2-1 ① DNA which contains chromosome 16 but not chromo-somes 9, 14 and 15 (Figure 5, lane 3). The same 5.6 kb band was found in the control human DNA of FLEB14-14. In order to confirm results of the karyotype analysis, the same sets of DNAs were hybridized with human cardiac actin (Gunning et al., 1984) and human myoglobin-2 (Nakamura et al., 1988), probes for genes which are located in chromosomes 15 and 16, respectively (Figure 5B and C).

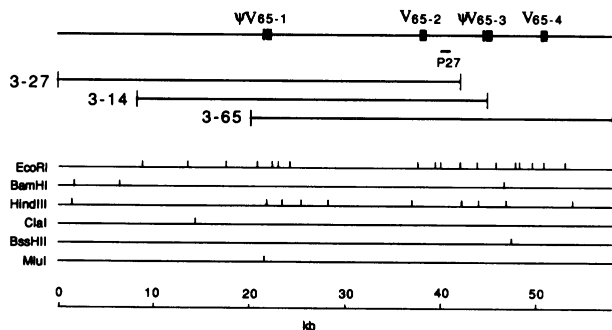

Fig. 4. Restriction map of the human V_{H-F} region. The V_{H-F} region is shown at the top with closed boxes indicating exons. The transcriptional orientations of the V_{65-1} and V_{65-3} segments are from left to right. Orientations of the V_{65-2} and V_{65-4} segments are not determined. Location of the V_{H-F} region specific probe (P27) is shown by a bar. Cosmid clone DNAs are shown by horizontal lines below. The restriction sites of *Eco*RI, *Bam*HI, *Hind*III, *Cla*I, *Bss*HII and *Mlu*I are shown at the bottom. *Sal*I did not have any restriction sites within this region.

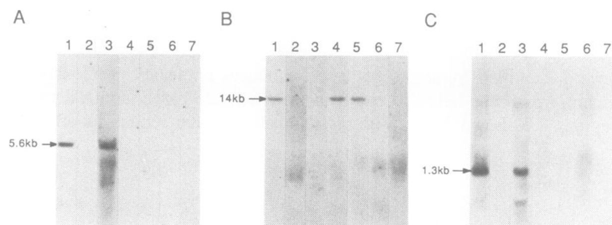

Fig. 5. Southern hybridization of DNAs from mouse–human hybrid cell lines with the V_{H-F} specific probe. Southern blot filters of *Hind*III **(A)**, *Eco*RI **(B)** and *Msp*I **(C)** digested DNAs of FLEB14-14, 2D5-A1, H/B 2-1①, H/B 1B1-43②, C/B CL-17, Rag/G04 and Rag cells were hybridized with the P27 probe (A), cardiac actin probe (B) or myoglobin-2 probe (C). Each lane contains 2 μg of DNA. Origins of DNAs are: **lane 1**, FLEB 14-14; **lane 2**, 2D5-A1; **lane 3**, H/B 2-1 ①; **lane 4**, H/B 1B1-43②; **lane 5**, C/B CL-17; **lane 6**, Rag/G04; **lane 7**, Rag.

Two V_H segments in the V_{H-F} region are potentially functional

We determined nucleotide sequences of four V_H segments (V_{65-1}, V_{65-2}, V_{65-3} and V_{65-4}) within the V_{H-F} region (Figure 6). Coding region sequences of the V_{65-1} and V_{65-3} segments were identified by comparison with those of the V_{35} segment of the V_{H-I} family (Matsuda et al., 1988). Similarly, the V_{65-2} and V_{65-4} sequences were homologous to the V_3 sequence of the V_{H-III} family (Matsuda et al., 1988). The V_{65-1} segment was a diverged pseudogene with the following mutations; several nonsense and frame-shift mutations in the coding region, and the diverged recombination signal sequences. The V_{65-3} segment had more divergence; the 3′ half (downstream from nucleotide 288) of the V_{65-3} segment was replaced by a totally unrelated sequence in addition to point mutations, insertions and deletions throughout the coding region (Figure 6). The polarities of the transcription of the two segments were identical by comparison of their nucleotide sequences and the restriction map of this region.

The nucleotide sequence of the 15-1 segment (Berman et al., 1988) was almost identical to that of the V_{65-3} segment except for several base substitutions. The *Bam*HI and *Hind*III restriction site maps of the 3′ half of the V_{H-F} region are similar to those of the V_{15} region (~28 kb) which carries one V_{H-I} family segment (15-1) and one V_{H-III} family segment (15-2B) with the same transcriptional polarity.

On the other hand, the V_{65-2} and V_{65-4} segments were potentially functional genes except for a slight change in the heptamer sequence from CACAGTG to CACAGCG in the V_{65-4} segment (Figure 6). The nucleotide sequence of the V_{65-4} segment was almost identical to that of the 15-2B segment (Berman et al., 1988). Computer assisted homology search could not find homologous V_H segments in any published Ig sequences. Although Lötscher et al. (1988) reported that some of human V_κ genes have also been transposed to chromosomes other than chromosome 2 where

Table I. Human chromosomes present in human–mouse hybrid cells

Hybrid cells	Human chromosomes[a] 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Y
2D5-A1	–	–	+	–	–	–	–	–	–	–	–	–	–	+	–	–	+	–	–	–	+	–	–	–
H/B 2-1①	+	–	–	–	(+)	+	–	–	–	+	+	+	+	–	–	+	+	–	+	[+]	+	–	+	–
H/B 1B1-43②	–	–	–	–	[+]	–	+	–	–	–	–	–	+	–	+	–	(+)	–	–	–	–	–	–	–
C/B CL-17	–	–	–	+	+	+	+	–	+	–	–	+	+	+	+	–	+	+	+	+	+	+	–	+

[a]Percentage of cells in total population containing the specific chromosome are indicated as follows: +, >30%; [+], 20–30%; (+), 10–20%; –, <10%.

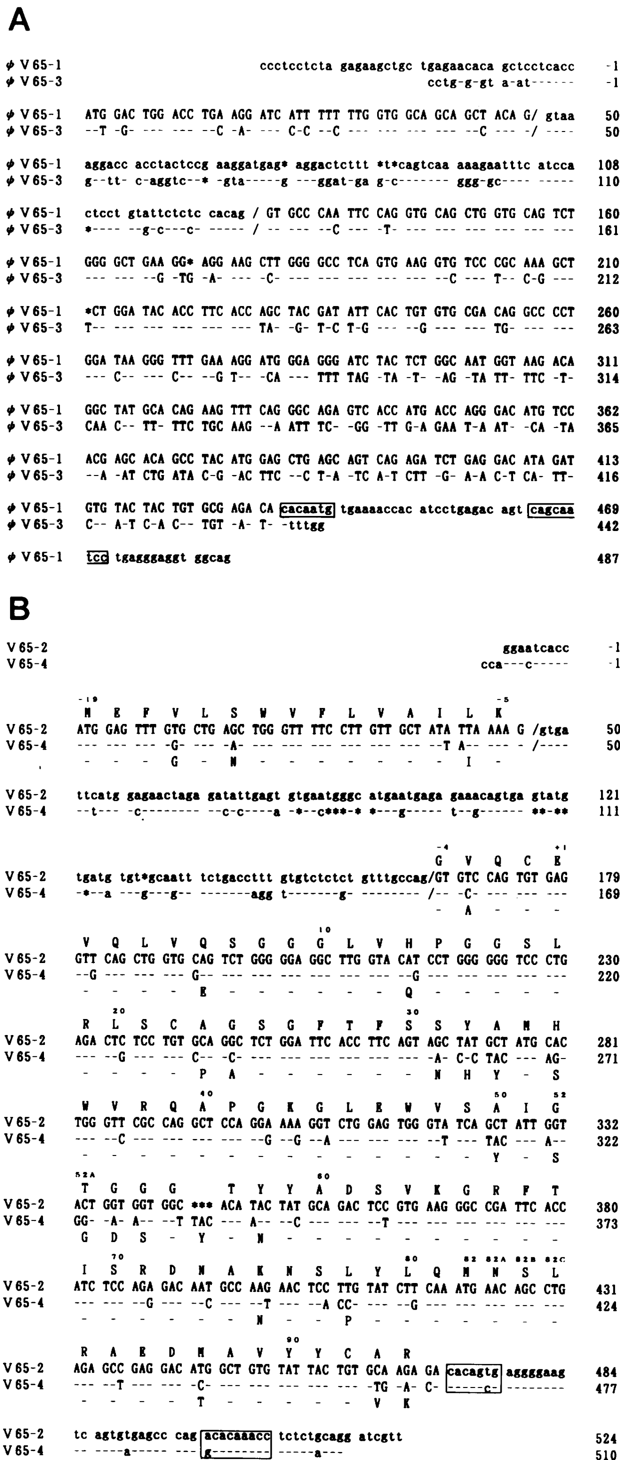

Fig. 6. Nucleotide sequences of the V_H segments of the $V_{H\text{-}F}$ region. **(A)** $V_{65\text{-}1}$ and $V_{65\text{-}3}$ sequences. **(B)** $V_{65\text{-}2}$ and $V_{65\text{-}4}$ sequences. Sequences that align with the protein coding sequences are given in triplets, and introns are shown in lower case letters. Deletions are shown by asterisks, and the signals for recombination are boxed. Nucleotide position +1 is the first letter of the initiation codon. The amino acid sequences of $V_{65\text{-}2}$ and $V_{65\text{-}4}$ segments deduced from the nucleotide sequences are shown above and below the nucleotide sequences, respectively. Numbers according to Kabat *et al.* (1987) are given above amino acid residues.

the human V_κ locus is located, all these translocated V_κ segments are pseudogenes.

There are several explanations for conservation of apparently functional V_H segments outside chromosome 14. First, translocation of the $V_{H\text{-}F}$ region to chromosome 16 was a recent event in evolution. Another possibility is that these V_H segments are under selective constraint through their usage by interchromosomal rearrangement like the formation of the chimeric T cell receptor gene by rearrangement between the human γ and δ loci (Tycko *et al.*, 1989). They found that the majority of the chimeric transcripts identified in normal human lymphoid cells had correct open reading frames, indicating that trans-chromosomal rearrangement took place between the two loci. Some correction mechanisms such as gene conversion might have been involved in conservation of orphon V_H segments.

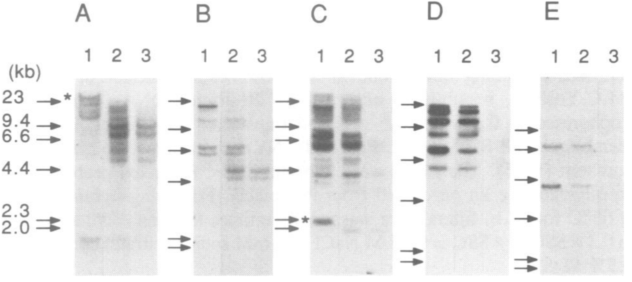

Fig. 7. Southern hybridization of DNA of human–mouse hybrid cell lines with the human V_H probes. *Bam*HI (A), *Hin*dIII **(B, D** and **E)** and *Eco*RI (C) digested DNAs from FLEB14-14 **(lane 1)**, Rag/G04 **(lane 2)** and Rag **(lane 3)** were Southern blotted and hybridized with human $V_{H\text{-}I}$ (A), $V_{H\text{-}II}$ (B), $V_{H\text{-}III}$ (C), $V_{H\text{-}IV}$ (D) and $V_{H\text{-}V}$ (E) family probes. Each lane contains 2 μg of DNA. Asterisks in A and C correspond to fragments containing $V_{65\text{-}1}/V_{65\text{-}3}$ and $V_{65\text{-}2}/V_{65\text{-}4}$ of the $V_{H\text{-}F}$ region, respectively. Arrows indicate the size markers of 23, 9.4, 6.6, 4.4, 2.3 and 2.0 kb from top to bottom.

There are many orphon V_H segments

We then tested if there are any other V_H segments which are located on chromosomes other than chromosome 14. Southern blot hybridisation of DNAs from FLEB14-14, Rag/G04 and Rag cells were carried out using the $V_{H\text{-}I}$, $V_{H\text{-}II}$, $V_{H\text{-}III}$, $V_{H\text{-}IV}$ and $V_{H\text{-}V}$ family probes. No significant difference except for the intensity of some bands was detected between FLEB14-14 and Rag/G04 DNAs when the $V_{H\text{-}IV}$ and $V_{H\text{-}V}$ probes were used (Figure 7). The results using the $V_{H\text{-}I}$, $V_{H\text{-}II}$ and $V_{H\text{-}III}$ probes were slightly complicated because these probes cross-hybridized with murine V_H segments. Nevertheless, when the *Bam*HI digested DNAs were hybridized with the $V_{H\text{-}I}$ probe, a band of >30 kb in size, which corresponds to the *Bam*HI fragment carrying $V_{65\text{-}1}$ and $V_{65\text{-}3}$, was detected in FLEB14-14 DNA but not in Rag/G04 DNA. A similar result was obtained when the *Eco*RI digested DNAs were hybridized with the $V_{H\text{-}III}$ probe. A few $V_{H\text{-}III}$ fragments in FLEB14-14 DNA, including a strong 2.0 kb band which carries $V_{65\text{-}2}$ and $V_{65\text{-}4}$ segments, were absent from Rag/G04 DNA. Similarly, only FLEB14-14 DNA produced an 18 kb *Hin*dIII fragment hybridizing with the $V_{H\text{-}II}$ probe. From these results we conclude that there are more V_H segments which are located on chromosomes other than chromosome 14.

Materials and methods

FLEB14-14 is an Epstein–Barr virus transformed pro-B cell line with the germ line context of the Ig gene as described before (Katamine *et al.*, 1984; Otsu *et al.*, 1987). Rag/G04 is a mouse–human hybrid cell line which carries

a single human chromosome with 14−X translocation t(X;14)(p22;q32) as described (Purrello *et al.*, 1987). The other four cell lines, H/B 2D5-A1, H/B2-1 ①, H/B 1B1-43 ② and C/B CL-17 are mouse−human hybrid cell lines containing various human chromosomes as indicated in Table I (M.C.Yoshida, unpublished observation). Restricted DNAs were electrophoresed in 0.7% agarose gels and transferred to Biodyne B nylon membrane (Pall Bio Support, East Hills, NY) according to the method of Southern (1975). The filters were hybridized to ^{32}P-labeled probe using an oligolabeling kit purchased from Pharmacia. Following hybridization at 65°C for 12 h, filters were washed three times (30 min each) at 65°C in 0.1×SSC (1×SSC is 0.15 M NaCl−15 mM sodium citrate) containing 0.5% SDS.

Plasmid DNA of cosmid clones was isolated by the alkaline lysis method as described (Maniatis *et al.*, 1982). Isolated restriction fragments were cloned into pUC18, pUC19, Bluescript KS or Bluescript SK vectors. The chain termination method (Sanger *et al.*, 1980; Hattori and Sakaki, 1986) was used for sequencing plasmid clones. Sequenase version 2.0 kit (US Biochemical Co.) was used for sequencing. Synthetic oligonucleotides 5′-AGGTGCAGCTGGTGCAGTCTG-3′, 5′-CCAGGGGCCTGTCGCA-CCC A-3′, 5′-CACTCCAGCCCCTTCCCTGGAGC-3′ and 5′CACTCCA-GACCCTTTCCTGGAGC-3′ were used as primers for sequencing V_H segments.

Origins of DNA probes of the $V_{H\text{-}I}$, $V_{H\text{-}II}$, $V_{H\text{-}III}$ and $V_{H\text{-}IV}$ families are V_{266BL} (Nishida *et al.*, 1982), $V_{CE\text{-}1}$ (Takahashi *et al.*, 1984), V_{HBV} (Kodaira *et al.*, 1986) and $V_{71\text{-}2}$ (Lee *et al.*, 1987), respectively. The $V_{H\text{-}V}$ family probe (5-IRI) was a gift from F.Alt (Berman *et al.*, 1988). D_5 ($D_{LR\text{-}5}$) probe has been described before (Zong *et al.*, 1988) and the other five D probes (D_{M2}, D_{XP1}, D_{A4}, D_{K1} and D_{N4}) were donated by Y.Kurosawa (Ichihara *et al.*, 1988b). Probes for human cardiac actin (pHRL83-IVS4) and human myoglobin-2 (pCMM65) were donated by P.Gunning and Y.Nakamura, respectively.

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