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Dispersed localization of D segments in the human immunoglobulin heavy-chain locus

Fumihiko Matsuda, Kwang Ho Lee, Sumiko Nakai[1], Takayuki Sato, Mieko Kodaira, Shu Qin Zong, Hiroshi Ohno[2], Shiro Fukuhara[2] and Tasuku Honjo

Department of Medical Chemistry and [2]Department of Medicine, Kyoto University Faculty of Medicine, Sakyo-ku, Kyoto 606 and [1]Department of Genetics, Osaka University School of Medicine, Osaka 560, Japan

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We have studied the organization of the human immunoglobulin heavy-chain genes by pulse field gel electrophoresis as well as by isolation of cosmid clones. The total length of the heavy-chain variable region locus was estimated to be ~3000 kb. We found that D segments including a recently isolated D_5 segment were dispersed among VH segments. We identified a pseudo V segment 18 kb 3′ to the D_5 segment in isolated cosmid clones. A 300 kb fragment produced by *Mlu*I digestion contained VH, D, JH segments and the distance between VH and D was estimated to be ~240 kb. Overlapping cosmid clones containing the human D_1, D_2, D_3, D_4, JH, Cμ and Cδ genes were isolated. Restriction maps of these regions indicated that the distance between D and JH is about 22 kb. A partial restriction map of the VH locus was constructed using the pulse field gel electrophoresis technique and deletion of VH segments in B cells.

Key words: cosmid clones/deletion map/nucleotide sequence/pulse field gel electrophoresis

Introduction

The immunoglobulin heavy chain consists of the variable (VH) region which recognizes antigens and the constant (CH) region which mediates physiological functions. The complete VH region gene is comprised of three germline segments, namely VH, diversity (D) and joining (JH) segments (reviewed by Honjo, 1983; Tonegawa, 1983; Honjo and Habu, 1985) which are located on chromosome 14 (14q32) in the human (Kirsch *et al.*, 1982) and chromosome 12 in the mouse (D'eustachio *et al.*, 1980). The number and organization of these segments provide the germline basis of the immunoglobulin repertoire and random recombination between separate sets of the three segments generates combinatorial diversity. Numbers of the VH and D segments in the human genome are unknown whereas there are six JH segments and 11 CH genes. The six human JH segments are clustered within a small region (3 kb) about 6 kb upstream to the Cμ gene (Ravetch *et al.*, 1981). Four human D segments were shown to be clustered (Siebenlist *et al.*, 1981). Although their location was not determined, it was assumed that the D segments were located between the VH segments and the JH segments from the physical linkage study in mouse (Wood and Tonegawa, 1983). To evaluate precisely the germline repertoire of the human immunoglobulin, it is essential to construct the physical map of the immunoglobulin heavy-chain locus.

We have initiated isolation of the entire human VH locus using a cosmid vector to elucidate the germline VH repertoire. We have isolated 18 independent cosmid clones containing 55 VH segments from a human genomic library (Kodaira *et al.*, 1986). Using these 18 clones we have shown that VH segments belonging to three different families (VH-I′, VH-II′ and VH-III) are interspersed among themselves. Subsequently, we have cloned and characterized a novel family (VH-IV) of human VH genes (Lee *et al.*, 1987). We have also isolated and sequenced the fifth D segment (D_5) which is not physically linked to the D_1-D_4 cluster (Zong *et al.*, 1988).

To elucidate the general organization of the human VH locus we employed the pulse field gel electrophoresis (PFG) technique, which allowed us to estimate that the size of the human VH locus is ~3000 kb. Using this method we showed that D segments including the D_5 segment were dispersed among VH clusters in agreement with our previous proposal which was based on evolutionary considerations (Honjo *et al.*, 1981). This finding was further confirmed by cloning and characterization of the 120 kb DNA fragments containing D_1-D_4, JH, Cμ and Cδ genes. We have constructed a partial restriction map of the human VH locus by a combination of PFG analysis and deletion of VH segments in B cell lines.

Results and discussion

General organization of the human VH locus

DNA from FLEB14-14, which is an Epstein–Barr virus (EBV)-transformed fetal liver cell line and has no rearrangement of the immunoglobulin genes (Katamine *et al.*, 1984; Otsu *et al.*, 1987), was digested with the restriction enzyme *Mlu*I, electrophoresed in PFG and then subjected to Southern blot analysis using the VHBV probe of the VH-III family (Kodaira *et al.*, 1986). This probe detected 300, 350, 700, 800 and >1000 kb DNA fragments (Figure 1A, lane 1). The same five bands were detected using V_{266BL} (VH-I family), V_{CE-1} (VH-II family) and V_{71-2} (VH-IV family) as probes (data not shown), indicating that the total length of the VH locus is ~3000 kb.

When the same filter was hybridized with the D_5 probe which cross-hybridized the D_1, D_2, D_3 and D_4 segments, three out of the five *Mlu*I bands were detected, namely the 300, 700 and >1000 kb bands (Figure 1A, lane 2). By contrast, the JH probe detected only the 300 kb *Mlu*I band (Figure 1A, lane 3). These results indicate that the 300 kb *Mlu*I fragment contains VH, D and JH segments and that D segments are dispersed among the VH clusters.

The D_5 probe detected eight distinct bands ranging from ~6 kb up to 240 kb in *Sfi*I-digested FLEB14-14 DNA (Fig-

ure 1B). However, *Bam*HI digestion of FLEB14-14 DNA produced only five bands (18, 7.4, 6.6, 1.8 and 1.6 kb) hybridizing with the D_5 probe (Figure 1C) in agreement with the previous reports (Siebenlist *et al.*, 1981; Zong *et al.*, 1988). The results suggest that large chromosomal regions containing multiple D segments were duplicated and that more unknown germline D segments were present in the human genome.

Physical mapping of the D_4–JH–Cδ region

Among 18 independent VH or JH containing cosmid clones from human genomic libraries (Kodaira *et al.*, 1985), two clones, U2-2 and U7, were shown to hybridize with both D and JH probes. Comparison of the restriction maps of the two clones showed that they were almost identical to each other and encompassed the region from ~5 kb upstream to the D_2 segment to 2 kb downstream to the JH cluster (Figure 2). These clones thus overlapped with previously isolated clones (D25, D31 and D48) containing the D_1, D_2, D_3 and D_4 segments (Zong *et al.*, 1988).

Another clone (64), which hybridized only with JH probe, encompassed the region from 5 kb upstream to the JH cluster to 20 kb downstream to the Cδ gene. Clone 64 had a 10 kb overlap with the clones U2-2 and U7. These six clones together cover the region (~12 kb) containing the D_1–D_4, JH, Cμ and Cδ genes (Figure 2). The distance between the most 3′ D segment (D_4) and the JH cluster was found to be ~22 kb. Clone 64 has only two *Mlu*I sites, one immediately 3′ to the JH cluster and the other between the Cμ and Cδ genes. Physical mapping studies on the isolated cosmid clones are consistent with the above PFG results that all of the four D segments (D_1–D_4) and the JH cluster are located in the 300 kb fragment produced by *Mlu*I digestion.

The distance between the 3′ VH and D segment is less than ~240 kb

A cosmid clone (35) was isolated by the VH-I probe. The 1.1 kb *Pst*I–*Pvu*II fragment hybridizing with the VH-I probe was isolated and sequenced (sequencing strategy not shown). The coding region sequence was identified by comparison with the $V_{71\text{-}5}$ sequence of the VH-I family (Kodaira *et al.*, 1986) as shown in Figure 3A. Their nucleotide and amino acid sequences were 87 and 77% homologous, respectively. The V_{35} gene was found to be potentially functional. The amino acid sequence of the V_{35} segment was also homologous (78.6%) to that of V_{266BL} (Nishida *et al.*, 1982; Kenten *et al.*, 1982).

The 1.3 kb *Eco*RI fragment (p35 probe) isolated from clone 35 hybridized to a unique fragment of human placenta DNA on Southern blot hybridization (data not shown). The p35 probe hybridized with the 300 kb *Mlu*I fragment fractionated by PFG (Figure 1A, lane 4). Because the four D segments and the entire JH cluster were present in the 300 kb *Mlu*I fragment, we concluded that the distance between the V_{35} segment and the D_4 segment was less than 240 kb.

Evidence for 5′-D–VH-3′ organization

We confirmed that the D_5 segment was dispersed in VH segments by physical mapping of clone D26, in which the D_5 segment had been found originally (Zong *et al.*, 1988). Clone D26 had a 30 kb overlapping region with another clone 3 isolated by the VHBV probe (Figure 4). These two clones had a common restricted fragment hybridized with

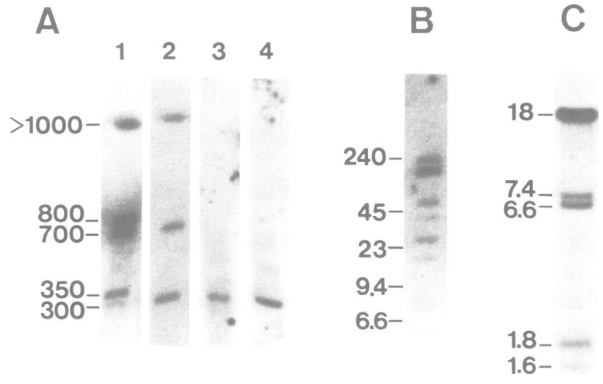

Fig. 1. PFG electrophoresis analysis of human VH locus. Southern blot filters of *Mlu* (**A**), *Sfi*I (**B**), *Bam*HI (**C**) digested DNA of FLEB14-14 were hybridized with probes indicated. Chromosomes of *Saccharomyces cerevisiae* were used as DNA size markers. Numbers in kb. Each lane contains ~1 μg (A,B) and ~2 μg (C) DNA respectively. Probes used for each lane are: (A) **lane 1**, VHBV (VH-III); **lane 2**, D_5; **lane 3**, JH; **lane 4**, p35; (B), (C) D_5.

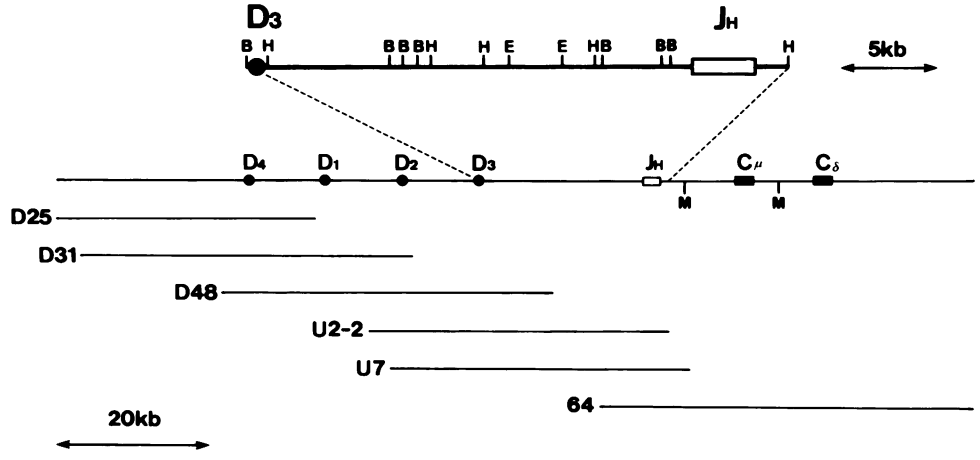

Fig. 2. Restriction map of the region containing human D–JH–Cμ–Cδ genes. The top line represents newly mapped 22 kb region. Second line represents ~120 kb region containing D_1–D_4 (●), JH cluster (□), Cμ and Cδ genes (■). The transcriptional orientation of all the segments is from left to right. Nucleases are abbreviated as follows: E, *Eco*RI; B, *Bam*HI; H, *Hin*dIII; M, *Mlu*I.

the VHBV probe. Since restriction mapping showed that the VH-hybridizing fragment was located ~20 kb 3′ to the D_5 gene, we determined the nucleotide sequence of the VH-hybridizing fragment (sequencing strategy not shown). The coding region sequences were identified by comparison with that of the $V_{71\text{-}1}$ gene of the VH-III family (Kodaira *et al.*, 1986) as shown in Figure 3B. The coding region of V_3 and $V_{71\text{-}1}$ were found to be 78% homologous with each other. There were six termination codons in frame with the initiator codon ATG in the V_3 sequence. Furthermore, the V_3 gene had eight insertion mutations and three deletion mutations in the coding region. VH pseudogenes seem to be classified into two distinct groups; those with a few mutations (conserved) and those with drastic changes (Kodaira *et al.*, 1986). The V_3 gene seems to be a member of the latter group.

We then tested if the D_5 segment is located in the

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V35   CTCATGAATA TGCAAATAAC CTGAGATTTA CTGAAGTAAA TACAGATCTG TCCTGTGCCC TGAGAGCATC ACCCAGCAAC
                                                                -19
                                                               Met Asp Trp Thr Trp Arg Ile Leu Phe
      CACATCTGTC CTCTAGAGAA TCCCCTGAGA GCTCCGTTCC TCACC        ATG GAC TGG ACC TGG AGG ATC CTC TTC
                          -5
      Leu Val Ala Ala Ala Thr G
      TTG GTG GCA GCA GCC ACA G/ gtaagaggct ccctagtccc agtgatgaga aagaggattg agtccagtcc aggga
                                      -4                  +1
                                      ly Ala His Ser Gln Val Gln Leu Val Gln Ser Gly Ala
      gatct catccacttc tgtgttctct ccacag/  GA GCC CAC TCC CAG GTG CAG CTG GTG CAG TCT GGG GCT
      10                                   20                                  30
      Glu Val Lys Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Gly
      GAG GTG AAG AAG CCT GGG GCC TCA GTG AAG GTC TCC TGC AAG GCT TCT GGA TAC ACC TTC ACC GGC
                                      40                                  50      52  52A
      Tyr Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met Gly Arg Ile Asn Pro
      TAC TAT ATG CAC TGG GTG CGA CAG GCC CCT GGA CAA GGG CTT GAG TGG ATG GGA CGG ATC AAC CCT
                                60                                  70
      Asn Ser Gly Gly Thr Asn Tyr Ala Gln Lys Phe Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser
      AAC AGT GGT GGC ACA AAC TAT GCA CAG AAG TTT CAG GGC AGG GTC ACC AGT ACC AGG GAC ACG TCC
                          80      82  82A 82B 82C                   90
      Ile Ser Thr Ala Tyr Met Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Val Val Tyr Tyr Cys Ala
      ATC AGC ACA GCC TAC ATG GAG CTG AGC AGG CTG AGA TCT GAC GAC ACG GTC GTG TAT TAC TGT GCG
      Arg
      AGA GA CACAGTG TGAAAACCCA CATCCTGAGG GTG TCAGAAACC CCAGGGAGGA GGCAG

V3       GATCAGCACT GAACACAGAG GGCTCACC  ATG GAG TTT GGG CTG AGC TGG GTT TTC CTT GTT GCT ATT

      TTA AAA G /gtgattcatg gagaaataga gagattgagt gtgaggggac atgagtgaga gaaacagtgg atttctgtgg

      cagtttctga ccagggtgtc tctgtgtttg cag/    GT GTC CAG TGT GAG GTG CAG CTG GTG GAG TCT GGG

      GGA GGC TTG GTC CAG CCT GGG GGT TCT CTG AGA CTC TCA TGT GCT ACC TCT GGA TTC ACC TTC AGT
                                                                                          A
      GAC CAC TAC ATG AGC TGG GTC CG- CAG GCT CAA GGG AAA GGG CAG -AG TTG GTA GGT TTA ATA AGA
        A  G    C      C   A                                    C
      ACA ACT AAA GTT AAC GCA GAA TAT GCT GCG TCT GTG AAA GGC AGA CTT ACC ATC TCA AGA GAG GAT
                                        A
      TCA AAG AAC ACG CTG TAT CT- CAA ATG GAC AGC CTG AAA ACC GAG GAC TTG GCC GTG TAT TAC TGT

      GCT AGA GA CACAGTG AGGGGAGGTC ACGTGAGCCC AG ACACAACCT CCCTGCAGGC
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Fig. 3. Nucleotide sequence of V_{35} (**A**) and V_3 (**B**) genes. Sequences that align with the protein coding sequence are given in triplets with numbering according to Kabat *et al.* (1987) and introns are shown in lower case letters. The amino acid sequence of V_{35} deduced from the nucleotide sequence is also shown above the nucleotide sequence. Inserted bases are placed above the next base and deletions are shown by dashes. The signals for VH−D joining are boxed. Exon and intron junctions are shown by slashes.

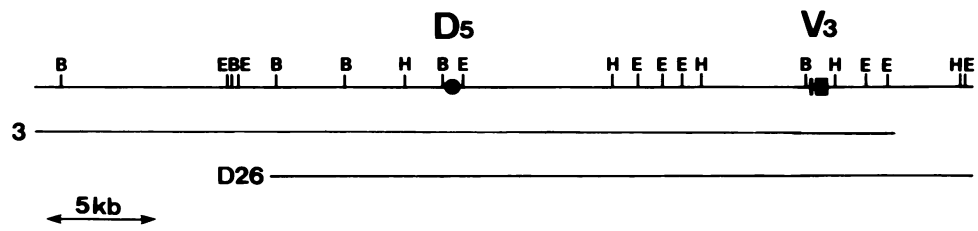

Fig. 4. Restriction map of the human D_5 locus. The transcriptional orientation of D_5 (●) and V_3 (■) genes is from left to right. Nucleases are abbreviated as follows: E, *Eco*RI; B, *Bam*HI; H, *Hin*dIII.

immunoglobulin heavy-chain locus (band 14q32) using the human− mouse cell hybrid Rag/Go4 line which carries a single human chromosome with 14-X translocation t(X;14) (p22;q32). The hybrid contained the immunoglobulin heavy-chain locus (band 14q32) which was translocated to the short arm of chromosome X (Purrello *et al.*, 1987). High mol. wt DNAs of Rag/Go4, FLEB14-14 (germline control) and Rag (mouse cell) were digested with *Bam*HI. Southern blot hybridization using D_5 probe showed the identical set of five bands, namely 18, 7.4, 6.6, 1.8 and 1.6 kb in both FLEB14-14 and Rag/Go4 DNAs. However, none of them was detected in Rag DNA (Figure 5). The D_5 probe did not hybridize with mouse−human hybrid cells which had human X chromosomes but not human chromosome 14 (data not shown). The results indicate that the D_5 segment is located in the heavy-chain locus of chromosome 14.

What mechanism brought the D segment upstream to the VH segment? The heavy-chain genes of horned shark, a primitive elasmobranch, was shown to have a unique organization; a 10 kb unit consisting of each copy of the VH, D, JH and CH segments, which might be an original form of the immunoglobulin gene, was tandemly repeated in the genome of this organism (Hinds and Litman, 1986). The human VH locus must have evolved through repeated duplication of VH, D and JH segments. Assuming that a unit of duplication contained the VH, D and JH segments, the D and JH segments could easily have been interspersed between VH segments.

In fact, many fragments in VH-containing cosmid clones were weakly hybridized with either the D or the JH probe (unpublished observations). The D segments, which are flanked by nonamer and heptamer recombination signal sequences at both sides, might have been capable of D−JH rearrangement even if located very far from the JH cluster. These D segments could be conserved by functional constraint. Since the sequences of the five human germline D segments thus far isolated are not found in any of the known V−D−J sequences, it is reasonable to assume that there may be more unknown functional D fragments in the human VH locus. The JH segments, which had been dispersed among VH clusters, were unable to participate in the formation of functional heavy-chain genes because of the large distance from the Cμ gene, and thus diverged rapidly without functional constraint. The fragment detected by the JH probe might be diverged pseudo-JH genes or their relicts.

Order of the MluI fragments of the human VH locus

We have tested whether EBV-transformed human B cell lines might have deleted some *Mlu*I fragments containing VH segments by VH−D−JH recombinations on both alleles. DNAs from these cells were digested with *Mlu*I, electrophoresed in PFG and subjected to Southern blot analysis using VH-III probe as shown in Figure 6. DNA from FLEB14-14 cells which gives the germline VH profile was used as control. The profiles of some cell lines were very complex and, therefore, did not allow us to draw any conclusions. Three cell lines, namely CESS, LCL-D and EBV5, showed deletions of some *Mlu*I fragments.

The deletion of the 300 and 800 kb fragments was observed in all of the three cell lines, suggesting that these fragments are located at the most 3′ part of the VH locus. Since the JH cluster is located in the 300 kb fragment as described above, the 800 kb fragment is likely to be located immediately upstream to the 300 kb fragment. The 350 kb fragment was deleted in two of them (LCL-D and EBV5)

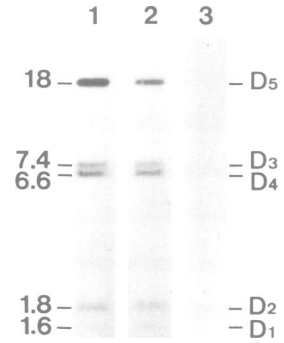

Fig. 5. Southern hybridization of the D_5 probe to DNA from a mouse−human hybrid cell line. Southern blot filters of *Bam*HI-digested DNAs of FLEB14-14, Rag/Go4 and Rag were hybridized with the D_5 probe. Each lane contains ~2 μg DNA. Origins of DNAs are: **lane 1**, FLEB14-14; **lane 2**, Rag/Go4; **lane 3**, Rag. Numbers are in kb.

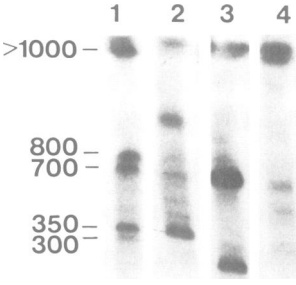

Fig. 6. Deletion of VH segments in human B cell lines. DNAs from the cell lines were digested with *Mlu*I, electrophoresed in PFG. Filters were hybridized with the VHBV (VH-III family) probe. DNA size markers are indicated in kb. Each lane contains ~1 μg of DNA. DNAs used for each lane are: **lane 1**, FLEB14-14; **lane 2**, CESS; **lane 3**, LCL-D; **lane 4**, EVB5.

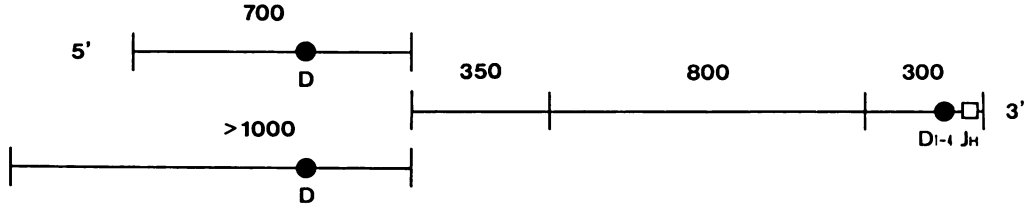

Fig. 7. Organization of human VH locus. Horizontal lines separated by vertical lines show the *Mlu*I fragments which contain VH segments. The number above each line represents the size of the fragment in kb. The symbols (●) and (□) indicate the D and JH segments respectively.

although it remained in the germline intensity in CESS cell line. None of the three cells showed complete loss of the >1000 and 700 kb fragments. As the >1000 and 700 kb fragments were retained in LCL-D and EBV5 cells which deleted the 800, 350 and 300 kb fragments, the >1000 and 700 kb fragments might be located 5′ to those deleted fragments. DNA fragments larger than 1000 kb were staggered after PFG and caused some non-specific binding. It was, therefore, very difficult to detect the change of the intensity of this fragment. Assuming that the rearrangements in human VH locus are mediated by DNA excision mechanism in these cells, we propose a tentative order of the *Mlu*I fragments of the VH locus as follows: 5′-(>1000, 700)−250−800− 300-3′ as shown in Figure 7.

Materials and methods

FLEB14-14, EBV5, CESS and LCL-D are EBV-transformed human cell lines. FLEB14-14, which is derived from a human fetal liver cell, has several B lineage markers but no rearrangements in the immunoglobulin gene (Katamine *et al.*, 1984; Otsu *et al.*, 1987). The other three lines are derived from human peripheral blood lymphocytes and have both alleles of the JH segment rearranged (Takahashi *et al.*, 1984; our unpublished data). Genomic DNAs used for PFG were prepared as described (Poustka *et al.*, 1987). Restriction endonculease digests of DNA were electrophoresed using Pulsaphor™ system (LKB) according to the manufacturer's directions. Gels were transferred to Genescreenplus™ nylon membrane (NEN) according to the method of Southern (1975) after treatment with 0.25 M HCl for 15 min. The filters were hybridized to ^{32}P-labeled nick translated probe as described (Honjo *et al.*, 1979). Filters were washed three times (30 min each) at 50°C in 0.1 M NaCl, 10 mM sodium citrate, 0.1% sodium dodecylsulfate.

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 and pUC19. The chain termination method (Sanger *et al.*, 1980; Hattori and Sakaki, 1986) was used for sequencing pUC clones. Origins of DNA probes are: V_{266BL} (Nishida *et al.*, 1982), $V_{CE\text{-}1}$ (Takahashi *et al.*, 1984), V_{HBV} and $V_{71\text{-}2}$ (Kodaira *et al.*, 1986), D_5 (Zong *et al.*, 1988) and JH (Otsu *et al.*, 1987).

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References

D'eustachio,P., Pravtceva,D., Marcu,K. and Ruddle,F.H. (1980) *J. Exp. Med.*, **151**, 1545−1550.
Hattori,M. and Sakaki,Y. (1986) *Anal. Biochem.*, **152**, 232−238.
Hinds,K.R. and Litman,G.W. (1986) *Nature*, **320**, 546−549.
Honjo,T. (1983) *Annu. Rev. Immunol.*, **1**, 499−528.
Honjo,T. and Habu,S. (1985) *Annu. Rev. Biochem.*, **54**, 803−830.
Honjo,T., Obata,M., Yamawaki-Kataoka,Y., Kataoka,T., Kawakami,T., Takahashi,N. and Mano,Y. (1979) *Cell*, **18**, 559−568.
Honjo,T., Nakai,S., Nishida,Y., Kataoka,T., Yamawaki-Kataoka,Y., Takahashi,N., Obata,M., Shimuzu,A., Yaoita,Y., Nikaido,T. and Ishida,N. (1981) *Immunol. Rev.*, **59**, 33−68.
Kabat,E.A., Wu,T.T., Reid-Miller,M., Perry,H.M. and Gottesman,K.S. (1987) *Sequences of Proteins of Immunological Interest.* NIH Publications, Washington, DC.
Katamine,S., Otsu,M., Tada,K., Tsuchiya,S., Sato,T., Ishida,N., Honjo,T. and Ono,Y. (1984) *Nature*, **309**, 369−371.
Kenten,J.H., Molgoard,H.V., Houghton,M., Derbyshire,R.B., Viney,J., Bell,L.O. and Gould,H.J. (1982) *Proc. Natl. Acad. Sci. USA.*, **79**, 6661−6665.
Kirsch,I.R., Morton,C.C., Nakahara,K. and Leder,P. (1982) *Science*, **216**, 301−303.
Kodaira,M., Kinashi,T., Umemura,I., Matsuda,F., Noma,T., Ono,Y. and Honjo,T. (1986) *J. Mol. Biol.*, **190**, 529−541.
Lee,K.H., Matsuda,F., Kinashi,T., Kodaira,M. and Honjo,T. (1987) *J. Mol. Biol.*, **195**, 761−768.
Maniatis,T., Fritsch,E.F. and Sambrook,J. (1982) *Molecular Cloning: A Laboratory Manual.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
Nishida,Y., Miki,T., Hisajima,H. and Honjo,T. (1982) *Proc. Natl. Acad. Sci. USA.*, **79**, 3833−3837.
Otsu,M., Katamine,S., Uno,M., Yamaki,M., Ono,Y., Klein,G., Sasaki, M.S., Yaoita,Y. and Honjo,T. (1987) *Mol. Cell. Biol.*, **7**, 708−717.
Poustka,A., Pohl,T.M., Barlow,D.P., Frischauf,A. and Lehrach,H. (1987) *Nature*, **325**, 353−355.
Purrello,M., Alhadeff,B., Whittington,E., Buckton,K.E., Daniel,A., Arnaud,P., Rocchi,M., Archidiacono,N., Filippi,G. and Siniscalco,M. (1987) *Cytogenet. Cell. Genet.*, **44**, 32−40.
Ravetch,J.V., Siebenlist,U., Korsmeyer,S., Waldmann,T. and Leder,P. (1981) *Cell*, **27**, 583−591.
Sanger,F., Coulson,B.G., Smith,A.J.H. and Roa,B.A. (1980) *J. Mol. Biol.*, **143**, 161−178.
Siebenlist,U., Ravetch,J.V., Korsmeyer,S., Waldmann,T. and Leder,P. (1981) *Nature*, **294**, 631−635.
Southern,E.M. (1975) *J. Mol. Biol.*, **98**, 503−517.
Takahashi,N., Noma,T. and Honjo,T. (1984) *Proc. Natl. Acad. Sci. USA*, **81**, 5194−5198.
Tonegawa,S. (1983) *Nature*, **392**, 575−581.
Wood,C. and Tonegawa,S. (1983) *Proc. Natl. Acad. Sci. USA.*, **80**, 3030−3034.
Zong,S.Q., Nakai,S., Matsuda,F., Lee,K.H. and Honjo,T. (1988) *Immunol. Lett.*, in press.

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