The EMBO Journal vol.7 no.7 pp.2003 - 2010, 1988

The use of chromosomal translocations to study human immunoglobulin gene organization: mapping D_H segments within 35 kb of the C_μ gene and identification of a new D_H locus

L.Buluwela, D.G.Albertson[1], P.Sherrington[2], P.H.Rabbitts[3,5], N.Spurr[4] and T.H.Rabbitts

Medical Research Council Laboratory of Molecular Biology, [1]Medical Research Council Molecular Genetics Unit, MRC Centre, [2]University of Cambridge Department of Haematological Medicine, MRC Centre, [3]Ludwig Institute for Cancer Research, MRC Centre, Hills Road, Cambridge CB2 2QH, and [4]Imperial Cancer Research Fund Clare Hall Laboratories, Blanche Lane, South Mimms, Potters Bar, Hertfordshire, UK

[5]Present address: Medical Research Council Clinical Oncology and Radiotherapeutics Unit, MRC Centre, Hills Road, Cambridge CB2 2QH, UK

Communicated by T.H.Rabbitts

We have studied the Burkitt's lymphoma cell line Daudi which carries the translocation t(8;14). The breakpoint of this translocation on the $14q^+$ chromosome occurs near to a rearranged D_H-J_H join, and the actual chromosome junction is a few hundred base pairs upstream of the joined D_H element. The nucleotide sequence of the rearranged D_H segment shows that it does not come from the previously described D cluster. Using this D_H sequence as a probe we have identified two separate D_H clusters. One of these is the major D_H cluster and is located only 20 kb upstream of the J_H segments. A pseudo-V_H (probably the first V_H segment) is also found ~98 kb from J_H. A second, minor D_H locus has been found which seems to be located on the distal side of the V_H locus on chromosome 14, since there is little evidence for rearrangement or deletion of this locus in any B cell DNA analysed. A single V_HIII subgroup gene is located within 25 kb of the newly identified D_H element: it is possible that this minor locus occurs near the limit of the Igh locus.

Key words: translocations/Burkitt's lymphoma/immunoglobulin/diversity segment/V genes

Introduction

The study of chromosomal abnormalities associated with antigen receptor genes is instructive not only from the point of view of tumor pathogenesis, but also in representing mutant chromosomes which allow the natural gene organization and rearrangement to be analysed. This aspect of the study of chromosome aberration has, for example, allowed a detailed analysis of the T cell receptor δ-α locus to be carried out (Baer *et al.*, 1988). The B cell tumours, Burkitt's lymphoma (BL), generally carry translocations involving chromosome 8q24 (at which site the c-*myc* proto-oncogene maps), with either chromosome 14q32, 2p13 or 22q11 (reviewed, Rabbitts, 1985). The latter positions encode the immunoglobulin (Ig) heavy (H) chain, kappa light chain and lambda light chain genes respectively, each of which is intimately involved in the chromosomal breakpoints.

In endemic BL, breakpoints at the Igh band on 14q32 frequently involve the joining (J) region segments (Haluska *et al.*, 1986) and the cell line Daudi has been shown to have a diversity (D)$-J_H$ join at the boundary of the translocation t(8;14) (q24;q32). We have studied in detail this chromosomal translocation junction, which has ultimately allowed us to define the location and sequence of two distinct clusters of D_H segments, one of which is only 20 kb from the J_H locus. In addition, the location of V_H segments relative to D_H has been elucidated.

Results

Characterization of the translocated J_H locus of the Burkitt's lymphoma Daudi

In order to study the t(8;14) breakpoint in the Burkitt's lymphoma Daudi, we have isolated bacteriophage λ clones using an Ig J_H probe from a genomic library prepared from Daudi DNA. The restriction map of two overlapping clones, λD39 and λD54, coming from the non-productive IgH allele is presented in Figure 1A and covers a region that contains all of the J_H locus and extends beyond the translocation breakpoint with chromosome 8 as defined by Haluska *et al.* (1987). A subclone, p54RH2, was prepared containing the J_H segments and DNA up to and beyond the breakpoint (Figure 1A). The nucleotide sequence, starting 3′ of J_H and extending beyond the chromosomal breakpoint, was determined from this material by the strategy indicated in Figure 1B. Comparison of part of this sequence with that of the germline J_H sequence (Ravetch *et al.*, 1981; Rabbitts, 1983) is shown in Figure 1C. Clearly the J_H region near the translocation join in Daudi is extensively mutated, featuring point mutations and extensive deletions, and insertions of random nucleotide sequences reminiscent of N region diversity. As previously noted (Haluska *et al.*, 1987), the chromosome translocation junction in Daudi is found upstream of a DJ_H gene rearrangement involving J_H4 and a D_H sequence that is distinct from the family originally defined (Siebenlist *et al.*, 1981). However, the new D_H element is closely related to a D_H family reported to be present at the junction of a t(14;18) rearrangement in a follicular lymphoma (Bakshi *et al.*, 1987). The sequence that we have determined for the rearranged D_H sequence differs from that reported (Haluska *et al.*, 1987) by the inclusion of two extra T nucleotides. We also present sequence upstream of the rearranged D_H and note that this is different to the conserved upstream DNA sequences of the main D_H family (Ravetch *et al.*, 1981). However, the upstream sequence does contain the promoter element (Reth and Alt, 1984) that has been shown to be used in DJ_H transcription; therefore, the chromosomal translocation does not break at the 5′ end of the D element, but rather this occurs ~600 bp upstream (as judged by hybridization experiments with the probe p54Stu/RI1 (Figure 1B, and see below). Thus the translocation has occurred somewhat upstream of the D−J join itself (Figure 1).

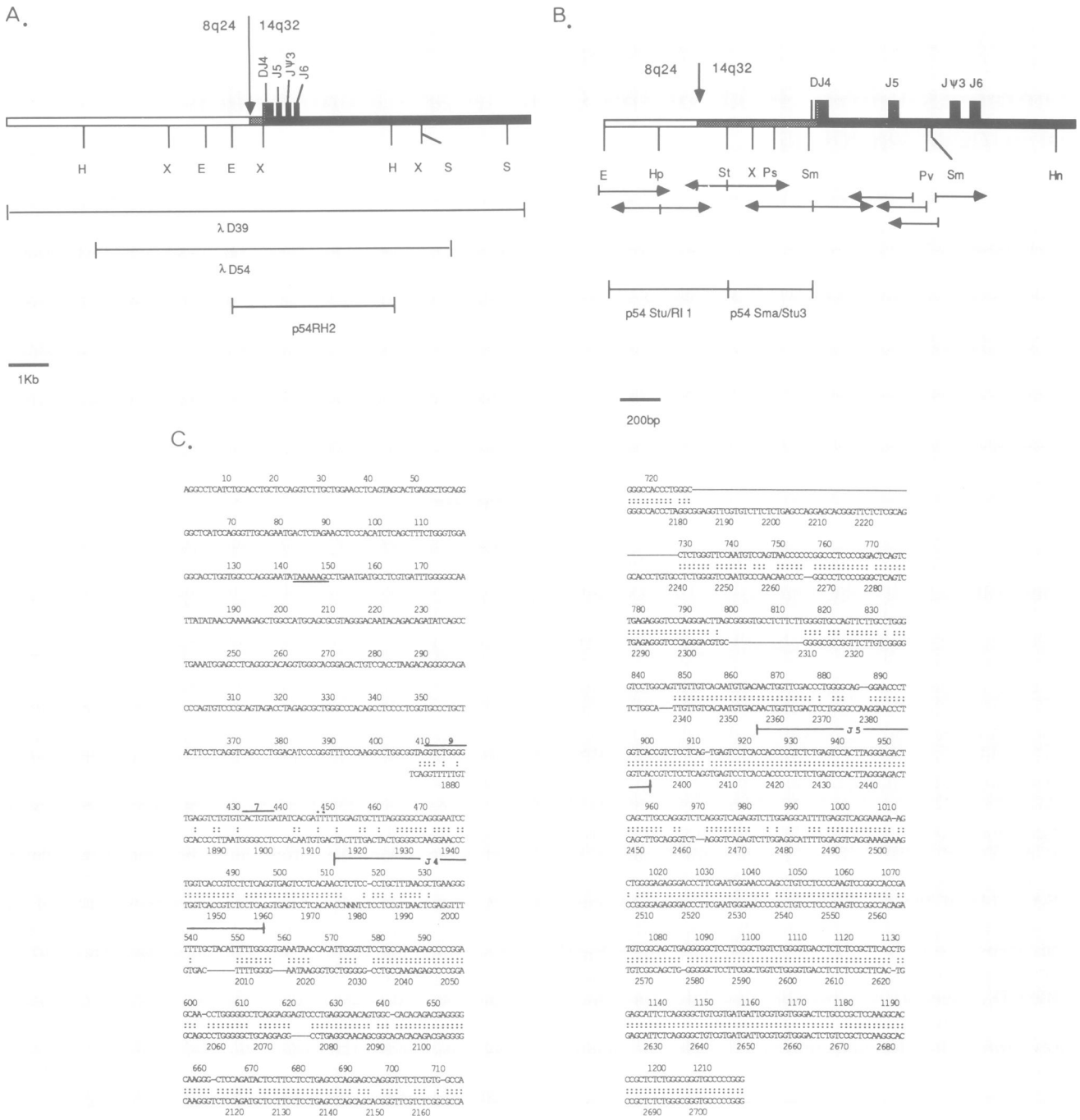

Fig. 1. Translocation t(8;14)(q24;q32) from the BL cell line Daudi. (**A**) Restriction map of translocation region. The top line shows a map, derived from the clones λD39 and λD54, of the breakpoint region. The open box derives from band 8q24, the stippled box from Igh D_H and closed box from the Igh J_H region. H, *Hin*dIII; X, *Xba*I; E, *Eco*RI; S, *Sac*I. (**B**) Sequencing strategy for breakpoint region. Arrows indicate site and direction of sequenced regions. For explanation of boxed regions see (A). Vertical arrow shows chromosomal breakpoint regions. E, *Eco*RI; Hp, *Hpa*II; St, *Stu*I; X, *Xba*I; Ps, *Pst*I; Sm, *Sma*I; Pv, *Pvu*II; Hn, *Hin*cII. The location of two subclones prepared in pUC vectors are shown. (**C**) Comparative sequence of translocated Daudi region and germline J_H segments. Top line shows rearranged Daudi sequence [obtained as shown in (B)], and the bottom line shows the germline J_H locus sequence (Ravetch *et al.*, 1981). Dots indicate identities and location of J_H4 and J_H5 are shown. Heptamer and nonamer recombination signals of the joined D_H are overlined and the putative D_H promoter is underlined (position 142).

Germline organization of the D_H family found in the D_HJ_4 rearrangement

We have sought to define the genomic organization of the unusual D_H family joined to J_H4 in Daudi by using DNA immediately upstream of the D_HJ_H4 rearrangement as a source of probes. From subclone p54RH2, two probes, p54Stu/RI1 and p54Sma/Stu3 were derived (Figure 1B) to assess if D_H associated sequences are present up to the translocation junction. The probe p54Stu/RI1 hybridizes to sequences amplified along with c-*myc* gene amplification in the cell line Colo32OHSR (Alitalo *et al.*, 1983) (Figure 2A) but in addition detects multiple single copy sequences that are also detected with p54Sma/Stu3 (Figure 2B). This suggests that D_H associated sequences are maintained up to the translocation junction, and that these D_H probes cross-hybridize to the various D_H family fragments. The

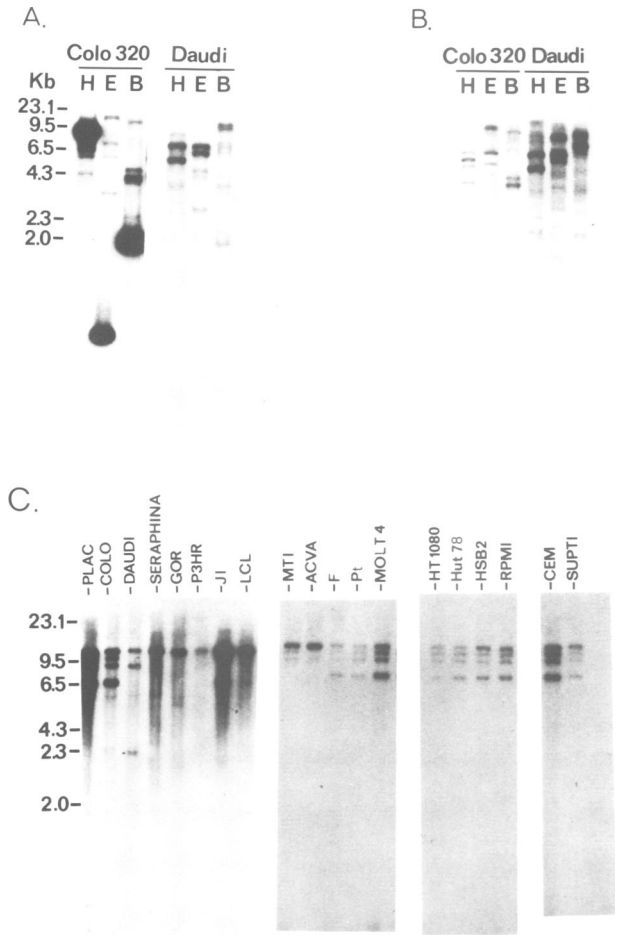

Fig. 2. Filter hybridization of genomic DNA with Daudi translocation breakpoint probes. 10 μg aliquots of DNA were digested with *Hin*dIII (H), *Eco*RI (E) or *Bam*HI (B) (2A and B) or *Hin*dIII (2C), fractionated and transferred as described. The hybridization probes are: **Panel A**, p54Stu/RI1; **Panels B** and **C**, p54Sma/Stu3. Sizes are determined by co-electrophoresis of λ *Hin*dIII markers. Panel C: Plac, Placenta; Colo, Colo320; LCL, DH-LCL (a lymphoblastoid cell line); F and Pt are T-CLL and T-PLL respectively; RPMI, RPMI 8402. (Samples on the right-hand three panels are T-cell leukaemias; Daudi, Seraphina, Gor, P3HRI and J1 are Burkitt's lymphomas.)

p54Sma/Stu3 probe was hence selected as a D_H probe. Hybridization of p54Sma/Stu3 to *Hin*dIII digested DNA from a variety of B and T cell lines is shown in Figure 2C, and from this it is seen that all fragments detected, with the exception of the 12 kb *Hin*dIII fragment, are extensively deleted in the B cells, but not in T cells; this presumably reflects D_H deletion occurring in the course of Igh rearrangement in B but not T cells.

To establish the germline organization of the D_H family detected by p54Sma/Stu3, we have isolated six cosmids that hybridize to this probe from a cosmid library prepared from the cell line Colo320HSR. Hybridization of the p54Sma/Stu3 probe to *Hin*dIII digests of these cosmids (Figure 3a) shows that all *Hin*dIII fragments detected by p54Sma/Stu3 in germline DNA are contained in these cosmids. The partial restriction maps of these cosmids are given in Figure 3f and show that two loci are defined, one containing only the 12 kb *Hin*dIII fragments detected by p54Sma/Stu3 (herein designated minor D_H locus). The restriction map of the major cluster (Figure 3f) shows that part of it is equivalent to the previously published D_H locus. The positions of D_H sequences in the major and minor loci were defined by using specific oligonucleotide probes for the two D_H families. Two oligonucleotide probes were used, one complementary to a D_H sequence from the previously described family (Siebenlist *et al.*, 1981) (oligo HD) and a second complementary to the D_H segment joined near the junction of the Daudi translocation t(8;14) (see Figure 1) (oligo DD). The sequences of these probes are given in the legend to Figure 3. The first oligonucleotide probe is perfectly matched to the sequence of D1, D2 and D4, but is mismatched at base 31 to the sequence of D3. Hybridization of these probes to digests of cosmids 21 and 23 (covering the major cluster) and 22 and 26 (covering the minor locus) are shown in Figure 3. Hybridization of the DD oligo detects six D_H members of the major cluster and a single member in the minor locus (Figure 3c). Hybridization of the IgHD oligo detects fragments that are compatible with the presence of D1, D2, D3 and D4 and an additional D_H-like sequence in Cos23 in the major cluster (Figure 3d). Hybridization to the minor locus reveals a related D_H sequence likely to be the previously detected D5 (Siebenlist *et al.*, 1981). Analysis of the hybridization of the IgHD oligo at increased stringency shows that the putative D5 sequence and additional D_H sequence in Cos23 are mismatched to the probe sequence and also serves to confirm the presence of D4 in Cos23 (Figure 3e). The locations of the various D_H elements in the major and minor clusters are illustrated in Figure 3f.

Nucleotide sequence of D_H family

Plasmid subclones containing *Hin*dIII fragments carrying D_H sequences were constructed from the two D_H clusters and *Pst*I restriction fragments carrying D_H sequences identified and subcloned in M13mp19. Clones in the correct orientation were selected for sequencing by hybridization to the DD oligonucleotide probe. The nucleotide sequence of the D_H elements was determined using a primer conserved to the 5′ end of this D_H family (see Figure 4 legend). The sequence of all the D_H elements was obtained (apart from the D_H contained in a 3 kb *Hin*dIII fragment in cosmid 21 (D21/3), not shown in Figure 3f) and are presented in Figure 4 together with the sequences of D1, D2, D3, D4 and DHQ52 (Siebenlist *et al.*, 1981; Ravetch *et al.*, 1981). Our sequence analysis confirms the presence of a second IgH D family within the major cluster [D sequences previously found in rearrangement with chromosome band 18q21 (Bakshi *et al.*, 1987) correspond to D21/0.5 and D21/7]. Sequence comparison of this D_H family with the sequences of D1, D2, D3 and D4 show poor sequence homology in the coding sequence, although coding sequence size is largely conserved at 28−31 bp (D21/10 contains a coding region of 37 bp). Both D_H families have the sequence GTGT preceding the first heptamer recombination motif. None of the germline D_H sequences correspond to the Daudi rearranged D_H element (D_HJ_4 sequence) and the only outstanding D_H, D21/3, does not apparently have a sequence homologous to the DD oligonucleotide primer. We therefore must conclude that the joined D_H in Daudi is mutated, with respect to its germline counterpart, as a result of D_H-J_H rearrangement and/or the chromosomal translocation.

Cosmids from the major D_H cluster contain the J_H and C_μ loci

Since cosmids 21, 24 and 25 cover ~42 kb of DNA downstream of D21/9 (the 3′-most D_H element defined in this

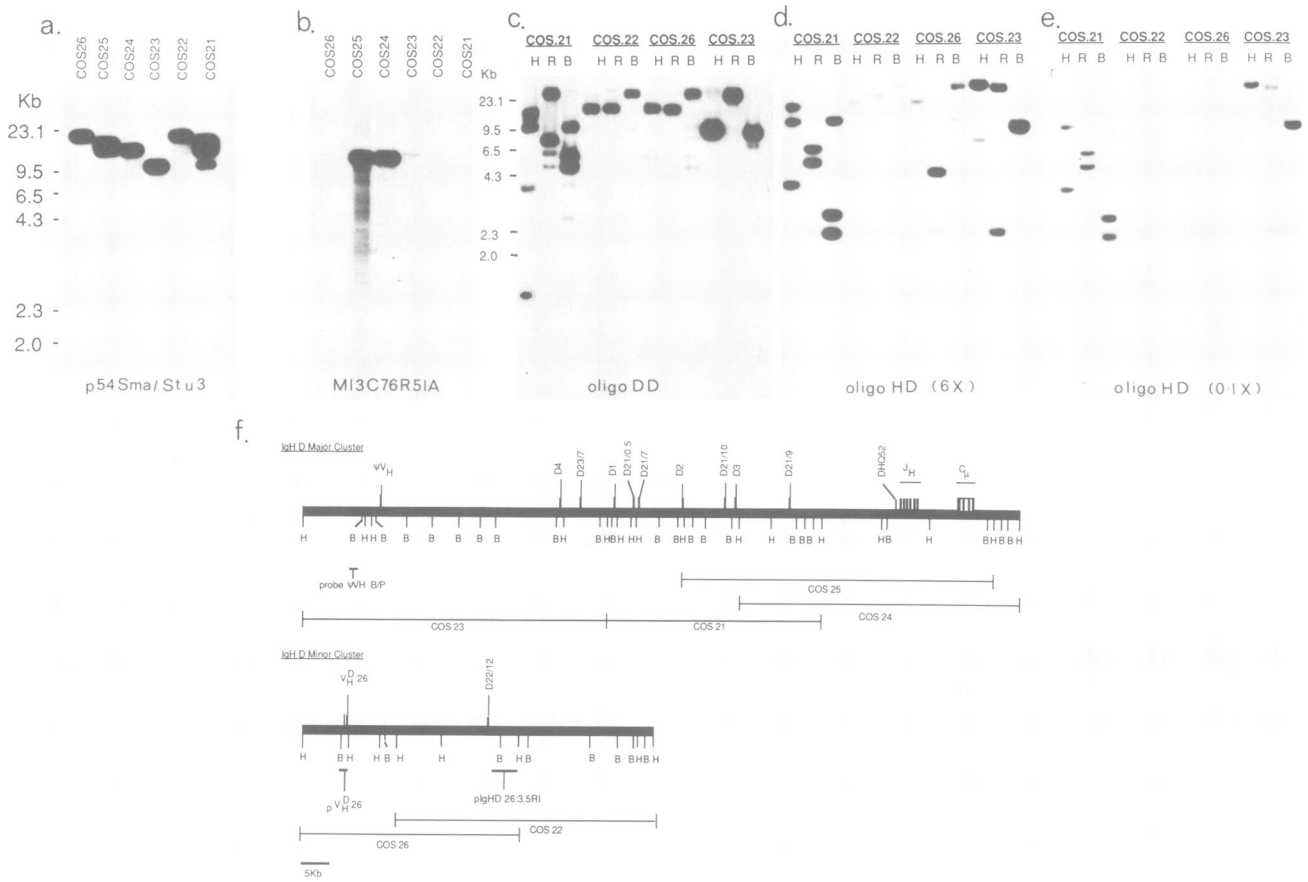

Fig. 3. Characterization of the two human D_H loci. Cosmid clones were isolated using the probe p54Sma/Stu3 derived from the Daudi translocation breakpoint region (Figure 1B). Hybridization characteristics of cosmid clones (a–c). (**a**) Probe: p54Sma/Stu3; cosmids digested with *Hin*dIII. (**b**) Probe: M13C76R51A (Flanagan and Rabbitts, 1982); cosmids digested with *Hin*dIII. (**c**) Probe: OligoDD (sequence: AGGTCTG$_4$TGAGGTCTGTGTCACTGTGATATCACGAT); (**d**) Probe: OligoHD (sequence: GGAT$_4$GTG$_5$CTCGTGTCACTGTGAGGATATTGT) washes at 6 × SSC, 65°C. (**e**) Probe: OligoHD, washes at 0.1 × SSC, 65°C. H, *Hin*dIII; R, *Eco*RI; B, *Bam*HI. (**f**) Restriction maps of D_H-containing cosmids. H, *Hin*dIII; B, *Bam*HI. *Bam*HI sites are incomplete in Cos24 and Cos25. Hybridization of the probe VHB/P to genomic digests confirm that the terminal end of Cos23 is in a germline configuration (data not shown). Locations of VH, DH, JH and C_μ are shown.

9		7		7		9	
GGATTTTGT	GGGGGCTCGTGT	CACTGTG	AGGATATTGTACTGGTGGTGTATGCTATACC	CACAGTG	ACACAGCCCCATT	CCCAAAGCC	D1
GGATTTTGT	GGGGGCTCGTGT	CACTGTG	AGGATATTGTAGTGGTGGTAGCTGCTACTCC	CACAGTG	ACACAGACCCATT	CCCAAAGCC	D2
GGATTTTGT	GGGGGCTCGTGT	CACTGTG	AGCATATTGTGGTGGTGATT GCTATTCC	CACAGTG	ACACAACCCCATT	CCTAAAGCC	D3
GGATTTTGT	GGGGGCTCGTGT	CACTGTG	AGGATATTGTAGTAGTACCAGCTGCTATGCC	CACAGTG	ACACAGCCCCATT	CCCAAAGCC	D4
GGTTTTTGG	CTGAGCTGAGAAC	CACTGTG	CTAACTGGGGA	CACAGTG	ATTGGCAGCTCT	ACAAAAACC	DHQ52
AGGTTTGAA	GTGAGGTCTGTGT	CACTGTG	GTATTACTATGATAGTAGTGGTTATTACTAC	CACAGTG	TCACAGAGTCCA	TCAAAAACT	D21/9
AGGTTTGAA	GTGAGGTCTGTGT	CACTGTG	GTATTATGATTACGTTTGGGGGAGTTATGCTTATACC	CACAGCA	TCACACGGTCCA	TCAGAAAAC	D21/10
AGGTTTGGG	GTGAGGTCTGTGT	CACTGTG	GTATTATGATTTTTGGACTGGTTATTATACC	CACAGTG	TCACAGAGTCCA	TCAAAAACC	D22/12
AGGTTTGGG	GTGAGGTCTGTGT	CACTGTG	GTATTAGCATTTTTGGAGTGGTTATTATACC	CACAGTG	TCACAGAGTCCA	TCAAAAACC	D23/7
AGGTTTAGA	ATGAGGTCTGTGT	CACTGTG	GTATTACGATATTTTGACTGGTTATTATAAC	CACAGTG	TCACAGAGTCCA	TCAAAAACC	D21/0.5
AGGTTTGGG	GTGAGGTCTGTGT	CACTGTG	GTATTACTATGTTCGGGGAGTTATTATAAC	CACAGTG	TCACAGAGTCCA	TCAAAAACC	D21/7
AGGTCTGGG	GTGAGGTCTGTGT	CACTGTG	ATATCACGATTTTTGGAGTGCTTTAG				DAUDI

Fig. 4. Comparison of nucleotide sequences of human Igh D_H elements. Heptamer and nonamer sequences on either side of D_H (spacing 12 bp) are boxed. Sequences of D1–D4, DHQ52 from Siebenlist *et al.* (1981). The sequence of the conserved primer used to obtain D_H sequences is (GGTCAGCCCTGGACATCCC).

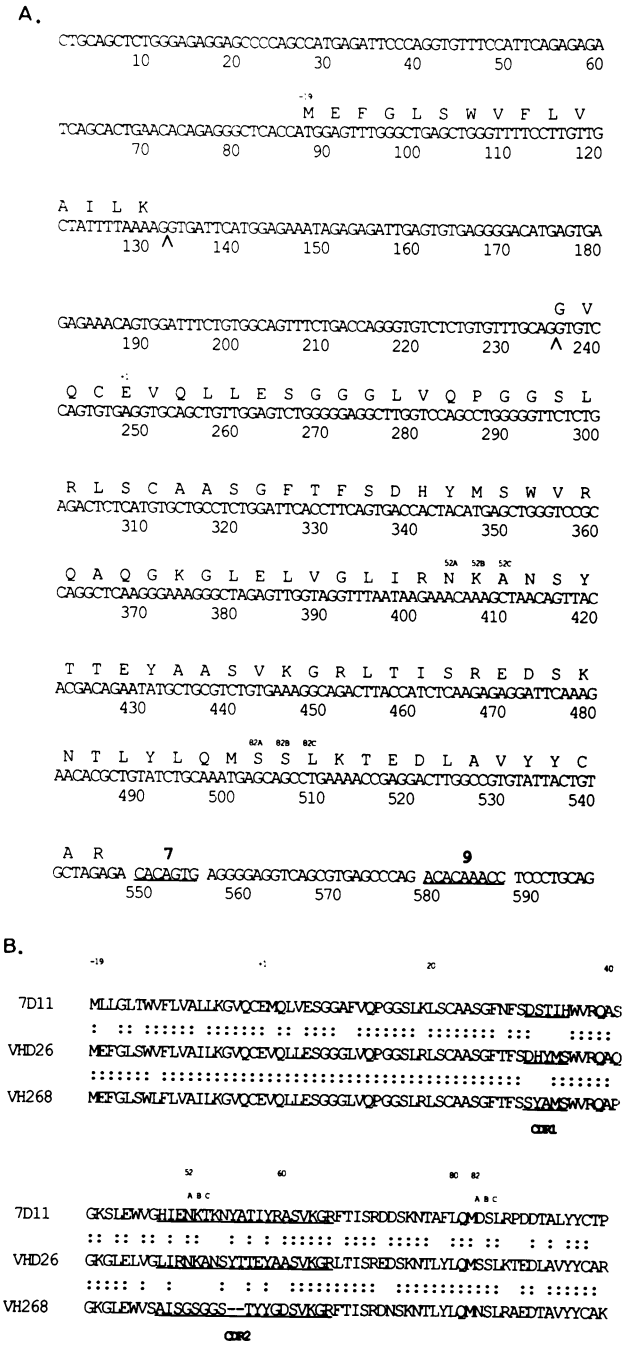

Fig. 5. Comparative sequences of the V_H gene located near to the minor D_H cluster. (**A**) Nucleotide and derived protein sequences (in single letter code) of V_H^D26 (Figure 3f) [amino acid numbering according to Kabat *et al.* (1987)]. (**B**) Comparison of amino acid sequences of V_H^D26, VH26-8 (Matthyssens and Rabbitts, 1980) and 7D11 (Cleary *et al.*, 1986).

study) (Figure 3f), we investigated the possible inclusion of the J_H locus in these cosmids. The result of hybridizing the J_H probe M13C75R51A (Flanagan and Rabbitts, 1982) to digests of all six cosmids studied is shown in Figure 3b and shows that the whole J_H locus is contained in cosmids 24 and 25. Nucleotide sequence data of this hybridizing region of Cos 24/25 confirm that the J_H segments are present (not shown). Further, the restriction map of these cosmids also shows that they contain the C_μ gene (Rabbitts *et al.*, 1981). This linkage places the D21/9 sequence ~20 kb upstream of J_H (and, therefore, also of D_HQ52).

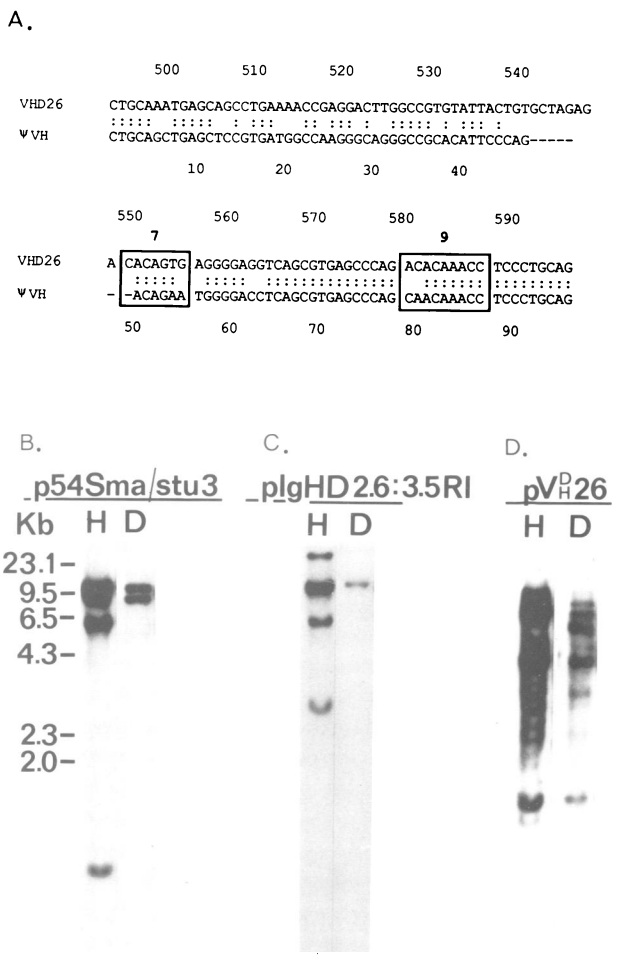

Fig. 6. Sequence at the end of the pseudo-V_H gene within the major D_H cluster. (**A**) The nucleotide sequence of the 3′ end of the pseudo-V_H (see Figure 3f for location) compared to the analogous region of V_H^D26 (Matthyssens and Rabbitts, 1980). The sequence was obtained from the *PstI* site at the 3′ end of this sequence. Heptamer and nanomer sequences are boxed. (**B**), (**C**) and (**D**) Hybridization of various probes to Daudi and control DNA. 10 μg of HeLa (H) or Daudi (D) DNA were digested with *Hin*dIII, fractionated and transferred to Hybond-N. Filters were hybridized to the probes indicated and sizes were assessed by co-electrophoresis of λDNA cleaved by *Hin*dIII.

Both major and minor D_H clusters contain V_H sequences

We have studied V_H sequences in the cosmids from the major and minor D_H clusters by hybridization to the V_HIII subgroup probe V_H268 (Matthyssens and Rabbitts, 1980). This probe detects hybridizing sequences in both major and minor cluster cosmids (cosmids 23 and 26 respectively) (data not shown). Both of these hybridizing V_H sequences have been subcloned and their nucleotide sequences determined. The V_H in cosmid 26 (V_H^D26) is located ~23 kb from D22/12 (Figure 3f) and its sequence shows that it is a member of the V_HIII subgroup. The whole V_H sequence is shown in Figure 5A, and includes a leader exon, a small intron and the bulk of the V_H domain; conserved heptamer and nonamer recombination signal sequences occur at the 3′ end of the V_H sequence. The derived amino acid sequence is unusual in that it has a CDR2 composed of 19 amino acids as opposed to the 17 amino acid size (Figure 5B) found in the majority of V_HIII genes described (Kabat

Table I. Segregation of human D_H clusters to chromosome 14

	D_H	Human chromosomes in hybrid cell lines																					
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X
SIF4A31	√			+	+	+	+								+		+							+
SIR74ii	√	+	+	w	+								+	+	+			+	tr			+	w	+
CTP41E3	√		+	+			+	+							+									+
CTP3B4	√	+	+	+		+	+	+	+				+		+		+	+	+					+
TWIN19-D12	√	+		+	+		+		+			+	+		+		+		+		+			
DUR4R4	√					+			+			+	+	+	+			+	+		+	+		
3W4CL5	√							+			+	+			+	+		+				+		+
SIR19A	√	+	+	+		+		+	+		+	+	+	+	+	+		+	+	+	+	+	tr	+
FG10	√	+	+			+	+				+				+		+			+	+	+		+
DT1.2.4	×			+								+				+			+			+		+
FST9/10	×			+	+		+		+		+		+	+		+			+				+	+
HORL:9D2R1	×											+												+

The table shows rodent−human somatic cell hybrids with known complements of human chromosomes. √ indicates positive hybridization with p54Sma/Stu3 (see Figure 1); × indicates no signal observed. tr = small proportion of cells carry the relevant chromosomes.
Hybrids are described in: Edwards *et al.* (1985); Heisterkamp *et al.* (1982); Jones *et al.* (1976); Kielty *et al.* (1982); Nabholz *et al.* (1969); Philips *et al.* (1985); Solomon *et al.* (1976); Swallow *et al.* (1977) and Whitehead *et al.* (1982).

et al., 1987). Only one other example of a 19 amino acid CDR2 region in a human V_HIII gene has been reported (Cleary *et al.*, 1986) (Figure 5B), but this CDR2 size is common in the murine V_HIII equivalent (Kabat *et al.*, 1987).

The V_H sequence in cosmid 23 is located ~33 kb from D4 (i.e. at the 5′ end of the D cluster) and 105 kb from the C_μ gene. The nucleotide sequence shows that it is a V_H pseudogene in which only the nonamer and 23 bp spacer have been conserved (Figure 6A). However, as V_H pseudogenes and active genes are closely interspersed (Baer *et al.*, 1985, 1987; Kodaira *et al.*, 1986), this result indicates that functional V_H sequences are likely to lie nearby and puts the distance between V_H and J_H sequences at ~98 kb.

The minor D_H cluster is towards the 5′ end of the V_H locus

Hybridization of p54Sma/Stu3 (Figure 1) to B cell DNAs shows that the minor D_H locus is not deleted during D_H rearrangements that delete the major D_H locus (Figure 2C). This result indicates that the minor locus might lie 3′ of the C_H genes, within the V_H locus or outside chromosome band 14q32 in which the IgH locus resides. However, hybridization of p54Sma/Stu3 to a panel of mouse-human somatic cell hybrids of known human karyotype places all of the D_H sequences on chromosome 14 (Table I). The position of the minor locus, relative to the J_H locus, was determined by *in situ* hybridization to chromosomes prepared from the cell line Raji [which contains a t(8;14) (q24;32) in which the junction is located in the C_γ locus (Hamlyn and Rabbitts, 1983)] and the cell line JVM-2 (Rabbitts *et al.*, 1988) [carrying a t(11;14) (q13;q32) chromosome with the junction in the J_H locus]. However, both Raji and JVM-2 retain the minor D_H locus in germline configuration (data not shown). The probe (IgHD26; Figure 3f) used in this analysis contains 3 kb DNA located 3′ of D22/12 and is found to hybridize predominantly to the minor D_H locus in HeLa DNA, plus a subset of loci deleted during D_H rearrangement in Daudi (Figure 6C). The results of the *in situ* hybridizations are summarised in Table II. An Ig C_γ probe hybridizes both to normal chromosome 14 and to the $14q^+$ chromosome in Raji and JVM-2 cells. However, both the D_H and V_H probes only seem to hybridize with the normal 14 chromosome in each cell line (Table II). These results show that all D_H loci and at least the vast majority of V_HIII genes are located upstream of J_H. Further, hybridization of pV_H^D26 to Daudi DNA shows that V_HIII genes are extensively deleted in this cell line (Figure 6D), but Daudi retains at least one copy of the minor D_H locus in germline configuration (Figure 6C). We therefore conclude that the minor D_H locus is located towards the 5′ end of the V_H locus at chromosome band 14q32.

Table II. *In situ* hybridization of Igh probes

Cell line	JVM-2		Raji		
Chromosome	14	$14q^+$	14	$14q^+$	$8q^-$
Probe					
p3.ORH4.2 (C_γ)	+	+	+	+	−
pV_H^D26	+	−	+	−	−
pIgHD26	+	−	+	−	+

Summary of *in situ* hybridization results.
Hybridization of p3.ORH4.2 [Igγ constant region probe (Krawinkel and Rabbitts, 1982)], pIgHD26 (see Figure 3f) or pV_H^D26 (Figure 3f) to metaphase chromosomes of Raji or JVM-2 cells. Hybridization of only normal chromosomes 14 or $14q^+$ are shown, plus the $8q^-$ chromosome of Raji.
Note: (1) the JVM-2 $14q^+$ chromosome is from a t(11;14)(q13;q32) and the Raji $14q^+$ chromosome from a t(8;14)(q24;q32).
+ = hybridization to at least 70% of relevant chromosomes examined.
− = no hybridization seen.
(2) pIgHD26 results with JVM-2 chromosomes hybridized to 9 out of 12 normal chromosomes 14 [scored +] and 1 out of 15 chromosomes $14q^+$ [scored −].
(3) No $11q^-$ chromosome could be found in JVM-2 cells (either by karyotype or by *in situ* hybridization) using a probe specific for 11p15 which hybridized to 90% of normal chromosome 11s.

Discussion

The translocation t(8;14) breakpoint in Daudi cells

The BL cell line Daudi carries a typical translocation t(8;14) (q24;q32) in which the translocation breakpoint occurs near the J_H locus (Haluska *et al.*, 1987). Detailed analysis of the rearrangement within the J_H locus reveals a D_H-J_H4 join,

just downstream from the translocation position. Notably, the rearranged D_H element retains its upstream recombination signal (Figure 1C) and therefore this signal sequence cannot be invoked in the translocation process. We also note that extensive somatic mutation has occurred in the region of the translocation–D–J join, as previously described for the first exon of the translocated c-*myc* gene in Daudi (Rabbitts *et al.*, 1984).

Comparison of the D_H element joined to the J_H4 revealed a D sequence which was not previously assigned to the D_H cluster. Since the D_H segment joined in Daudi retains its germline sequences for ~600 bp upstream (i.e. up to the translocation position), we were able to derive a probe enabling us to study the immunoglobulin heavy chain D clusters in detail. These studies, therefore, were facilitated by the existence of the mutant chromosome in Daudi cells.

Mapping the major D_H cluster

The results described in this paper show that the major D_H cluster (i.e. the one apparently utilized predominantly in the assembly of V_H genes in B cell gene rearrangements) starts very close to the J_H locus, at a distance of ~20 kb. This close proximity of D_H and J_H was first shown in mouse DNA (Wood and Tonegawa, 1983) and presumably reflects the recombinase mechanism recognizing proximal elements for rearrangement. In the context of the initial rearrangement process, the location and extent of the V_H locus is important, particularly where the first V_H segment is located. A partial answer to this question is provided here, because we have located a pseudo-V_H segment ~98 kb from the J_H cluster (Figure 2). Although V_HII and V_HIII subgroup probes failed to recognize other hybridizing V_H segments in our D_H-containing cosmids, it seems likely that V_H segments will be found in the vicinity (perhaps members of other, non-cross hybridizing subgroups) and, therefore, that this represents the start of the V_H locus.

A new minor D_H locus within the Igh locus

The D_H element rearranged to J_H4 in the translocated chromosome of Daudi cells has helped to define a new D_H locus, containing at least one sequenced D_H element, in the Igh locus. This minor D_H locus appears to be rarely, if ever, used in B cells (or in T cells) as we failed to detect rearrangement or deletion of this locus. This presumably means that the minor D_H locus is located near to the end of the V_H locus. Certainly, that part of the minor D_H locus described here is associated with a V_HIII subgroup gene and, therefore, is not entirely outside the Igh locus.

Finally, it is now beginning to appear feasible to obtain a complete map of the human Igh locus as more landmarks become available. Thus the C_H genes mark one end of the map and the minor D_H locus may help to delineate the distal end. In addition, methods of rapid mapping (Coulson *et al.*, 1986) might be used to establish an ordered cosmid map from which the individual V_H and other elements may be placed and then sequenced. This is particularly feasible in the Igh locus, since V_H subgroup genes seem to be interspersed (Baer *et al.*, 1985, 1987; Kodaira *et al.*, 1986) and initial experiments on screening a cosmid library suggest that many of the V_H containing cosmids cross-hybridize with several distinct V_H probes (unpublished results).

Materials and methods

Characterization of Daudi translocation breakpoint

A phage recombinant library from Daudi genomic DNA [in λ1059 (Karn *et al.*, 1984)] was screened with the Igh J_H probe, M13C7651A (Flanagan and Rabbitts, 1982) and clones analysed for the presence of V_H sequences. Two clones, λD39 and λD54, failed to hybridize and were analysed for the presence of translocated DNA. Subcloning was carried out into M13 and pUC vectors (Vieira and Messing, 1982) and nucleotide sequences obtained by dideoxy chain termination procedure in M13, and analysed by computing methods (Sanger *et al.*, 1980; Staden, 1986).

Isolation and characterization of cosmid clones

A cosmid library of Colo32OHSR DNA was prepared in the Lorist 6 vector (Gibson *et al.*, 1987). Colo320 cells were embedded in agar plugs, partially digested with *Hin*dIII and DNA of ~50 kb isolated from a field inversion electrophoresis gel (Tunnacliffe *et al.*, 1987). This material was ligated to *Hin*dIII cleaved Lorist 6. Hybridization of unamplified cosmid recombinants was carried out at 6 × SSC (65°C) with purified insert from the clone p54Sma/Stu3 (Figure 1 legend). Cosmids were made by standard means and subclones prepared in pUC or M13 vectors.

Hybridization procedures

Genomic DNA was analysed by filter hybridization (Southern, 1975) using random oligonucleotide labelling (Feinberg and Vogelstein, 1983). Conditions of hybridizations were as previously described (Lefranc *et al.*, 1986).

Hybridization of biotinylated probes to metaphase spreads was conducted according to methods described in Albertson (1984, 1985) with the following modification. After RNase treatment, metaphase spreads that had been prepared by conventional methods were rinsed in 2 × SSC at 65°C for 15 min, dehydrated and air-dried. Denaturation was in 70% formamide at 70°C for 3.5 min. Hybridization and washing of slides were as described previously, except that washing times were reduced to 10 min.

Acknowledgements

We thank U.Siebenlist for access to λD_H clones and A.Forster and R.Fishpool for expert technical help. L.B. was the recipient of an MRC Training Fellowship and D.G.A. was the recipient of a Du Pont Fellowship.

References

Albertson,D.G. (1984) *EMBO J.*, **3**, 1227–1234.
Albertson,D.G. (1985) *EMBO J.*, **4**, 2493–2498.
Alitalo,K., Schwab,M., Lin,C.C., Varmus,H.E. and Bishop,J.M. (1983) *Proc. Natl. Acad. Sci. USA*, **80**, 1707–1711.
Baer,R., Chen,K.-C., Smith,S.D. and Rabbitts,T.H. (1985) *Cell*, **43**, 705–713.
Baer,R., Forster,A. and Rabbitts,T.H. (1987) *Cell*, **50**, 97–105.
Baer,R., Boehm,T., Yssel,H., Spits,H. and Rabbitts,T.H. (1988) *EMBO J.*, **7**, 1661–1668.
Bakshi,A., Wright,J.J., Graninger,W., Seto,M., Owens,J., Cossman,J., Jensen,J.P., Goldman,P. and Korsmeyer,S.J. (1987) *Proc. Natl. Acad. Sci. USA*, **84**, 2396–2400.
Cleary,M.L., Meeker,T.C., Levy,S., Lee,E., Trela,M., Sklar,J. and Levy,R. (1986) *Cell*, **44**, 97–106.
Coulson,A., Sulston,J., Brenner,S. and Karn,J. (1986) *Proc. Natl. Acad. Sci. USA*, **83**, 7821–7825.
Edwards,Y.H., Parkar,M., Povey,S., West,L.F., Farnington,F.M. and Solomon,E. (1985) *Annu. Hum. Genet.*, **49**, 101–109.
Feinberg,A.P. and Vogelstein,B. (1983) *Anal. Biochem.*, **132**, 6–13.
Flanagan,J.G. and Rabbitts,T.H. (1982) *EMBO J.*, **1**, 655–660.
Gibson,T.J., Rosenthal,A. and Waterston,R.H. (1987) *Gene*, **53**, 283–286.
Haluska,F.G., Finger,S., Tsujimoto,Y. and Croce,C.M. (1986) *Nature*, **324**, 158–161.
Haluska,F.G., Tsujimoto,Y. and Croce,C.M. (1987) *Proc. Natl. Acad. Sci. USA*, **84**, 6835–6839.
Hamlyn,P.H. and Rabbitts,T.H. (1983) *Nature*, **304**, 135–139.
Heisterkamp,N., Groffen,J., Stephenson,J.R., Spurr,N.K., Goodfellow,P.N., Solomon,E., Carritt,B. and Bodmer,W.F. (1982) *Nature*, **299**, 747–749.
Jones,E.A., Goodfellow,P.N., Kennett,R.H. and Bodmer,W.F. (1976) *Somat. Cell Genet.*, **2**, 483–496.
Kabat,E.A., Wu,T.T., Reid-Miller,M., Perry,H.M. and Gottesman,K.S. (1987) *Sequences of Proteins of Immunological Interest* (Vol. 4), US Dept of Health Services.

Karn,J., Matthes,H.W.D., Gait,M.J. and Brenner,S. (1984) *Gene*, **32**, 217–224.
Kielty,C.M., Povey,S. and Hopkinson,D.A. (1982) *Annu. Hum. Genet.*, **46**, 307–327.
Kodaira,M., Kinashi,T., Unemura,I., Matsuda,F., Noma,T., Ono,Y. and Honjo,T. (1986) *J. Mol. Biol.*, **190**, 529–541.
Krawinkel,U. and Rabbitts,T.H. (1982) *EMBO J.*, **1**, 403–407.
Lefranc,M.-P., Forster,A., Baer,R., Stinson,M.A. and Rabbitts,T.H. (1986) *Cell*, **45**, 237–246.
Matthyssens,G. and Rabbitts,T.H. (1980) *Proc. Natl. Acad. Sci. USA*, **77**, 6561–6565.
Nabholz,M., Miggiano,V. and Bodmer,W.F. (1969) *Nature*, **223**, 358–363.
Philips,J.R., Shepard,E.A., Povey,S., Davis,M.B., Kelsey,G., Monteiro,M., West,L.F. and Cowell,F. (1985) *Annu. Hum. Genet.*, **49**, 267–274.
Rabbitts,T.H. (1983) *Biochem. Soc. Trans.*, **11**, 119–126.
Rabbitts,T.H. (1985) *Trends Genet.*, **1**, 327–331.
Rabbitts,T.H., Forster,A. and Milstein,C.P. (1981) *Nucleic Acids Res.*, **9**, 4509–4524.
Rabbitts,T.H., Forster,A., Hamlyn,P. and Baer,R. (1984) *Nature*, **309**, 592–597.
Rabbitts,P.H., Douglas,J., Fischer,P., Nacheva,E., Karpas,A., Catovsky, D., Melo,J.V., Stinson,M.A. and Rabbitts,T.H. (1988) *Oncogene*, in press.
Ravetch,J.V., Siebenlist,U., Korsmeyer,S., Waldmann,T. and Leder,P. (1981) *Cell*, **27**, 583–591.
Reth,M.G. and Alt,F.W. (1984) *Nature*, **312**, 418–423.
Sanger,F., Coulson,A.R., Barrell,B.G., Smith,A.J.H. and Roe,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.
Solomon,E., Bobrow,M., Goodfellow,P.N., Bodmer,W.F., Swallow,D.M., Povey,S. and Noel,B. (1976) *Somat. Cell Genet.*, **2**, 125–140.
Southern,E.M. (1975) *J. Mol. Biol.*, **98**, 503–517.
Staden,R. (1986) *Nucleic Acids Res.*, **14**, 217–231.
Swallow,D.M., Solomon,E. and Pajunen,E. (1977) *Cytogenet. Cell Genet.*, **18**, 136–148.
Tunnacliffe,A., Buluwela,L. and Rabbitts,T.H. (1987) *EMBO J.*, **6**, 2953–2957.
Vieira,J. and Messing,J. (1982) *Gene*, **19**, 259–268.
Whitehead,A.S., Solomon,E., Chambers,S., Bodmer,W.F., Povey,S. and Fey,G. (1982) *Proc. Natl. Acad. Sci. USA*, **79**, 5012–5025.
Wood,C. and Tonegawa,S. (1983) *Proc. Natl. Acad. Sci. USA*, **80**, 3030–3034.

Received on March 15, 1988; revised on April 15, 1988