The EMBO Journal vol.7 no.6 pp.1661 - 1668, 1988

Complex rearrangements within the human $J_\delta - C_\delta/J_\alpha - C_\alpha$ locus and aberrant recombination between J_α segments

R.Baer[2], T.Boehm, H.Yssel[1], H.Spits[1] and T.H.Rabbitts

Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK, and [1]Laboratory of Immunology, UNICET, Dardilly, France

[2]Present address: Department of Microbiology, University of Texas Health Science Center, 5323 Harry Hines Boulevard, Dallas, TX 75235, USA

Communicated by T.H.Rabbitts

We have examined DNA rearrangements within a 120 kb cloned region of the human T cell receptor $J_\delta - C_\delta/J_\alpha - C_\alpha$ locus. Three types of pattern emerge from an analysis of T cell lines and clones. Firstly, cells with two rearrangements within $J_\delta - C_\delta$; secondly, cells with one rearrangement within $J_\delta - C_\delta$ and one or more $J\alpha$ rearrangements, and finally, cells with rearrangements within $J\alpha$ and consequential deletion of the δ locus. Further analysis by cloning of rearrangements within the J_α locus show that, in addition to $V_\alpha - J_\alpha$ joins, $J_\alpha - J_\alpha$ aberrant recombinations occur and rearrangement data indicate that such events are frequent. A model is presented to account for such recombinations.

Key words: T cell receptor/C_α/C_δ/J_δ/J_α/gene rearrangements

Introduction

The structure of the human T cell receptor (TCR) α, β and γ chains has been elucidated in considerable detail in recent years. The J segments in TCRβ and γ chain genes reside a short distance (<5 and 10 kb, respectively) upstream of the exons encoding the constant domains (Toyonaga *et al.*, 1985; Tunnacliffe and Rabbitts, 1985; Lefranc *et al.*, 1986a; Quertermous *et al.*, 1987; Tighe *et al.*, 1987). The organization of the J_α region, however, is unique in that the J_α segments are dispersed over a considerable distance; estimates range from 35 kb in humans (Yoshikai *et al.*, 1985) to 65 kb in mouse (Hayday *et al.*, 1985; Winoto *et al.*, 1985). Recently, another TCR gene has been localized 5′ to the TCR J_α region in mouse (Chien *et al.*, 1987a) and found to encode the δ chain of the second T cell receptor δ/γ heterodimer (Band *et al.*, 1987; Hata *et al.*, 1987; Born *et al.*, 1987; Bonyhadi *et al.*, 1987; Loh *et al.*, 1987). The human homologue of the mouse C_δ gene has recently been identified by its association with a chromosomal translocation present in a T cell line (Boehm *et al.*, 1988). Sequencing of the human gene reveals about 76% homology between mouse and human C_δ genes in the first exon (Hata *et al.*, 1987; Loh *et al.*, 1987; Boehm *et al.*, 1988).

The biological significance of the extensive δ/α gene locus is not known. We have isolated a region of about 120 kb that encompasses the human $J_\delta - C_\delta$ and $J_\alpha - C_\alpha$ gene segments, in order to study the patterns of rearrangements at these two genes in T cells of different phenotypes. The results show complex patterns of rearrangement involving J_δ and J_α segments. In addition, a unique situation occurs in the J_α locus where aberrant $J_\alpha - J_\alpha$ joining events are found. In view of these data, a hypothetical scheme is presented which invokes switching of rearranged V_α segments between J segments following joining.

Results

A molecular map of the human $J_\delta - C_\delta/J_\alpha - C_\alpha$ locus

We have isolated overlapping λ phage clones that contain a region of the TCRδ/α chain locus that includes the Cα gene plus ~80 kb of the J_α locus immediately upstream of this gene. These clones also encompass the C_δ gene and at least two J_δ gene segments. A restriction map of this region is illustrated in Figure 1 along with the positions of various single-copy DNA probes which can be used to monitor gene rearrangement within the δ/α chain locus. We also indicate 17 J_α gene segments whose genomic positions have been localized (Figure 1); these represent only a subset of the total J_α repertoire since cDNA cloning studies predict the presence of 50 - 100 J_α segments in the human α chain gene (Yoshikai *et al.*, 1986; Kimura *et al.*, 1987; Klein *et al.*, 1987). The human C_δ gene occurs 83 kb from C_α (Figure 1) (Boehm *et al.*, 1988), and therefore the upper size limit of the J_α region is 83 kb.

The J_δ region is less well defined; the most upstream J_δ segment so far known (Jδ1) is located about 13 kb upstream of the first exon of C_δ (Figure 1) (Boehm *et al.*, 1988). There is at least one other J_δ element, designated J_δ2, located about 7 kb upstream of C_δ (manuscript in preparation).

Rearrangements at the $J_\delta - C_\delta$ complex

We have studied TCRδ rearrangement in T cell lines and T cell leukaemias using the probe JδS16 (Boehm *et al.*, 1988) (Figure 1). In *Eco*RI cleaved DNA, this probe detects two germ-line fragments; a 6.4 kb fragment containing the J_δ1 segment, and a 5.5 kb fragment containing sequences 3′ of J_δ1. Thus, only the former fragment is altered in size following a rearrangement using J_δ1. As shown in Figure 2A, in seven T cell samples (14 alleles) studied for δ rearrangement, 12 out of 14 alleles are detectably rearranged, while two are deleted for J_δ1. Among the former, MOLT 13 and MOLT14 carry similar rearrangements; this is presumably due to the fact that they represent two isolates from the same leukaemia patient (Minowada *et al.*, 1982). However, Peer, NL5, MOLT 13/14 and DND41 also share similar rearranged restriction fragments, about 4.0 kb in length. As shown in Table I, these cells are positive for the CD3 structure, suggesting the presence of a receptor complex on their cell surface. The Peer cell line has indeed been shown to carry a δ/γ heterodimer (Brenner *et al.*, 1987) and

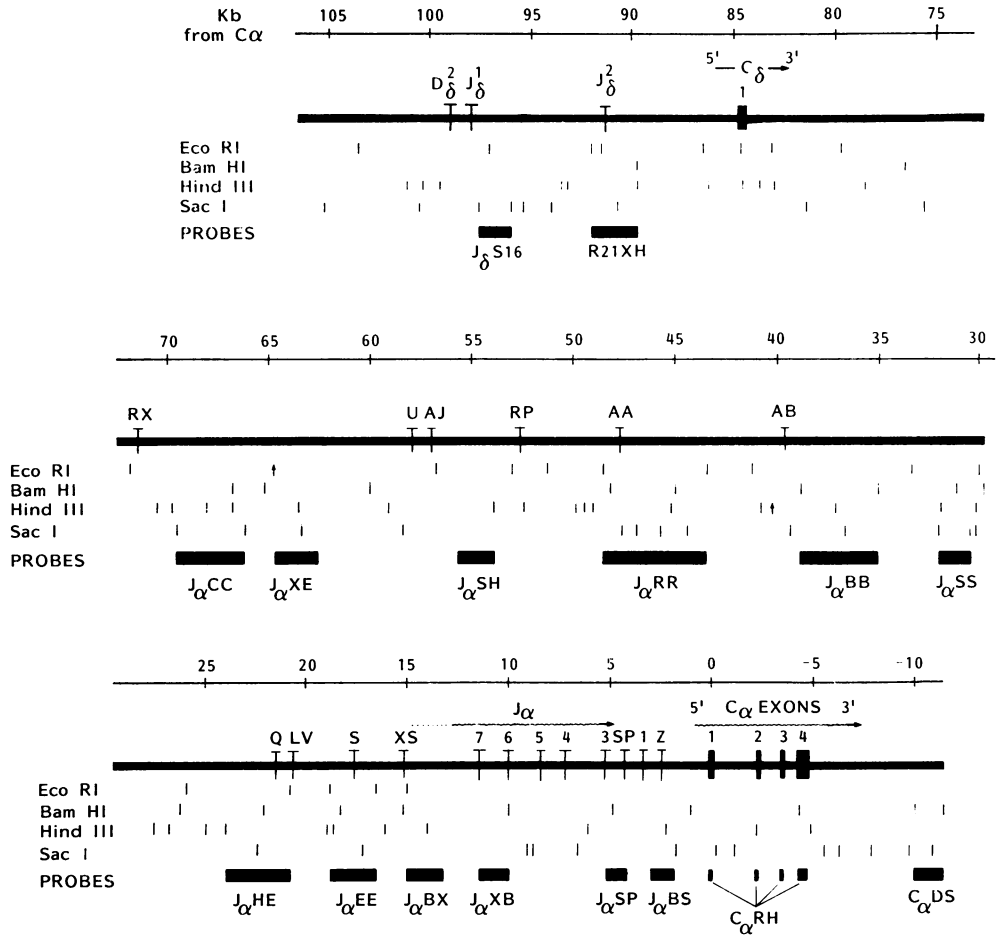

Fig. 1. Molecular structure of the human $J_\delta-C_\delta/J_\alpha-C_\alpha$ locus. The restriction map was deduced from analyses of overlapping recombinant λ clones obtained from various genomic libraries (Lefranc *et al.*, 1986a; Baer *et al.*, 1987b; Boehm *et al.*, 1988). The maps of the recombinants and their overlaps were determined by restriction analysis and cross-hybridization, and confirmed by Southern analysis of DNAs from non-T cell sources. J_δ and J_α segments are indicated. J_α segments Z, 1 and SP were localized by nucleotide sequence analysis of the relevant 3.6 kb *Bam*HI fragment (Yoshikai *et al.*, 1986; Baer *et al.*, 1986). We have also sequenced the adjacent 5.1 kb *Bam*HI fragment (data not shown) containing three J_α segments (3, 4 and 5). The J_α^6 segment was identified by anlaysis of sequences immediately upstream of the 5.1 kb *Bam*HI fragment; thus, only seven J_α segments are located in the 10 kb region immediately upstream of C_α. Other J_α segments have been previously identified as follows: J_αSP (Yoshikai *et al.*, 1985. Baer *et al.*, 1986; Sim *et al.*, 1984; Croce *et al.*, 1985; Rabbitts *et al.*, 1985); J_αZ (Yoshikai *et al.*, 1985); J_α1 (Yoshikai *et al.*, 1985; Baer *et al.*, 1986); J_α5 (Baer *et al.*, 1987b); J_α7 (Baer *et al.*, 1987a); J_αXS (Yoshikai *et al.*, 1985; Baer *et al.*, 1985); J_αS (Yoshikai *et al.*, 1986; Mengle-Gaw *et al.*, 1987); J_αLV (Mengle-Gaw *et al.*, 1987); J_αQ (Yoshikai *et al.*, 1986; Mengle-Gaw *et al.*, 1987); J_αRP (this paper and Leiden *et al.*, 1986); J_αAJ (Baer *et al.*, 1987b); J_αV (Yoshikai *et al.*, 1985; Baer *et al.*, 1987b); and J_δ1 (Boehm *et al.*, 1988). Various single-copy sequences that can be used as probes to monitor gene rearrangement are illustrated with closed boxes. Vertical lines represent sites for *Eco*RI, *Bam*HI, *Hin*dIII and *Sac*I restriction enzymes; arrows indicate polymorphic restriction sites. The single-copy probes indicated represent a useful addition to those already used in clonality determinations of haematological malignancies. In particular, T cell tumours of very early stages of differentiation, without evidence of TCRβ or γ rearrangements, can now be classified and their clonality assessed.

the analysis of cDNA clones encoding the δ chain has revealed a V−D−J−C structure (Hata *et al.*, 1987; Loh *et al.*, 1987). This data implies that most of the rearrangements that we observe into J_δ1 are in fact rearrangements involving V regions, rather than simple D−J joins. (V genes rearranging to J_δ segments, as opposed to J_α, are herein designated V_δ.) Therefore, our results suggest little variability of human $V_\delta-J_\delta$1 rearrangements, as described for mouse (Chien *et al.*, 1987b). In order to support this finding, thymus DNA from human fetuses of 18 and 20 week gestation was analysed. As shown in Figure 2B, two faint, but distinct rearranged bands are consistently seen. Their sizes are 6.6 and 6.8 kb, and the latter coincides with one of the rearrangements found in MOLT 13/14 T cell leukaemias (Figure 2A), also indicating a rather restricted variability of TCRδ rearrangements. It also appears that the $J_\delta-C_\delta$ complex is not deleted in the majority of the thymus cells, as shown by comparison to the DNA from spleen of the same individuals (Figure 2B), suggesting that rearrangements in the TCRδ region predominate over those in J_α at this stage of thymic development.

In contrast to T cells showing bi-allelic rearrangements at TCRδ, our hybridization data for the Peer cell line, which produces a γ/δ heterodimer (Brenner *et al.*, 1987) and has a productive $V_\delta-J_\delta$1 rearrangement (Loh *et al.*, 1987), shows a J_δ1 rearrangement on one allele but deletion of $J_\delta-C_\delta$ on the other (Figure 2A). The deleted allele is rearranged within the J_α locus as shown by Southern hybridization with the J_αSS probe (Figure 3C). Thus, recombination within both J_δ and J_α regions can occur within the same cell, implying that V regions can join to J_δ and J_α segments on the homologous chromosomes 14.

The occurrence of TCRγ and TCRβ rearrangements has also been studied in the panel of cells used here and in all

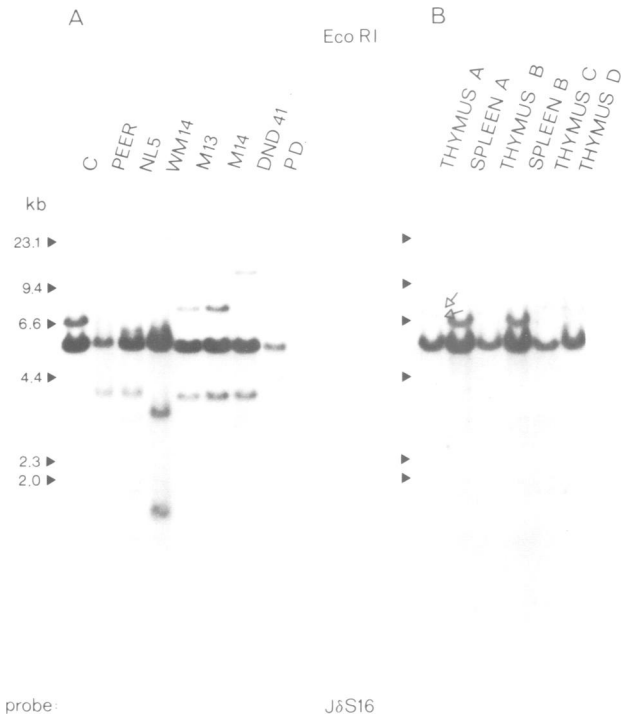

Fig. 2. Rearrangements of the $J_\delta 1$ segment. (**A**) Southern filter hybridization of T cell DNAs with the human J_δ probe, $J_\delta S16$. The germ-line pattern in lane C is from Colo320. (**B**) Southern filter hybridization of human fetal thymus DNA with $J_\delta S16$. thymus samples A and B were taken at 20 weeks gestation, and are compared to their corresponding spleen DNA. Thymus samples C and D were obtained at 18 weeks gestation. The rearranged fragments are highlighted by open arrowheads. Sizes were determined by co-electrophoresis of λ-DNA cut with *Hin*dIII and are given in kb.

cases, TCRγ and TCRβ rearrangements were found in association with TCRδ rearrangement (Table I).

Rearrangements in the J_α region are clustered

In addition to the mixed J_δ/J_α rearrangements discussed above, rearrangements within J_α show two further features, i.e. clustering and internal rearrangements. As shown in Figure 1, J_α segments are scattered over a region of ~83 kb. When analysing a panel of 14 T cell lines, we found that all have at least one rearrangment in J_α, but these rearrangements were unevenly distributed over the J_α region. Figure 3 and Table II summarize these results. Of 33 rearrangements detected, two (6%) occur upstream of J_αRR; 15 (45%) with J_αRR; seven (21%) with J_αBB; three (9%) with J_αSS or J_αHE, and six (18%) were located downstream of J_αHE. A downstream cluster of rearrangements had been previously noted to oocur at J_αSP (Baer *et al.*, 1986). Rearrangements at J_αRR and J_αBB therefore account for more than half of the J_α rearrangements in this T cell panel.

Deletion and inversion within the J_α locus generated by $J_\alpha - J_\alpha$ recombination

Detailed mapping of various rearrangements within J_α revealed a rather more complex picture. As shown in Table II, seven out of 14 T cell samples studied contain more than two rearrangements detectable by J_α probes. MOLT 17, for example, carries two rearranged alleles at J_αRR, shows a single rearranged fragment at J_αBB (Figure 3B) and has yet another rearrangement at J_αHE, together with a germ-line fragment (Table II). This data and the results with six other T cell samples (P.W., Pt, MOLT 3, MOLT 26, RPMI 8402, AT5-B1), suggest the presence of internal J_α rearrangements (Figure 3A and B and Table II). The molecular structure of these internal rearrangements in the J_α locus was studied by cloning of the breakpoints from two different sources, namely the T-cell leukaemia line RPMI 8402 and the T-CLL tumour AT5-B1. The RPMI 8402 cell line has

Table I. Summary of TCR gene status in T cell lines studied

Line	Phenotype						
	CD3	CD4	CD8	J_γ	J_β	J_δ	J_α
Peer	+	−	−	R/R	R/?[a]	R/D	R/G
NL5	+	−	−	R/?	ND	R/R	G/G
WM14	+	−	−	R/?	R/?	R/R	G/G
Molt 13/14	+	−	−	R/?	R/?	R/R	G/G
DND41	+	+	−	R/R	R/?	R/R	G/G
P.De	ND	ND	ND	R/R	ND	R/D	?/G[b]
P.3	+	ND	+	R/R	R/R	D/G	R/R
CCRF-CEM	+	+	−	R/R	R/R	D/D	R/R
KE37	−	+	−	R/R	ND	D/D	?/R
JM	+	+	+	R/R	R/R	D/D	R/R
P.Wi	+	+	+	ND	ND	D/G	R/R
Pt	ND	ND	ND	R/G	R/?	D/G	R/R
MT-1	−	−	−	R/R	R/R	D/D	R/R
Molt 3	−	+	+	R/R	R/R	D/D	R/R
Molt 16	+	−	−	R/R	R/D	D/D	R/R
Molt 17	+	−	−	R/R	R/D	D/D	R/R
HUT102	−	+	−	R/R	R/R	D/D	?/R
RPMI 8402	−	−	−	R/R	R/R	R/D	R/R
AT5-B1	+	ND	+	R/R	R/R	ND	R/R

[a]At least one allele rearranged.
[b]Only a germ-line fragment is detectable; dosage analysis was not attempted.
ND = Not determined.

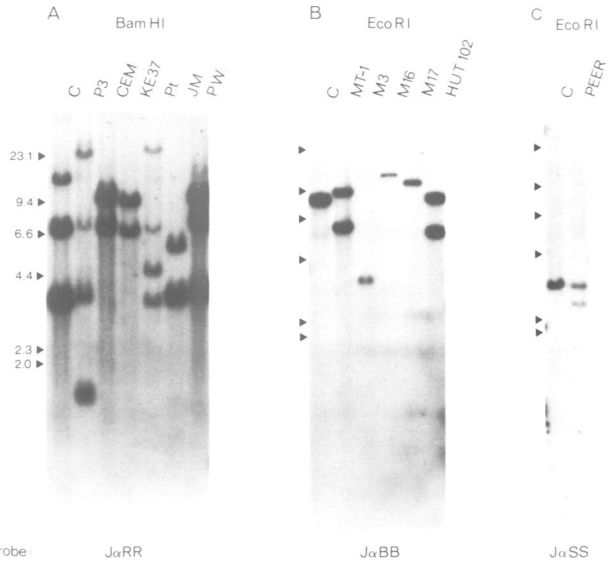

Fig. 3. Rearrangement of the J_α locus in T cell lines. The results of filter hybridizations of control (C, Colo320 DNA) and various T cell DNAs (cleaved with various restriction enzymes) using J_αRR, J_αBB and J_αSS probes (Figure 1) as indicated. (**A**) Rearrangements at J_αRR. (**B**) Rearrangements at J_αBB. (**C**) Detection of a J_α rearrangement in the Peer cell line at J_αSS.

Table II. Rearrangements at J_α segments in T cell leukaemias as determined by Southern analysis

Sample	Status					
	J_αSE	J_αRR	J_αBB	J_αSS	J_αHE	Others
P3	?/G[a]	R/R	?/G	?/G	?/G	ND
CCRF-CEM	D/D	R/D	?/G	?/G	?/G	R/G (J_αBS)[b]
KE37	ND	R/D	?/G	?/G	ND	ND
JM	D/D	R/D	?/G	?/G	?/G	R/G (J_αSP)[b]
P.W.	?/G	R/D	?/G	R/G	?/G	ND
Pt	?/G	R/R	?/G	?/G	D/D	R/D (J_αEE); R/G (J_αBS)[c]
MT-1	D/D	D/D	R/R	?/G	?/G	ND
Molt 3	D/D	D/D	R/D	D/D	?/G	R/G (J_αBX)[b]
Molt 16	D/D	R/R	R/D	?/G	?/G	ND
Molt 17	D/D	R/R	R/D	?/G	R/G	ND
HUT102	D/D	R/G	R/G	?/G	?/G	ND
RPMI 8402	ND	R/R	?/G	?/G	?/G	R/D (J_δS16)[d]; R/D (J_αCC)
AT5-B1	R/D	ND	R/D	ND	ND	R/G (J_αBS)[b]
Peer	?/G	?/G	?/G	R/G	?/G	R/D (J_δS16)

[a]Only a germ-line fragment is detectable; dosage analysis was not attempted.
[b]Baer *et al.* (1986).
[c]Mengle-Gaw *et al.* (1987).
[d]Boehm *et al.* (1988).
ND = Not determined.

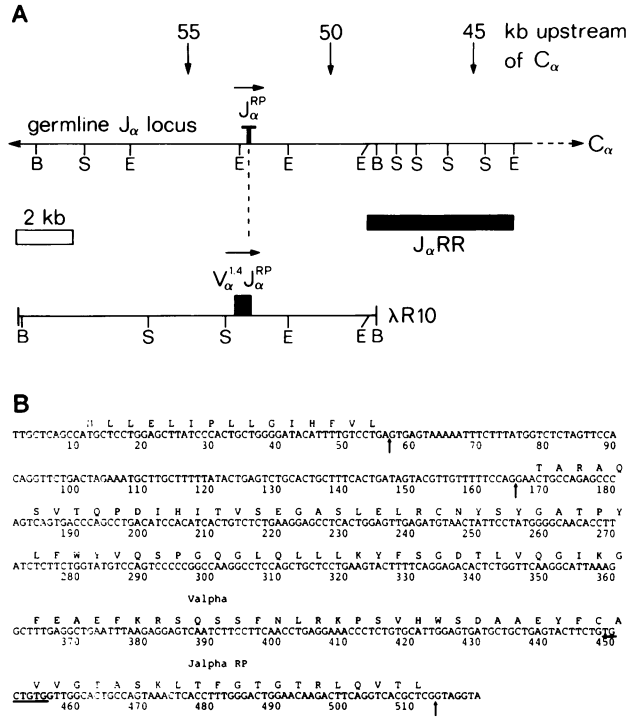

Fig. 4. Productive genomic $V_\alpha-J_\alpha$ rearrangement in RPMI 8402 DNA. (**A**) Restriction map of λR10, a recombinant phage made from RPMI 8402 genomic DNA compared to the equivalent region of the unrearranged J_α locus. The probe J_αRR is indicated (see Figure 1 for the position relative to C_δ and C_α) by a closed box underneath the J_α region. J_α^{RP} and $J_\alpha^{1.4}$ positions are shown and the position of divergence of unrearranged and rearranged maps are indicated by vertical dotted line. B = *Bam*HI; S = *Sac*I, E = *Eco*RI. (**B**) Nucleotide sequence of region of joining $J_\alpha^{1.4}$ and J_α^{RP} from λR10. The derived amino acid sequence is given in the single letter code. Note the presence of a cryptic heptamer sequence, TGCTGTG (underlined) encompassing the cysteine residue at the 3′ end of the V_α sequence.

three rearrangements of the $J_\delta-J_\alpha$ locus. One of them is a t(11;14)(p15;q11) chromosome translocation (Huang *et al.*, 1974; LeBeau *et al.*, 1986), involving $J_\delta1$ (Boehm *et al.*, 1988). Two other DNA rearrangements were detected in RPMI 8402 DNA with the J_αRR probe, each presumably derived from a different allele of the TCRα chain gene. To isolate these latter rearrangements, we screened a λ phage library of RPMI 8402 DNA with the probe JαRR. Restriction mapping of the hybridizing λ clones revealed two types, respresentative members (λR10 and λR15) of which are presented in Figures 4 and 5, and both types have rearrangements at the J_α^{RP} gene segment.

Nucleotide sequence analysis shows that the gene rearrangement in λR10 represents a $V_\alpha-J_\alpha$ join to J_α^{RP} gene segment (Figure 4B); the V_α segment is a previously unidentified member of the V_α^1 family [designated $V_\alpha^{1.4}$ in accordance with the nomenclature of Yoshikai *et al.* (1986)]. The $V_\alpha^{1.4}-J_\alpha^{RP}$ join is potentially productive in that the amino acid translations of $V_\alpha^{1.4}$ and J_α^{RP} are in-frame with respect to one another.

The restriction map of λR15 also varies from that of the germ-line α chain gene upstream of J_α^{RP} (Figure 5A). However, in this, case, the restriction pattern upstream of J_α^{RP} in λR15 is identical to that of the J_α locus beginning ~18 kb upstream of J_α^{RP} when viewed in the opposite orientation. This indicates that the λR15 rearrangement is the consequence of a local 18 kb inversion within the J_α locus. Nucleotide sequence analysis confirms that λR15 diverges from the germ-line J_α locus at a position immediately upstream of J_α^{RP} (Figure 5B). The upstream sequence in λR15 is a different J_α gene segment (designated J_α^{RX}) present on the complementary strand which has fused, in a head-to-head fashion, with a J_α^{RP}. In its normal configuration, J_α^{RX} resides upstream of, and shares the same transcriptional orientation as J_α^{RP}. Recombination between J_α^{RX} and J_α^{RP} would generate the 18 kb inversion of the J_α locus of the RPMI 8402 cells, and produce the $J_\alpha^{RX}-J_\alpha^{RP}$ fusion observed in λR15. Since the recombining J_α segments are both presumably associated with 12 bp spacer recombination signals (Early *et al.*, 1980; Sakano *et al.*, 1980), the $J_\alpha^{RX}-J_\alpha^{RP}$ rearrangement appears not to obey the 12/23 bp rule for rearrangement.

The three $J_\delta-J_\alpha$ rearrangements observed in RPMI 8402 (a translocation at $J_\delta1$, a normal $V_\alpha-J_\alpha$ join, and an unusual, small inversion involving two J_α segments) represent examples of both the mixed δ/α rearrangements and of multiple rearrangements within the $J_\delta/_\alpha$ locus. The hybridization data summarized in Table II indicate that the leukaemia sample AT5-B1 is an example of multiple J_α rearrangements. We have previously described a normal $V_\alpha-J_\alpha$ join on one allele in AT5-B1 DNA and an inversion chromosome breakpoint on the other (Baer *et al.*, 1987b). Yet a third J_α rearrangement is found in AT5-B1 DNA using J_αBB (Table II). The restriction map of a λ phage clone (λA30) encompassing this third rearrangement of AT5-B1 is shown in Figure 6A. Comparison with germ-line J_α locus reveals that this rearrangment is the consequence of a local deletion of 8.1 kb (Figure 6A). The deletion point of λA30 lies within the 0.8 kb *Sac*I−*Bam*HI fragment (Figure 6A). This fragment contains a J_α gene segment (designated J_α^{AB}) which diverges at its 5′ end from that of the germ-line J_α^{AB} segment (Figure 6C and D). Sequence analysis in this region of λA30 identifies the sequence recombined with J_α^{AB} (Figure 6B), and shows that these rep-

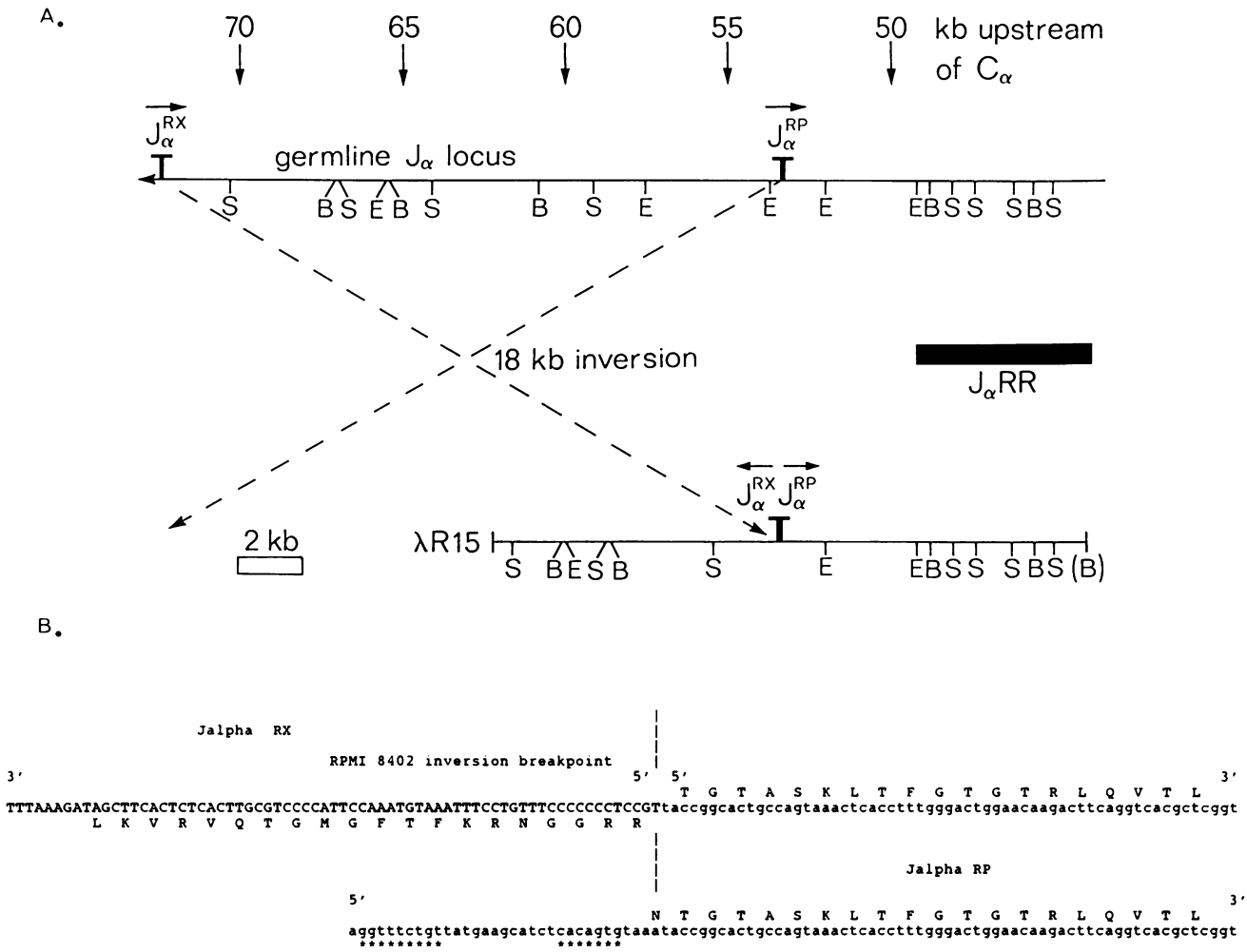

Fig. 5. A $J_\alpha - J_\alpha$ inversion found in RPMI 8402 DNA. (**A**) Comparative restriction maps of the unrearranged J_α locus shows J_α^{RX} and J_α^{RP} segments and the clone made from RPMI 8402 DNA (λR15) which carries the downstream inversion junction in which J_α^{RX} and J_α^{RP} are fused back to back. The probe J_αRR used to isolate λR15 is indicated. S = *Sac*I, B = *Bam*HI, E = *Eco*RI (the *Bam*HI site at the right hand of λR15 is derived from λ2001 cloning vector site). (**B**) Nucleotide and derived protein sequences (protein is given in the single letter code) or unrearranged J_α^{RP} (bottom line) and the junction of J_α^{RP} and J_α^{RX} in the inversion DNA. Heptamer and nonamer recombination signals are shown by dots.

resent, in their germ-line configuration, the 5′ flank of a distinct J_α gene segment, designated J_α^{AA} (Figure 6B). Thus, the 8.1 kb deletion in λA30 was generated by a recombination involving J_α^{AA} and J_α^{AB}.

Discussion

The structure of the $J_\delta - C_\delta / J_\alpha - C_\alpha$ locus

The molecular map presented in this paper shows that the human $J_\delta - C_\delta$ and $J_\alpha - C_\alpha$ genes are arranged in tandem in a manner analogous to that of the mouse TCRδ/α locus (Chien *et al.*, 1987a). A similar tandem arrangement of J−C regions has also been described for the human TCRβ (Sims *et al.*, 1984; Toyanaga *et al.*, 1985) and γ (Lefranc and Rabbitts, 1985; Quertermous *et al.*, 1987) loci; however, in the latter cases, the respective duplicated constant genes are highly homologous and presumably arose in evolution by recent gene duplication events (Lefranc *et al.*, 1986b; Tunnacliffe *et al.*, 1985). The most unusual feature of the TCR J_δ and J_α regions is their dispersed organization. The J_δ region is at least 13 kb in length, contrasting to the rather short J regions in TCRβ (Toyonaga *et al.*, 1985; Tunnacliffe and Rabbitts, 1985) gene and the immunoglobulin genes (Honjo, 1983). This paper shows the human J_α region spans most of the 85 kb that separates the C_δ and C_α exons (Figures 1 and 2). Structurally important is the fact that V genes are rearranged to both J_δ and J_α segments with rearrangements to J_α segments resulting in C_δ deletion (Table II).

Since one set of T cells (Figure 2) displays J_δ rearrangements on both alleles (Figure 2A), it suggests that the J_δ segments are the first target for V−J recombinations at this locus (Chien *et al.*, 1987a; Lindsten *et al.*, 1987). This early rearrangement of TCRδ is supported by the analysis of foetal thymus samples (Figure 2B) and our recent observation of two T-ALL tumours which have rearrangements of δ but not γ and β (L.Foroni *et al.*, manuscript submitted). Another set of T cells have J_α rearrangements on both alleles, with concomitant deletion of J_δ / C_δ. The Peer cell line represents another set of T cells showing rearrangements in both J_δ and J_α regions on the homologous chromosomes 14 (Figure 2A and 3C). It is thus likely that the series of rearrangments at the TCRδ/α locus is initiated by rearrangement of $V_\delta - J_\delta$. If δ rearrangement is productive, and a productive TCRγ rearrangement also occurs, then the cell develops into a δ/γ-bearing member of the $CD3^+$, $CD4^-$, $CD8^-$ subset

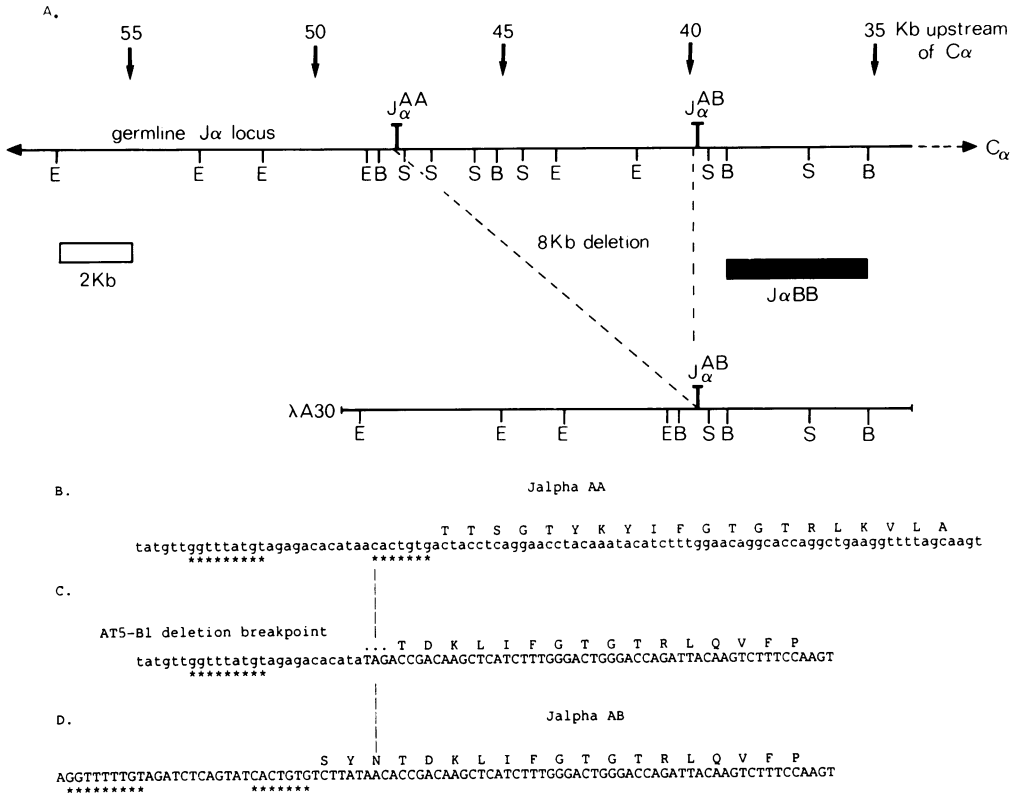

Fig. 6. An 8.1 kb deletion in AT5-B1 tumour DNA resulting from $J_\alpha - J_\alpha$ recombination. (**A**) Restriction map of λA30 (a recombinant clone derived from AT5-B1 genomic DNA). This map is compared to the corresponding J_α locus region. The probe J_αBB (indicated by the black box) was utilized as the probe to isolate λA30 compared to germ-line J_α is indicated by dotted lines. E = *Eco*RI; B = *Bam*HI; S = *Sac*I. Nucleotide and derived protein sequences of J_α^{AA} in germ-line configuration (**B**), after AT5-B1 deletion (**C**) and of germ-line J_α^{AB} (**D**). The heptamer and nonamer recombination signals are starred, points of rearrangement indicated by vertical dashed lines and J_α segments are translated into the putative protein product (given in the single letter code). Three nucleotides of presumptive N-region diversity at the deletion point (C) are shown by dots. Note that the breakpoint occurs one nucleotide upstream of the J_α^{AA} heptamer and nine nucleotides downstream of the J_α^{AB} heptamer.

(Brenner *et al.*, 1987). However, non-productive $V_\delta - J_\delta$ rearrangement can be superseded by $V_\alpha - J_\alpha$ joining (productive $V_\delta - J_\delta$ joining might be expected to halt further rearrangement at the $\delta - \alpha$ locus); since rearrangement of TCRβ should proceed independently, $\alpha - \beta$ bearing cells would appear from those that have undergone productive α and β recombinations. The fate of the TCRγ locus seems to be irrelevant to the decision about the final differentiation pathway of the T cell since productive γ gene rearrangements occur in the absence of γ RNA expression (Lefranc *et al.*, 1986a; Tighe *et al.*, 1987). Furthermore, the Peer cell line expresses both γ/δ surface receptors and intracellular β chains (Koning *et al.*, 1987), supporting the view that TCRβ rearrangement can occur in the absence of γ RNA expression (Lefranc *et al.*, 1986a; Tighe *et al.*, 1987) and that the β-chain gene can be irrelevantly expressed. Thus, the decision to differentiate along 'γ/δ' or 'α/β' lineages is likely to rest at the stage of δ/α rearrangement, as previously hypothesized (Allison and Lanier, 1987). It follows from this that expression of productive γ alleles is merely a biological consequence of TCRδ expression, and expression of productive β alleles the mere consequence of rearrangement. Therefore, the unique genetic structure of the TCRδ/α locus not only ensures an ontogenetically controlled hierarchy of gene rearrangements, but also the assignment of T cells to either the 'γ/δ' or 'α/β' lineages.

How do the internal rearrangements frequently seen within the J_α locus (Figures 5 and 6 and Table II) fit with this scheme of T cell differentiation? It would seem most likely that the $J_\alpha - J_\alpha$ recombinations are aberrant versions of some physiologically significant event within TCRδ/α, as is, for example the t(11;14)(p15;q11) in RPMI 8402 cells which appears to have occurred during an attempt at $V_\delta - D_\delta$ rearrangement (Boehm *et al.*, 1988).The internal J_α rearrangements examined by cloning, appear to have occurred via recombinase recognition signals. V, D and J rearrangements normally occur only between pairs of gene segments in which one bears a 12 bp spacer recombination signal (heptamer and nonamer) and the other a 23 bp signal. The $J_\alpha - J_\alpha$ joins that we have described are illegitimate not only in the sense that they are non-productive rearrangments of the TCRα chain gene, but also because they seem to be in violation of the 12/23 bp rule for immunoglobulin gene recombination. In other respects, however, $J_\alpha - J_\alpha$ rearrangements are reminiscent of normal V−J rearrangements that accompany lymphoid development, for example the $J_\alpha^{AA} - J_\alpha^{BB}$ rearrangement of AT5-B1 mimics deletional V−J joining. Interestingly, the site of recombination with respect to J_α^{AA} is unusual in that it occurs on the distal side of the conserved heptamer (i.e. upstream of the heptamer) rather than in the normal position between the heptamer and the J_α^{AA} segment (i.e. downstream of the heptamer). This again resembles deletional V−J joining, since the recombination site adjacent to the recombining V gene segment would also fall upstream of its associated heptamer.

Although the recombining J_α segments are associated with complete heptamer–nonamer signals, the $J_\alpha - J_\alpha$ rearrangements do not follow the 12/23 bp rule and hence are likely to have been mediated solely by the heptamers. Gene recombination at isolated heptamers is by no means unprecedented; examples of immunoglobulin gene rearrangements mediated by isolated heptamers include V_H gene replacement (Reth *et al.*, 1986; Kleinfield *et al.*,1986) aberrant rearrangement of the κ locus (Höchtl and Zachau, 1983; Kelley *et al.*, 1985; Seidman and Leder, 1980) and C_κ deletion in λ-producing cells (Durdick *et al.*, 1984; Siminovitch *et al.*, 1985). One hypothesis is that these apparently aberrant $J_\alpha - J_\alpha$ rearrangements reflect a natural process of 'switching' between J_α segments following $V_\alpha - J_\alpha$ joins, which would maintain the rearranged V_α while changing J_α. This switching could occur as follows. A V_α segment joins to a J_α segment with consequential loss of recombination signals from both V_α and J_α segments. However, like immunoglobulin V_H segments, TCR V_α sequences have a 'cryptic' heptamer placed at the end of the V region (encompassing the cysteine codon, see for example Figure 4). This cryptic V_α heptamer might then provide a target for the switching process which would move the productively rearranged V_α from one J_α to one further downstream. However, the heptamer-mediated V_H rearrangement (Reth *et al.*, 1986; Kleinfield *et al.*, 1986) results in the incoming V_H segment displacing the previously rearranged V_H segment but maintaining the full V_H sequence. For this to be true in this putative α 'switch', there would need to be considerable leeway in the precision of joining. Thus only the switches which result in productive V_α domains would be expressed as receptor. In this respect, it may be significant that imprecise joining with respect to heptamer sequences can be seen in the $J_\alpha^{AA} - J_\alpha^{AB}$ deletion event of AT5-B1 DNA (Figure 6). In addition, if J_α switching does occur, the inherent imprecision and consequential low rate of successful joins may be reflected in the high turnover of cells in the thymus. Such putative J_α switching might be important for such processes as the acquisition of tolerance to self-antigens, in which thymocytes are educated within the thymus to maintain a tolerant 'V_α' segment while switching to other J_α segments to increase receptor variability. Additionally, such a putative physiological process might explain the unusual multiplicity and highly dispersed nature of both murine and human J_α segments.

Materials and methods

Isolation and analysis of recombinant clones

λ recombinant clones were isolated from genomic libraries of human DNA, made in λ2001 vector (Karn *et al.*, 1984). Libraries were prepared with DNA from RPMI 8402 (Boehm *et al.*, 1988), SH (a lymphoblastoid cell line (Lefranc *et al.*, 1986a) and AT5-B1 (Baer *et al.*, 1987b). Restriction maps were prepared by single and double digests of λ DNA and of subclones prepared in pUC and M13 vectors (Vieira and Messing, 1982). Filter hybridization analyses were carried out as described previously (Boehm *et al.*, 1988). Nucleotide sequencing was conducted in M13 vectors using the dideoxy chain termination method (Sanger *et al.*, 1980; Bankier *et al.*, 1987). All sequences were compared, aligned and analysed using automatic computing procedures (Staden, 1986).

Characterization of TCRβ and γ loci

The status of TCRβ and γ loci was determined using a C_β probe (Sims *et al.*, 1984) and a $J_\gamma 1$ probe (Lefranc and Rabbitts, 1985).

Cell clones and lines

The characterization of cell lines has been previously reported (Minowada *et al.*, 1982; Alarcon *et al.*, 1987); NL5 and WM-14 are cloned normal lymphocytes and NL5 was derived from PBL depleted of $CD4^+/CD8^+$ T cells (H.Yssel and H.Spits, unpublished).

Acknowledgements

T.B. is a recipient of a Mildred Scheel Fellowship. We thank A.Forster for expert technical help, Dr M.Dyer for provision of leukaemia sample of patient De. We also thank Dr Litman and Professor J.Minowada for Peer cells.

References

Alarcon,B., de Vries,J., Pettey,C., Boylston,A., Yssel,H., Terhorst,C. and Spits,H. (1987) *Proc. Natl. Acad. Sci. USA*, **84**, 3861–3865.
Allison,J.P. and Lanier,L.L. (1987) *Immunol. Today*, **8**, 293–296.
Baer,R., Chen,K.-C, Smith,S.D. and Rabbitts,T.H. (1985) *Cell*, **43**, 705–713.
Baer,R., Lefranc,M.-P., Minowada,J., Forster,A., Stinson,M.A. and Rabbitts,T.H. (1986) *Mol. Biol. Med.*, **3**, 265–277.
Baer,R., Forster,A. and Rabbitts,T.H. (1987a) *Cell*, **50**, 97–105.
Baer,R., Heppell,A., Taylor,A.M.R., Rabbitts,P.H., Boulier,B. and Rabbitts,T.H. (1987b) *Proc. Natl. Acad. Sci. USA*, **84**, 9069–9073.
Band,H., Hochstenbach,F., McLean,S., Hata,S., Krangel,M.S. and Brenner,M.B. (1987) *Science*, **238**, 682–684.
Bankier,A.T., Weston,K.M. and Barrell,B.G. (1987) *Methods Enzymol.*, **155**, 51–93.
Boehm,T., Baer,R., Lavenir,I., Forster,A,, Waters,J.J., Nacheva,E. and Rabbitts,T.H. (1988) *EMBO J.*, **7**, 385–394.
Bonyhadi,M., Weiss,A., Tucker,P.W., Tigelaar,R.E. and Allison,J.P. (1987) *Nature*, **330**, 574–576.
Born,W., Miles,C, White,J., O'Brien,R., Freed,J.H., Marrack,P., Kappler,J. and Kubo,R.T. (1987) *Nature*, **330**, 572–574.
Brenner,M.B., McLean,J., Scheft,H., Riberdy,J., Ang,S.-L., Seidman,J.G., Devlin,P. and Krangel,M.S. (1987) *Nature*, **325**, 689–694.
Chien,Y.-H., Iwashima,M., Kaplan,K.B., Elliott,J.F. and Davis,M.M. (1987a) *Nature*, **327**, 677–682.
Chien,Y.-H., Iwashima,M., Weettstein,D.A., Kaplan,K.B., Elliott,J.F., Born,W. and Davis,M.M. (1987b) *Nature*, **330**, 722–725.
Croce,C.M., Isobe,M., Palumbo,A., Puck,J., Ming,J., Tweardy,D., Erikson,J., Davis,M. and Rovera,G. (1985) *Science*, **227**, 1044–1047.
Durdick,J., Moore,M.W. and Selsing,E. (1984) *Nature*, **307**, 749–752.
Early,P., Huang,H., Davis,M., Calame,K. and Hood,L. (1980) *Cell*, **19**, 981–992.
Gascoigne,N.R,, Chien,Y.-H., Becker,D.M., Kavaler,J. and Davis,M.M. *Nature*, **310**, 387–391.
Hata,S., Brenner,M.B. and Krangel,M.S. (1987) *Science*, **238**, 678–682.
Hayday,A.C., Diamond,D.J., Tonigawa,G., Heilig,J.S., Folsom,V., Saito,H. and Tonegawa,S. (1985) *Nature*, **316**, 828–832.
Höcht,J. and Zachau,H.G. (1983) *Nature*, **302**, 260–263.
Honjo,T. (1983) *Annu. Rev. Immunol.*, **1**, 449–528.
Huang,C.C., Hou,Y., Woods,L.K., Moore,G.E. and Minowada,J. (1974) *J. Natl. Cancer Inst.*, **53**, 655–660.
Karn,J., Matthes,W.D., Gait,M.J. and Brenner,S. (1984) *Gene*, **32**, 217–224.
Kelley,D.E., Wiedemann,L.M., Pillet,A.-C., Straus,S., Nelson,K.J., Davis,J., van Ness,B. and Perry,R.P. (1985) *Mol. Cell Biol.*, **5**, 1660–1675.
Kimura,N., Toyonaga,B., Yoshikai,Y., Du,R.-P. and Mak,T.W. (1987) *Eur. J. Immunol.*, **17**, 375–383.
Klein,M.H., Concannon,P., Everett,M., Kim,L.D.H., Hunkapiller,T. and Hood,L. (1987) *Proc. Natl. Acad. Sci., USA*, **84**, 6884–6888.
Kleinfield,R., Hardy,R.R., Tarlinton,D., Dangl,J., Herzenberg,L.A. and Weigert,M. (1986) *Nature*, **322**, 843–846.
Koning,F., Maloy,L.W., Cohen,D. and Coligan,J.E. (1987) *J. Exp. Med.*, **166**, 595–600.
Le Beau,M.M., McKeithan,T.W., Shima,E.A., Goldman-Leikin,R.E., Chan,S.J., Bell,G.I., Rowley,J.D. and Diaz,M.O. (1986) *Proc. Natl. Acad. Sci. USA*, **83**, 9744–9748.
Lefranc,M.-P. and Rabbitts,T.H. (1985) *Nature*, **316**, 464–466.
Lefranc,M.-P., Forster,A., Baer,R., Stinson,M.A. and Rabbitts,T.H. (1986a) *Cell*, **45**, 237–246.
Lefranc,M.-P., Forster,A. and Rabbitts,T.H. (1986b) *Proc. Natl. Acad. Sci. USA*, **83**, 9596–9600.

Leiden,J.M., Fraser,J.D. and Strominger,J.L. (1986) *Immunogenetics*, **24**, 17–23.
Lindsten,T., Fowlkes,B.J., Samelson,L.E., Davis,M.M. and Chien,Y.-H. (1987) *J. Exp. Med.*, **166**, 761–775.
Loh,E.Y., Lanier,L.L. Turck,C.W., Littman,D.R., Davis,M.M., Chien,Y.-H. and Weiss,A. (1987) *Nature*, **330**, 569–572.
Mengle-Gaw,L., Willard,H.F., Smith,C.I.E., Hammarstrom,L., Fischer,P., Sherrington,P., Lucas,G., Thompson,P.W., Baer,R. and Rabbitts,T.H. (1987) *EMBO J.*, **6**, 2273–2286.
Minowada,J., Minato,K., Srivostava,B.J.S., Nakazawa,S., Kubonishi,I., Tatsumi,E., Ohnuma,T., Ozer,H., Freeman,A.I., Henderson,E.S. and Gallo,R.C. (1982) In Serrou,B. *et al.* (eds), *Current Concepts in Human Immunobiology*. Elsevier, Amsterdam, pp. 74–84.
Quertermous,T., Strauss,W.M., van Dongen,J.J.M. and Seidman,J.G. (1987) *J. Immunol.*, **138**, 2687–2690.
Rabbitts,T.H., Lefranc,M.-P., Stinson,M.A., Sims,J.E., Schroder,J., Steinmetz,M., Solomon,E. and Goodfellow,P.N. (1985) *EMBO J.*, **4**, 1461–1465.
Reth,M., Gehrmann,P., Petrac,E. and Wiese,P. (1986) *Nature*, **322**, 840–842.
Sakano,H., Maki,R., Kurosawa,Y., Roeder,W. and Tonegawa,S. (1980) *Nature*, **285**, 676–683.
Sanger,F., Coulson,A.R., Barrell,B.G., Smith,A.J.H. and Roe,B.A. (1980) *J. Mol. Biol.*, **143**, 161–178.
Sangster,R.N., Minowada,J., Sucia-Foca,N., Minden,M. and Mak,T.W. (1986) *J. Exp. Med.*, **163**, 1491–1508.
Seidman,J.G. and Leder,P. (1980) *Nature*, **286**, 779–783.
Sim,G.K., Yague,J., Nelson,J., Marrack,P., Palmer,E., Augustin,A. and Kappler,J. (1984) *Nature*, **312**, 771–775.
Siminovitch,K.A., Bakhshi,A., Goldman,P. and Korsmeyer,S.J. (1985) *Nature*, **316**, 260–262.
Sims,J.E., Tunnacliffe,A., Smith,W.J. and Rabbitts,T.H. (1984) *Nature*, **312**, 541–545.
Staden,R. (1986) *Nucleic Acids Res.*, **14**, 217–231.
Tighe,L., Forster,A., Clark,D., Boylston,A., Lavenir,I. and Rabbitts,T.H. (1987) *Eur. J. Immunol.*, **17**, 1729–1736.
Toyonaga,B., Yoshikai,Y., Vadasz,V., Chin,B. and Mak,T.W. (1985) *Proc. Natl. Acad. Sci. USA*, **82**, 8624–8628.
Tunnacliffe,A. and Rabbitts,T.H. (1985) *Nucleic Acids Res.*, **13**, 6651–6661.
Tunnacliffe,A., Kefford,R., Milstein,C., Forster,A. and Rabbitts,T.H. (1985) *Proc. Natl. Acad. Sci. USA*, **82**, 5068–5072.
Vieira,J. and Messing,J. (1982) *Gene*, **19**, 259–268.
Winoto,A., Mjolsness,S. and Hood,L. (1985) *Nature*, **316**, 832–836.
Yoshikai,Y., Kimura,N., Toyonaga,B. and Mak,T.W. (1986) *J. Exp. Med.*, **164**, 90–103.
Yoshikai,Y., Clark,S.P., Yalor,S., Sohn,U., Wilson,B., Minden,M.D. and Mak,T.W. (1985) *Nature*, **316**, 837–840.

Received on February 17, 1988