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# The mechanism of chromosomal translocation t(11;14) involving the T-cell receptor Cδ locus on human chromosome 14q11 and a transcribed region of chromosome 11p15

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**A chromosomal translocation t(11;14) (p15;q11) is described in a human acute T-cell leukaemia of immature phenotype ($CD3^-$, $CD4^-$, $CD8^-$). The translocation occurs at a T-cell receptor joining Jδ segment, 12 kb upstream of the constant Cδ gene and 98 kb upstream of the Cα gene at chromosome band 14q11. Nucleotide sequencing shows that both Jδ and Cδ are very conserved between mouse and man. The region of chromosome 11 involved in the translocation is transcriptionally active and produces a 4-kb mRNA. The DNA sequence at the chromosome 11 junction shows a perfect match to a recombinase signal sequence implying that this translocation occurred by recombinase error. The occurrence of the translocation breakpoint at the Cδ locus, normally rearranged in immature T cells, and the structure of the translocation junctions suggests that the translocation occurred during an attempt at normal rearrangement of the Jδ segment in an early thymocyte.**

*Key words:* translocation/T-cell receptor/leukaemia

## Introduction

Recent research has shown that chromosomal abnormalities found in human lymphoid tumours are often associated with the rearranging genes which normally produce the B- and T-cell antigen receptors. This has been particularly well described in some B-cell tumours, notably Burkitt's lymphoma (reviewed, Rabbitts, 1985), follicular lymphoma and prolymphocyte B-cell leukaemia (reviewed, Croce and Nowell, 1986) where the immunoglobulin genes are found at the junctions of the various specific chromosomal translocations. T-cell tumours have also been shown to possess chromosome abnormalities and these frequently involve T-cell receptor genes. Interestingly, the majority of these tumours have translocations or inversions involving the T-cell receptor α locus (TCRα) at chromosome band 14q11. The aberrant chromosomes include t(8;14) (q24;q11) in which the TCRα gene becomes resituated to a position downstream of the c-*myc* proto-oncogene on chromosome 8 (Erikson *et al.*, 1986; Finger *et al.*, 1986; Shima *et al.*, 1986; McKeithan *et al.*, 1986); this bears analogy with the B-cell tumour Burkitt's lymphoma which features c-*myc* gene activation via chromosomal translocation. Recently, a new locus has been studied in T-cell tumours carrying inversion of chromosome 14 inv(14) (q11;q32) or translocation t(14;14) (q11;132) (Mengle-Gaw *et al.*, 1987). This locus has been called the 14q32.1 locus (Baer *et al.*, 1987a) corresponding to a region downstream of the immunoglobulin locus where a number of different T-cell tumour breakpoints have been localized. T-cell tumours bearing chromosome 11 translocations have also been described. These fall into two groups; one group having breakpoints at 11p13 (Harbott *et al.*, 1986; Williams *et al.*, 1984; White *et al.*, 1984) and the other at 11p15 (Le Beau *et al.*, 1986; Takasaki *et al.*, 1987) [t(11;14) (p13;q11) and t(11;14) (p15;q11) respectively]. Both types of translocation split the TCRα locus at 14q11 (Erikson *et al.*, 1985; Le Beau *et al.*, 1986; Lewis *et al.*, 1985) and appear to be present in rather immature T-cell types.

Clearly, the consistent occurrence of these chromosomal aberrations in tumour cells suggests a casual relationship to the T-cell malignancies, presumably by influencing an oncogene at or near the junction of the aberration. In addition, the propensity of these alterations involving T-cell receptor genes presumably reflect errors in the normal mechanism of creation of the mature receptor genes. In this paper we describe a T-cell malignancy of an immature thymocyte cell line carrying a translocation [t(11;14) (p15;q11)]. The translocation occurs between a transcribed gene located on chromosome 11p15 (designated the 11p15 locus) and the human equivalent of the recently described mouse T-cell receptor Cδ gene (Chien *et al.*, 1987). The data indicate that the translocation resulted from an abortive attempt to rearrange at a Jδ, located just upstream of Cδ segment which may result in the pathogenic activation of the 11p15 locus in this T-cell tumour.

## Results

### *Identification of chromosomal translocation (t11;14) junction*

The t(11;14) (p15;q11) abnormality has recently been described in a leukaemia patient (Takasaki *et al.*, 1987) and in a T-cell line RPMI 8402 (Le Beau *et al.*, 1986) which was derived from a 16-year-old female patient with acute T-cell leukaemia and has the surface antigen phenotype of an immature thymocyte (Minowada *et al.*, 1982). The DNA of the cell has undergone both TCRβ and TCRγ gene rearrangement (Greenberg *et al.*, 1986; Lefranc and Rabbitts, 1985; Sangster *et al.*, 1986), and expresses mRNA for γ and β but not TCRα chains (Sangster *et al.*, 1986; Le Beau *et al.*, 1986). We have recently studied the TCRα locus in the RPMI 8402 cell line and derived a probe located ~92 kb from the TCR Cα gene in unrearranged DNA (Baer *et al.*, submitted). This probe (R21XS) was made from a recom-

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binant λ phage clone isolated from a λ phage library of RPMI 8402 genomic DNA (λR21, Figure 1A). The phage library of RPMI 8402 DNA was screened with R21XS and a single clone, λR28 (Figure 1B), was isolated. Analysis of this clone showed the complete absence of highly repetitive sequences (data not shown). Accordingly, a subclone, designated pR28XE7.2 and derived from the 5′ end of λR28 was used as a probe for Southern filter hybridization experiments. This probe detected an 8-kb *Bam*HI restriction fragment in HeLa DNA (Figure 1C) and in addition to this unrearranged restriction fragment, we observed a rearranged band of ~19 kb in RPMI 8402 DNA (Figure 1C). Since we have already detected rearrangements of both alleles within the Jα locus of RPMI cells, it seemed possible that this third, upstream rearrangement corresponded to the chromosomal translocation junction. The chromosomal origin of the R28XE7.2 probe was therefore investigated by hybridization with DNA isolated from human−rodent somatic cell hybrids. Two hybrids were used in this experiment; F1R5R3 carrying only human chromosomes 14 and 18 (Solomon *et al.*, 1983) and HORL9D2R1 carrying only human chromosome 11+x (Goodfellow *et al.*, 1982). The results, illustrated in Figure

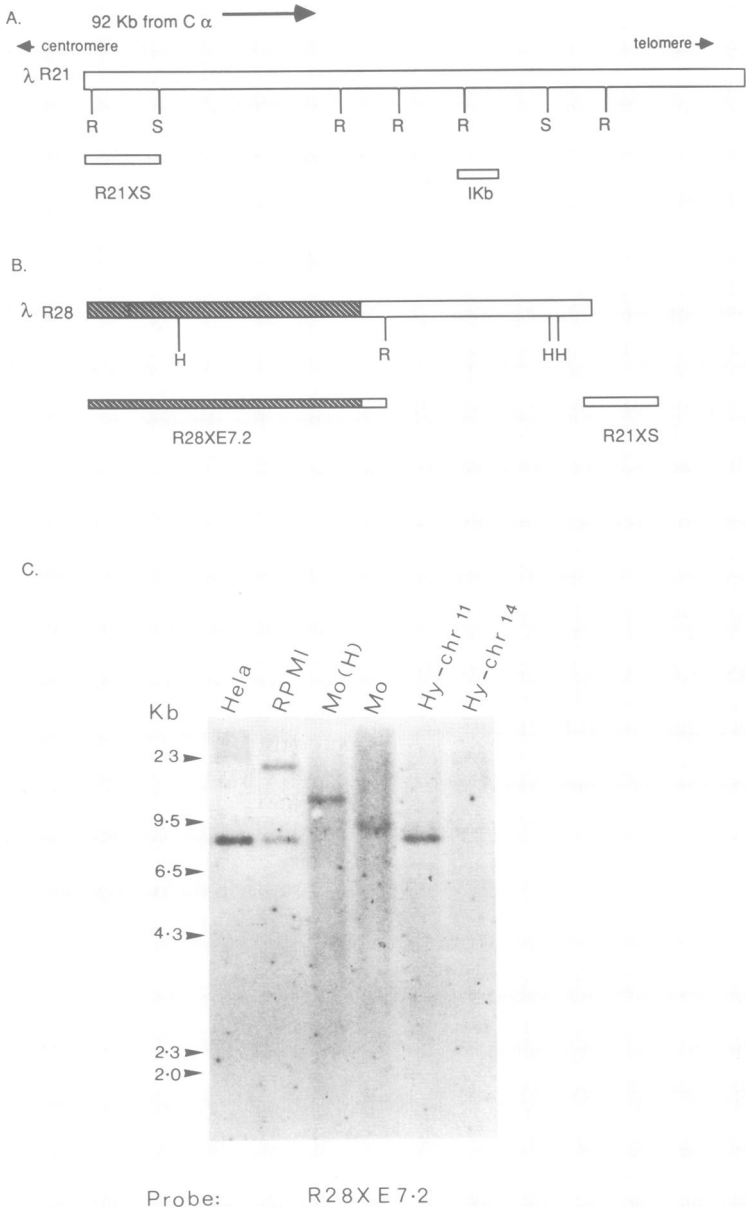



**Fig. 1.** Isolation of the chromosomal breakpoint of translocation t(11;14). (**A**) Restriction map of λR21. λR21 corresponds to part of the unrearranged Jα locus. The probe R21XS prepared form this clone is situated 92 kb upstream of Cα. This probe was used to isolate λR28 from the λ phage library of RPMI 8402 DNA. R = *Eco*RI, S = *Sac*I. (**B**) Restriction map of λR28. This λ clone was isolated from RPMI 8402. The white boxed region corresponds to the chromosome 14q11 Jα region and the shaded box to rearranged DNA at the 5′ end of the clone. R28XE7.2 represents a 7.2-kb *Xho*I−*Eco*RI fragment subcloned into pUC18X (see Materials and methods). The *Xho* site in this clone comes from the polylinker sequence of λ2001. H = *Hin*dIII, R = *Eco*RI. (**C**) Southern filter hybridization of somatic cell hybrids with R28XE7.2. 10 μg of the genomic DNAs (20 μg was used for mouse lanes) were completely digested with *Bam*HI except for the Mo(H) lane which was digested with *Hin*dIII, fractionated on 0.8% agarose and transferred to cellulose nitrate. After hybridization in 6× SSC as described in Materials and methods, the filter was washed at 65°C in 6× SSC, 0.1% SSC. Mo = mouse liver, RPMI = RPMI 8402, Hy-chr-11 = HORL9D2R1, Hy-chr-14 = FIR5R3.

1C, show that the 8-kb restriction fragment, detected by R28XE7.2, is present only in HORL9D2R1 and not in F1R5R3 DNA [the parental mouse DNA also shows a strong hybridizing fragment but of a different mol. wt (see below)] and we, therefore, conclude that the R28XE7.2 probe contains sequences derived from chromosome 11. Thus, the clone λR28 represents the junction of the t(11;14) (p15;q11) from the RPMI 8402 cell line. The orientation of the TCRα locus on chromosome 14 is centromere-Vα-Jα-Cα-telomere (Baer *et al.*, 1985; Erikson *et al.*, 1985; Lewis *et al.*, 1985). Therefore since the site of breakage occurs within the Jα locus and ~100 kb upstream of Cα (i.e. on the centromeric side of Cα), λR28 must come from the $11p^+$ chromosome (i.e. the derivative chromosome 11 containing the translocated long arm of chromosome 14).

The assignment of R28XE7.2 to chromosome 11 and to the short arm of this chromosome at band p15 was confirmed by *in situ* hybridization studies. Figure 2 shows the result of grain accumulation over metaphase chromosomes from normal PHA-stimulated T cells after hybridization with R28XE7.2. The number of grains counted over 61 metaphases was normalized to the lengths of individual chromosomes (i.e. adjusted for random association) and this analysis indicated a peak of hybridization over the short arm of chromosome 11 (Figure 2A). Analysis of all chromosomes 11 (from 40 metaphase) showed a localization of the hybridization to the tip of the short arm, broadly assigned to band p15 (which is the cytogenetic site of translocation).

The sensitivity of *in situ* hybridization does not allow a more accurate assessment of chromosomal localization to be made. A regional assignment was made by use of somatic cell hybrids derived from an aniridia patient with an interstitial deletion on chromosome 11p, cytogenetically defined as 11p12-p15.1 (Mannens *et al.*, 1987). The chromosome 11 complement of these hybrids is described in Table IA, the crucial hybrid being A3Bi-7c which carries the del (11p) chromosome as the only relevant human chromosome. The hybridization data of various probes appear in Table IB. Probes such as the insulin-like growth factor II (IGF-II) gene and the catalase (CAT) gene, which are known to map to chromosome 11p15 and 11p13 respectively, show different hybridization patterns with these hybrids; viz. IGF-II sequences are present in the A3Bi-7c hybrid but CAT sequences are not, confirming previous results (Mannens *et al.*, 1987). Probes derived from chromosome 11 centromeric and telomeric to the RPMI 8402 t(11;14) junction (pB1 and pB2, see below) detect sequences in the A3Bi-7c hybrid. This demonstrates that the probes lie outside of the deletion of del (11p) chromosome. We conclude, therefore that the breakpoint on chromosome 11 in the t(11;14) is on the telomeric side of the 11p15.1 band defined by the deletion point of the aniridia patient.

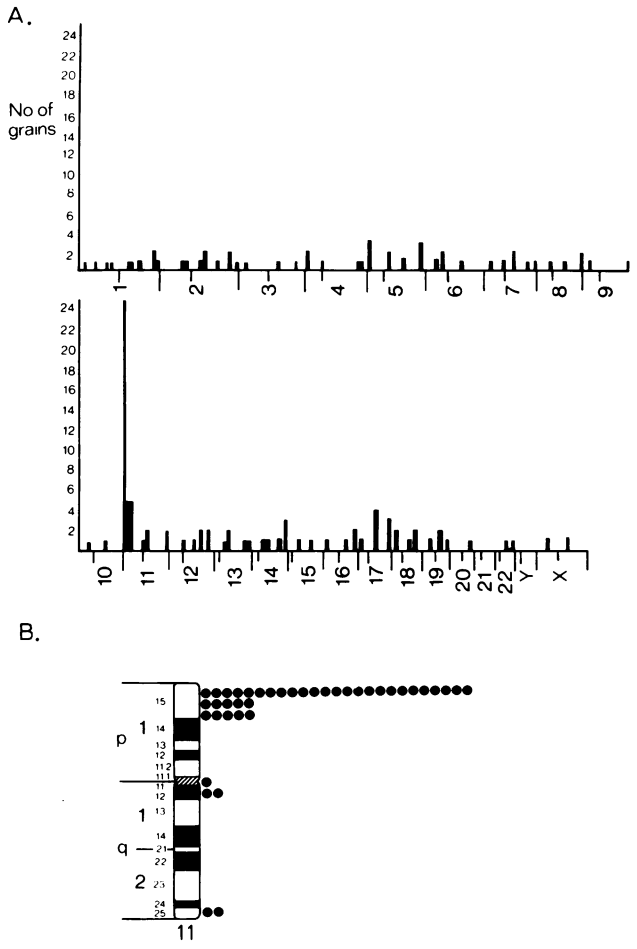



**Fig. 2.** *In situ* hybridization of normal metaphase chromosomes with R28XE7.2. (**A**) G-banded metaphase chromosomes of PHA-stimulated T cells were hybridized to $^3$H-labelled R28XE7.2 and silver grains counted. A histogram of 141 grains counted over 61 metaphase spread is shown. (**B**) Distribution of silver grains over 40 chromosomes 11.

### *Isolation of the reciprocal chromosomal junctions*

An insight into the mechanism of chromosomal aberration can be obtained by analysis of sequences at the various chromosomal junctions. We have therefore attempted to determine how the t(11;14) arose by studying the structure of the relevant DNA in a set of λ recombinant clones corresponding to the germ-line of chromosomes 11 and 14 and to the translocation sites. Figure 3 shows restriction maps of selected λ clones. The λR28 clone described above, carrying the breakpoint of the $11p^+$ chromosome (Figure 3B), was used to prepare a probe (R28EX4.9) which in turn was used to isolate an unrearranged segment (λREX29) of the corresponding region of the Jα locus [using a phage library prepared from B-cell DNA (SH DNA, Lefranc *et al.*, 1986)]. Similarly, the probe R28XE7.2 was used to isolate an unrearranged chromosome 11p15 segment λ11R2, shown in Figure 3D, from the RPMI 8402 λ phage library. A

**Table I.** Regional assignment of RPMI 8402 t(11;14) breakpoint

| | Hybrid cell | | |
|---|---|---|---|
| A | A3Bi-4c | A3Bi-7c | A3Bi-9b |
| del(11) (p12-p15.1) | − | + | − |
| normal 11 | + | − | − |
| B | | | |
| Probe | A3Bi-4c | A3Bi-7c | A3Bi-9b |
| pB1[a] | + | + | − |
| pB2[b] | + | + | − |
| CAT[c] | + | − | − |
| IGF-II[d] | + | + | − |
| INS[e] | + | + | − |

The probes were as follows: [a]pB1 see Figure 3D, [b]pB2 see Figure 3D, [c]pC24 a catalase cDNA clone (Bruns *et al.*, 1984), [d]IGF-II, a cDNA clone of the human insulin-like growth factor gene (Dull *et al.*, 1984), [e]phins96, a 1.7-kb genomic fragment encompassing the human insulin gene (Bell *et al.*, 1982).

chromosome 11 probe (pB1, Figure 3D) was prepared from this λ clone and used to isolate a recombinant phage containing the reciprocal translocation breakpoint (Figure 3C) from RPMI 8402. Thus λ11R2 corresponds to the derivative $14q^-$ chromosome.

Restriction mapping of the λ clones shown in Figure 3 and genomic filter hybrdization with various fragments enabled us to locate the translocation junctions in λR28 and λRB11 together with the corresponding regions of λREX29 and λ11R2 (data not shown). The nucleotide sequence of these four areas was determined (Figure 4). By comparing sequences from the translocation junction clones λR28 ($11p^+$ chromosome) and λRB11 ($14q^-$ chromosome) (Figure 4B and C respectively) with the unrearranged chromosome 11 sequence of λ11R2 (Figure 4D), we were easily able to determine the precise location of the chromosomal translocation junction (indicated by the vertical dotted lines in Figure 4 between residues 28 and 29 of λR28 and λRB11) since there was no loss (or gain) of material from chromosome 11 during the translocation process.

### *A J segment and recombination signals at the translocation junction*

Examination of the sequence from chromosome 14 near the translocation breakpoint identified features which probably influenced the translocation process. Analysis of the derived protein sequence from chromosome 14 in λR28 clearly identifies the presence of a joining or J segment, by virtue of certain conserved amino acids (e.g. Phe-Gly-X-Gly). The corresponding J segment is also present in the unrearranged chromosome 14 (λREX29, Figure 4A). However, the latter unrearranged J segment possesses the 5′ heptamer and nanomer recombination signal sequences normally associated with unrearranged J segments and which are usually deleted during J-segment rearrangements. In fact, the sequences of the translocated clone λR28 shows that these signal sequences have been lost. Instead, λR28 contains an additional 38 residues revealed by the comparison of the various breakpoint and germ-line sequences (Figure 4). This region of unknown origin at the translocation junction presumably derives from chromosome 14 since the breakage and rejoining of chromosome 11 was an entirely reciprocal process. An obvious possibility, therefore, is that this 38-bp region corresponds to a diversity or D element fused to the J segment prior to the translocation event. This would imply that the chromosome 14 segments in the reciprocal translocation clone, λRB11, corresponds to the 5′ end of this putative D element. In support of this view is the presence of sequences in the λRB11 showing a good match to consensus heptamer and nanomer signals on the chromosome 14 side of the breakpoint (indicated by # in Figure 4C). These sequences might derive from the 5′ signals of the D element, at the point of the translocation, back to back with chromosome 11 sequences (Figure 4C). These structural data therefore suggest that in the progenitor of the tumour cell, a D–J joining event preceeded chromosomal translocation which subsequently took place at the junction of this D element.

Why should the chromosomal translocation have occurred at that particular position of chromosome 11? Examination of the region of chromosome 11 at the junction reveals a sequence which would be strongly implicated in this process, viz. a heptamer sequence CACAGTG which is a perfect match to the consensus heptamer utilized in V–J joining. Although no reasonable match to a nanomer sequence exists on this piece of chromosome 11, the coincidence of the chromosomal junction with the heptamer, together with the back to back arrangement of the chromosome 11 heptamer and the putative D-element heptamer strongly implies their involvement in the translocation event.

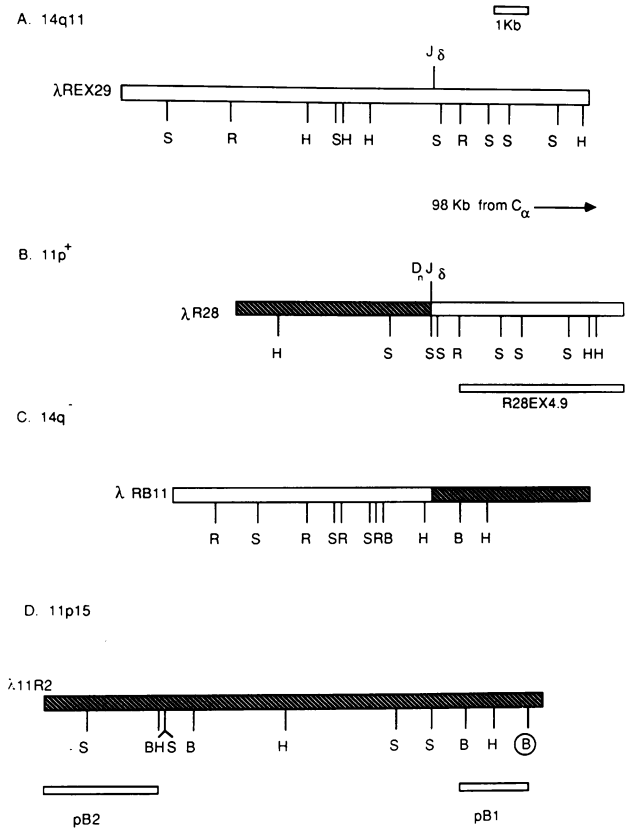



**Fig. 3.** Restriction maps of λ clones covering the unrearranged and equivalent translocation breakpoints. λREX29 (**A**) was isolated from SH B cell λ phage library while λR28 (**B**), λRB11 (**C**) and λ11R2 (**D**) were isolated from the RPMI 8402 λ phage library. λREX29 was prepared using R28EX4.9 as the probe (an *Eco*RI–*Xho*I subclone, in pUC18X, from the end of λR28), λ11R2 was prepared using R28XE7.2 and λRB11 was isolated using pB1 (a *Bam*HI subclone derived from λ11R2). S = *Sac*I, R = *Eco*RI, H = *Hin*dIII, B = *Bam*HI. The circled *Bam*HI site of λ11R2 is a polymorphic site. The *Hin*dIII sites in λRB11 are incomplete.

### *Chromosomal translocation involves the Jδ–Cδ locus*

J elements are readily recognizable by the presence of a number of conserved residues, thereby allowing the J segment adjacent to the translocation point in RMPI 8402 to be identified by comparison with other Jα segments. Recently, a new J–C region has been identified in mouse DNA, designated Jx–Cx, upstream of the Cα gene, which rearranges early in T-cell ontogeny (Chien *et al.*, 1987) and which may correspond to the δ gene, thought to be the partner for the γ gene (Brenner *et al.*, 1986). This correspondence of Cx with Cδ has recently been confirmed (P.Tucker, personal communication). Since the J segment identified above, in this human tumour, occurs ~98 kb upstream of Cα and the tumour phenotype is that of an immature T cell, it seemed possible that the involved J segment is the human equivalent of mouse Jδ. Figure 5 shows a comparison of protein (Figure 5A) and nucleotide (Figure 5B) sequences of mouse Jδ (Chien *et al.*, 1987) and the J

segment involved in the RPMI 8402 translocation breakpoint. This comparison illustrates very high conservation between these J sequences at both protein and nucleotide level (81 and 85% respectively). In addition, the recombination signal sequences have been almost completely conserved at the end of the human J compared with the mouse Jδ. These data lead us to conclude that the J segment at the RPMI 8402 junction is the human Jδ sequence.

The mouse Jδ segment is situated ~13 kb upstream of a constant region segment Cδ, to which it becomes spliced after transcription (Chien *et al.*, 1987). The location of the human Cδ segment was determined by hybridizing

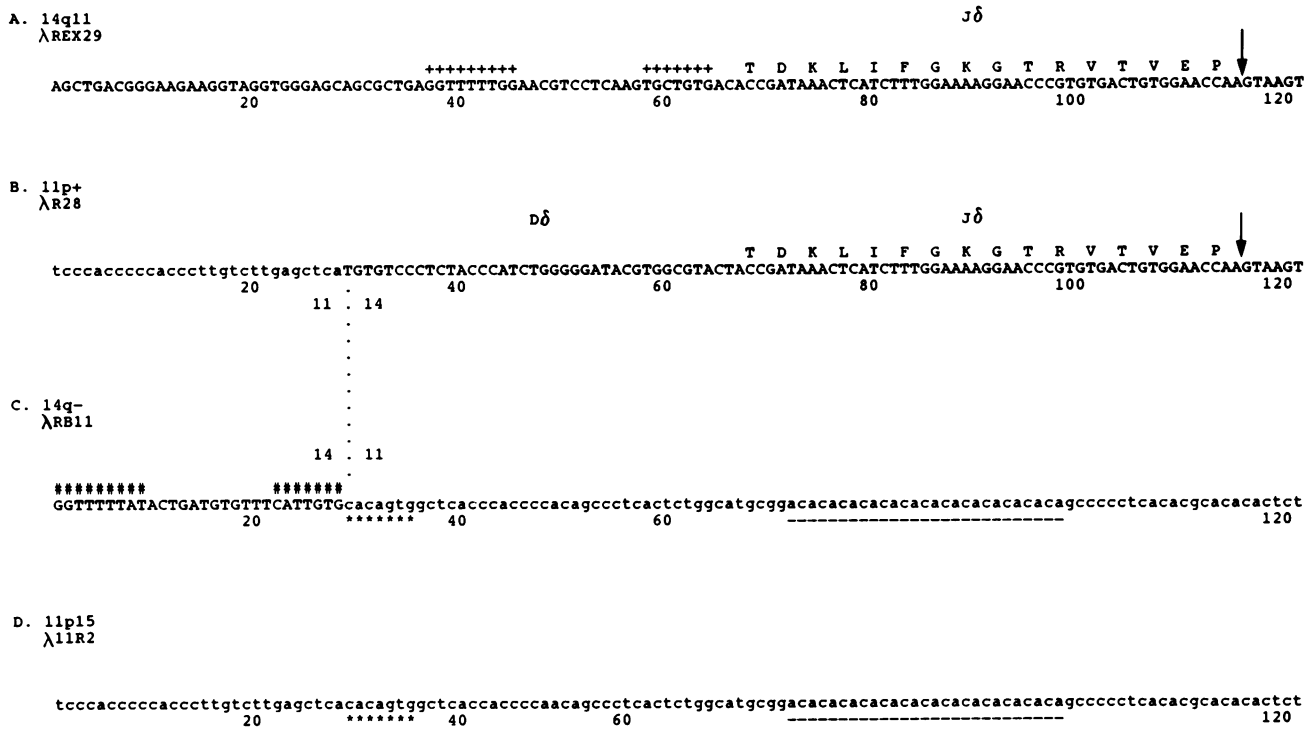



**Fig. 4.** Comparative nucleotide sequence at the 11;14 translocation junctions and corresponding germ-line sequences. The nucleotide and appropriate protein sequences at the breakpoints from λR28 (**B**) and λRB11 (**C**) are shown together with the corresponding germ-line regions [λREX29 (**A**) and λ11R2 (**D**)]. Nucleotides derived from chromosome 14 are given in upper case letters and from chromosome 11 in lower case letters (the region of a putative D element is in upper case in λR28). RNA splice sites at the end of Jδ are arrowed. Putative heptamer and nanomer recombination signals from chromosome 14 are shown by # and + and from chromosome 11 by *. A region of potential Z-DNA (alternating purine and pyrimidine residues) on chromosome 11 is underlined.

A.

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| human Jδ | T | D | K | L | I | F | G | K | G | T | R | V | T | V | E | P |
| mouse Jδ | . | . | . | . | V | . | . | Q | . | . | Q | . | . | . | . | . |

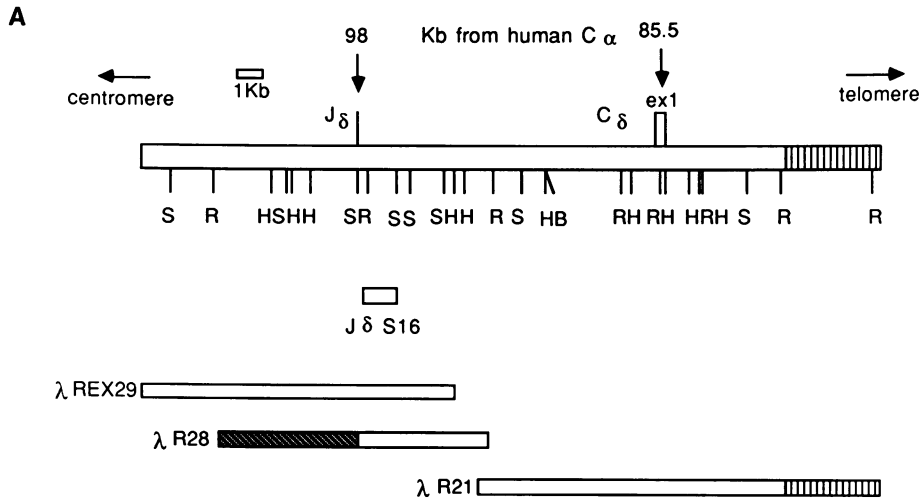
B.

```
human Jδ   GGTTTTTGGAACGT CCTCAAGTGCTGTGACACCGATAAACTCATCTTTGGAAAAGGAACCCGTGTGACTGTGGAACCAAGTAAGT
           #########       #######         40        50        60        70        80

mouse Jδ   ...........T.GG.....GTA......CT.....C......G........C.........AA.....................
           #########       #######
```

**Fig. 5.** Comparison of human and mouse Jδ segments. (**A**) Comparison of protein sequences. The full human putative δ protein sequence is given in the single letter code. Similar residues in the mouse Jx sequence (Chien *et al.*, 1987) are indicated by dots. (**B**) Comparison of nucleotide sequences. The 5′ recombination signal sequences are indicated by + and the 3′ RNA splice donor site by an arrow. Identical nucleotides in the mouse sequence compared with human are shown by dots. (A single space has been introduced at residue 15 of the human sequence to allow best alignment of two sequences.)

oligonucleotide probes [corresponding to the mouse Cx sequence (Chien *et al.*, 1987)] with recombinant λ clones from regions of DNA downstream of Jδ. This approach enabled us to locate the human Cδ gene within the recombinant clone λR21 ~12.5 kb downstream of the Jδ (in a position very analogous to that of the equivalent mouse gene) (data not shown). The region of human DNA covering Jδ and Cδ is shown in Figure 6A together with relevant recombinant λ clones covering this area. Nucleotide sequence data was obtained for the first exon of the human Cδ gene in order to confirm that this locality actually corresponded to the Jδ–Cδ locus and to assess the degree of conservation with the mouse gene. This sequence is shown in Figure 6B and the derived protein sequence was obtained by comparison with the mouse cDNA clone (Chien *et al.*, 1987); the exon/intron boundaries were also thus obtained, together with identification of




B

Human Cδ exon1

```
                G S Q P H T K P S V F V M K N G T N V A © L V K E F
TGATTATATTTGCAGGAAGTCAGCCTCATACCAAACCATCCGTTTTTGTCATGAAAAATGGAACAAATGTCGCTTGTCTGGTGAAGGAAT
      10     ▲ 20        30        40        50        60        70        80        90

  Y P K D I R I N L V S S K K I T E F D P A I V I S P S G K Y
TCTACCCCAAGGATATAAGAATAAATCTCGTGTCATCCAAGAAGATAACAGAGTTTGATCCTGCTATTGTCATCTCTCCCAGTGGGAAGT
      100       110       120       130       140       150       160       170       180

  N A V K L G K Y E D S N S V T © S V Q H D N K T V H S T D F
ACAATGCTGTCAAGCTTGGTAAATATGAAGATTCAAATTCAGTGACATGTTCAGTTCAACACGACAATAAAACTGTGCACTCCACTGACT
      190       200       210       220       230       240       250       260       270

  E V K T D S T
TTGAAGTGAAGACAGATTCTACAGGTAGGCC
      280       290     ▲ 300
```

C

```
                10                  20                  30
Hu    G S Q P H T K P S V F V M K N G T N V A © L V K E F Y P K D I
Mo    K . . . P A . . . . . I . . . . . . . . . . D . . . . E V

                40                  50                  60
Hu    R I N L V S S K K I T E F D P A I V I S P S G K Y N A V K L G
Mo    T . S . R . . . . . V . . . . . . . . . . . . S . . . . . .

                70                  80                  90
Hu    K Y E D S N S V T © S V Q H D N K T V H S T D F E V K T D S T
Mo    Q . G . . . . . N S E . . . . . P Y A N . F
```

**Fig. 6.** Location and nucleotide sequence of the human Cδ gene. (**A**) Restriction map of the region of human chromosome 14 band q11 containing Cδ. The direction of the telomere and centromere is indicated, together with distances of Jδ and Cδ from the Cα gene. The position of JδS16 is indicated (a 1.5-kb *Sac*I subclone in pUC). The relative location of λ phage clones covering the region are shown; the cross-hatched area of λR28 comes from chromosome 11 and the shaded part on the left-hand side of λR21 derives from an internal deletion within the Jα locus of RPMI 8402 (Baer *et al.*, submitted). S = *Sac*I, R = *Eco*RI, H = *Hin*dIII, B = *Bam*HI. (**B**) Nucleotides and derived protein sequence of first exon from human Cδ. Exon/intron boundaries are indicated by arrows and the derived protein sequence [derived in comparison to the mouse Cx sequence (Chien *et al.*, 1987)] is shown in single-letter code. Cysteines probably involved in disulphide bonding are circled. (**C**) Comparison of protein sequences of human and mouse Cδ exon 1. A comparison is shown of human Cδ (this paper) and mouse Cx/Cδ (Chien *et al.*, 1987). The human Cδ was derived entirely by comparison of nucleotide sequences with those of the mouse cDNA clone sequences and is shown in single-letter code. Identities in the mouse sequence are shown by dots. Cysteines probably involved in disulphide bonding are circled.

presumptive RNA splice sites (Staden, 1986). The first exon contains 95 codons, of which two code for putative intrachain disulphide linkage cysteines. Considerable homology exists between the human Cδ and the corresponding protein sequence of mouse Cδ (Figure 6C) illustrating the overall conservation of the Jδ-Cδ-Jα-Cα locus in these two species.

### *The translocated region of chromosome 11 is transcriptionally active*

Frequently, regions adjacent to chromosomal aberrations are transcriptionally active, a feature which may facilitate the creation of the abnormality (Kirsch *et al.*, 1985) and may reflect transcription of a gene involved in the aetiology of the particular tumour. Our hybridization results using the probe R28XE7.2 indicated the possible existence of a gene on chromosome 11 near the translocation junction, since this human probe detects a strongly hybridizing homologous sequence in mouse DNA (Figure 1B). This type of interspecies sequence conservation is a feature of genes, since protein coding regions tend to be more conserved than spacer regions. We have, therefore, investigated the existence of a gene in this region of chromosome 11 using Northern filter hybridizations with a probe pB1 made from λ11R2 (Figure 4D). mRNAs prepared from three different T cells were used in these experiments; RPMI 8402 was used since this carries the t(11;14) and this was compared with two thymocyte lines SUP-T1 (Smith *et al.*, 1986) and HPB-ALL (Minowada *et al.*, 1982), both of which are $CD3^+$, $CD4^+$ and $CD8^+$. All three lines have been shown to express TCRβ mRNA and this was confirmed in control hybridizations with a β-chain probe (Figure 7B). The same three mRNA preparations show different characteristics with the chromosome 11 probe pB1 (shown at the bottom of Figure 7A). This probe detects a 4-kb mRNA in RPMI 8402 cells but no equivalent mRNA was detectable in either of the other T-cell lines (Figure 7A). Therefore, we conclude that a gene exists at the translocation junction of chromosome 11 and this gene is active in the production of mRNA in the cell line which carries the translocation. However, this gene may have a restricted expression pattern in the T-cell lineage since we only detect it in RPMI 8402 and not in two more mature thymocytes. Clearly, more studies on this locus, designated the 11p15 locus, are needed to ascertain its genetic structure and relevance to tumour pathogenesis. However, it is interesting to note at this stage that the probe used to detect the 4-kb mRNA comes from the $14q^-$ chromosome, i.e. the segment of chromosome 11 which is translocated to chromosome 14, and therefore, the region of the 11p15 locus which is now located downstream of the Vα locus at chromosome 14 band q11 after translocation.

## Discussion

### *Mechanism of the translocation t(11;14)*

Chromosomal aberrations may arise either by chance events between chromosomes or they may occur by usurping pre-

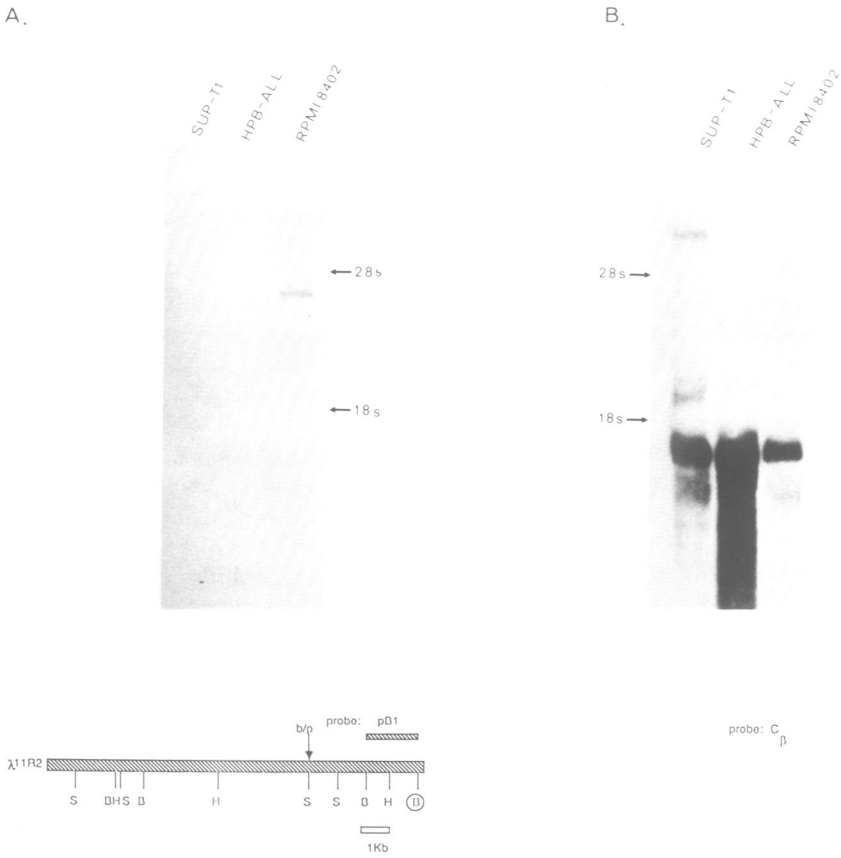



**Fig. 7.** RNA filter hybridization of T-cell RNA with chromosome 11 breakpoint probe. 2 μg poly(A)$^+$ mRNA of the three T-cell lines indicated was glyoxylated (Thomas *et al.*, 1980), fractionated on 1.4% agarose and transferred to Hybond-N. Duplicate filters were prepared. One filter was hybridized with (**A**) pB1 (shown at the bottom of the figure) or (**B**) M131B10BB1 (Cβ probe) (Sims *et al.*, 1984). Filters were washed with 0.1% × SSC, 0.1% SDS at 65°C prior to autoradiography. b/p denotes the breakpoint of the translocation.

existing mechanisms for chromosomal rearrangement. Obviously, aberrations found in B or T cells associated with rearranging antigen receptor genes can fall into the latter category. Furthermore, the creation of a chromosomal abnormality utilizing a rearranging gene locus can occur by any of three processes: (i) by random association of these latter loci with any other chromosomal region, or (ii) be directed to a particular region by the specific use of the heptamer and nanomer recombination signals, as recently demonstrated for an inversion 14 chromosome (Baer *et al.*, 1985; 1987b), or (iii) by use of sequences related to the heptamer and nanomer sequences but which are not themselves apparently normally involved in DNA rearrangement (Haluska *et al.*, 1987). The t(11;14) seen in RPMI 8402 seems to have arisen by the third of these possibilities. A perfect match to a heptamer consensus sequence occurs on chromosome 11, exactly at the borders of the translocation breakpoint, and this heptamer is back to back with a heptamer sequence from chromosome 14, which is separated by 12 bp from a nanomer sequence. This back to back organization of reciprocal rearrangements is reminiscent of the residual DNA resulting from normal V−D−J joins. The strong implication of this sequence arrangement being that the recombinase, normally involved in V−D−J rearrangement, was involved in the chromosomal translocation process by artefactually recognizing the chromosome 11 heptamer as that downstream of a V segment. Translocation thereby resulted between chromosomes rather than the normal intra-chromosomal rearrangement. The probable presence of D-element sequences at the translocation breakpoint supports this general mechanism. Certainly the sequence data shown in Figure 4 suggest an element fused to the Jδ segment. However, if this were one D element it would be extremely large, therefore it seems more likely that two or more D elements have been joined together. In support of this view are the nanomer and heptamer sequences at the breakpoint in λRB11, indicative of the 5′ end signal of sequences of an element joined to the Jδ sequence.

The chromosomal translocation in RPMI 8402 cells takes place near to the Jδ sequence. This is the human equivalent of the mouse element (Chien *et al.*, 1987) which has been shown to be rearranged and expressed at early times in T-cell development. RPMI 8402 cells do not express CD3, CD4 or CD8 on their surface and can be regarded as being derived from an early thymocyte. The association of the chromosomal translocation t(11;14) with the Jδ segment and the data discussed above suggest that normal rearrangement was taking place at the Jδ locus in the early thymocyte precursor of the tumour cell probably firstly with a Dδ−Jδ fusion. Then by a mistake, mediated through the conserved chromosome 11 heptamer, the recombinase generated an aberrant inter-chromosome rearrangement instead of completing a V−Dδ−Jδ fusion.

### *Conservation of the human and mouse Cδ genes*

A corollary of the putative rearrangement of Jδ in the thymocyte progenitor of the RPMI 8402 tumour is that the human equivalent of the mouse Cδ gene is active in early human T cells and therefore that this gene retains a conserved function in T-cell development. The protein sequence of human and mouse Jδ are highly conserved (three differences out of 16 residues). Further, the conserved nature of the Cδ genes is illustrated when the derived protein sequence of human Cδ is compared with the mouse sequence (Figure 6C). The derived protein sequences from the first exons of human and mouse Cδ share 71 residues out of 93 (76.3% homology), with the substitutions being fairly evenly scattered over the length of this domain. Therefore, we conclude that the Cδ gene, like the Cγ gene, has a conserved function in T-cell activity and that its location at the 5′ end of the extended Jα region is functionally important.

### *The 11p15 locus in RPMI 8402*

The idea that chromosome aberations have some basis in the formation of cancers stems from the consistent observation of these changes in primary tumours. The involvement of the V−J recombinase in the creation of such abnormalities poses some problems if it is assumed that a heptamer sequence (as discussed above) at the translocation breakpoint is involved. Assuming a random occurrence, a given heptamer sequence should appear every $1.6 \times 10^4$ bp or $1.8 \times 10^5$ times in a haploid genome. Clearly this frequency would result in an enormous error rate if the mere presence of this sequence were necessary to facilitate translocation. However, it seems likely that the chromosomal configuration at such sequences determines their accessibility for the recombination enzyme(s). In addition, the reasonable assumption must be made that chromosomal aberrations do occur on a sporadic basis but that the vast majority of these are innocuous. However, the rare aberration which occurs in a crucial chromosomal region will provide the afflicted cell with a growth advantage or immortality allowing it to progress to an overt tumour. The 11p15 locus may be one such locus since it is affected by the RPMI 8402 translocation. The interesting feature of this locus is that its transcription occurs in RPMI cells but not in two more mature thymocytes. Thus the gene may be a T-cell stage-specific gene which is perpetually activated by the chromosomal translocation. This complies with previous hypotheses that some proto-oncogenes are involved in cell division, while others may be involved in differentiation programmes (Harris, 1986). Thus the perpetual activation would prevent progression through the differentiation cycle and hold the cell in a fixed state of cell division. The 4-kb mRNA synthesized from the 11p15 locus is therefore of interest in this respect for further study.

### *The Jδ−Jα locus is a hot spot for chromosomal abnormality*

The Jδ segment in humans is 98 kb from Cα and may define the end of this region. Molecular studies of T-cell leukaemias have shown that this 100-kb region is a hot spot for involvement in a number of different chromosomal abnormalities apparently involved in different T-cell leukaemias. These include T-cell prolymphocytic leukaemia (Mengle-Gaw *et al.*, 1987), chronic lymphocytic leukaemia (Mengle-Gaw *et al.*, 1987; Baer *et al.*, 1987a) and acute lymphocytic leukaemia (Erikson *et al.*, 1986; Finger *et al.*, 1986; McKeithan *et al.*, 1986; Kagan *et al.*, 1987). The range of T-cell types involved covers a wide spectrum, ranging from very immature cells such as RPMI 8402 described in this paper, to $CD3^+$, $CD4^+$ (Mengle-Gaw *et al.*, 1987) and $CD3^+$, $CD8^+$ cell types (Baer *et al.*, 1987a). Rearrangements at the TCRα locus take place both at early (Jδ−Cδ) and late (Jα−Cα) stages of T-cell differentiation. To date, molecular cloning has been described in four different

chromosomal abnormalities involving chromosome band 14q11. These are inv(14) (q11;q32) (Mengle-Gaw *et al.*, 1987; Baer *et al.*, 1987a), t(14;14) (q24;q11) (Mengle-Gaw *et al.*, 1987), t(11;14) (p15;q11) (this paper) and t(8;14) (q24;q11) (Erikson *et al.*, 1986; Finger *et al.*, 1986; McKeithan *et al.*, 1986; Mathieu-Mahul *et al.*, 1985). Each of these breakpoints involves joining with the 5′ end of J segments spread throughout the Jδ−Jα locus and which eliminate the recombination signals at the site of inversion or translocation. Therefore, the involvement of the V−J recombinase in all of these rearrangements seems likely, some with the use of specific recombination signals and some with the use of sequences homologous to recombination signals.

## Materials and methods

### *Isolation and analysis of clones*

λ recombinant clones were isolated from genomic libraries, made in λ2001 vector (Karn *et al.*, 1984), using *Sau*3A partially digested DNA. Libraries were prepared from RPMI 8402 and SH (a B-cell lymphoblastoid cell line) (Lefranc *et al.*, 1986). Restriction maps were prepared by single and double digests of λDNA and of subclones prepared in pUC and M13 vectors (Vieira and Messing, 1982). A new vector pUC18X was prepared from pUC18 by converting the *Sal*I site by mutagenesis to an *Xho*I site (GTCGAC → CTCGAG); other polylinker sites remain the same. Hybridizations were carried out as previously described (Lefranc *et al.*, 1986) using cellulose nitrate filters for Southern hybridization (Southern *et al.*, 1975) or Hybond N (Amersham) for Northern hybridization (Thomas *et al.*, 1980). Hybridizations were done in 6 × SSC, 4 × Denhardt's solution (Denhardt, 1966), 50 μg/ml salmon sperm DNA and 0.1% SDS at 65°C, using random oligonucleotide labelled probes (Feinberg and Vogelstern, 1983). Washes were carried out using various salt concentrations (see figure legends) plus 0.1% SDS at 65°C followed by autoradiography at −70°C with prefogged film (Laskey and Mills, 1971).

Nucleotide sequencing was carried out in M13 vectors using the dideoxy chain termination method (Sanger *et al.*, 1977, 1986). M13 clones for sequencing were generated either by asymmetric enzyme cloning or random cloning of sonicated DNA. All sequences were compared, aligned and analysed using automatic computing procedures (Staden, 1986).

### *In situ hybridization*

*In situ* hybridization was carried out as described (Zabel *et al.*, 1983) using $^{3}$H-labelled, nick-translated (Rigby *et al.*, 1977) R28XE7.2 probe. Grains were counted over metaphase spreads of PHA-stimulated normal T lymphocytes; 141 grains were counted over 61 metaphases.

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## Note added in proof

Evidence has now been published that Cx and Cδ are the same:
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