Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 4161–4165, June 1989
Genetics

Clustering of breakpoints on chromosome 10 in acute T-cell leukemias with the t(10;14) chromosome translocation

(genetic recombination/T-cell receptor δ chain/*TCL3* gene/oncogene activation)

J. KAGAN*, L. R. FINGER*, J. LETOFSKY*, J. FINAN†, P. C. NOWELL†, AND C. M. CROCE*

*The Fels Institute for Cancer Research and Molecular Biology, Temple University School of Medicine, 3420 North Broad Street, Philadelphia, PA 19140; and †Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA 19104

Contributed by P. C. Nowell, February 21, 1989

ABSTRACT **The T-cell receptor (TCR) α/δ chain locus on chromosome 14q11 is nonrandomly involved in translocations and inversions in human T-cell neoplasms. We have analyzed three acute T-lymphoblastic leukemia samples carrying a t(10;14)(q24;q11) chromosome translocation by means of somatic cell hybrids and molecular cloning. In all cases studied the translocation splits the TCR δ chain locus. Somatic cell hybrids containing the human 10q+ chromosome resulting from the translocation retain the human terminal deoxynucleotidyltransferase gene mapped at 10q23-q24 and the diversity and joining, $D_δ2$–$J_δ1$, regions of the TCR δ chain, but not the $V_α$ region (variable region of the TCR α chain), demonstrating that the split occurred within the $V_α$–$D_δ2$ region. Molecular cloning of the breakpoint junctions revealed that the TCR δ chain sequences involved are made from the $D_δ2$ segment. The chromosome breakpoints are clustered within a region of ≈263 base pairs of chromosome 10. The results suggest that the translocation of the TCR δ chain locus to a locus on 10q, which we have designated *TCL3*, results in deregulation of this putative oncogene, leading to acute T-cell leukemia.**

Chromosomal abnormalities, translocations, inversions, and deletions are nonrandomly associated with certain types of human leukemias and lymphomas (1). In T-cell tumors, many such abnormalities involve the T-cell receptor (TCR) α chain locus (TCRα) at chromosome band 14q11 (2–5). The TCR δ chain locus (TCRδ) was identified between the 5′ portion of the α chain joining segments and the TCRα variable region ($V_α$) segments (6, 7). By means of somatic cell hybrids and molecular cloning of the entire δ locus (3, 8), this laboratory (2) and others (8, 9) were able to demonstrate the direct involvement of the $J_δ$–$D_δ$ segments, where $D_δ$ is the δ chain diversity region and $J_δ$ is the δ chain J region, in t(11;14) and t(8;14) chromosome translocations in T-cell malignancies.

The t(10;14)(q24;q11) chromosome translocation has been described in acute T-cell leukemias and high-grade T-cell lymphomas (10–12). We have analyzed cells derived from three patients with acute T-cell leukemia and have demonstrated that the breakpoint in this translocation occurs within the TCRα locus in the region between the TCRα constant region ($C_α$) and $V_α$ genes on chromosome 14 (12). Further analysis was performed employing probes derived from the TCRδ joining region ($J_δ$).

MATERIALS AND METHODS

Tumor Samples and Hybrids. Neoplastic cells were obtained from three patients: DW, a patient with T-cell lymphoblastic leukemia/lymphoma with a karyotype 46,X,Y, t(10;14)(q24;q11); JM, a patient with acute T-cell leukemia with a karyotype of 47,X,Y,12p−,+12p−,t(10;14)(q24;q11); and VB, a patient with T-cell non-Hodgkin lymphoma with a karyotype of 46,X,Y,−9,+der(9),t(9;16),t(10;14),−18,−19, −20,+der(20),+mar,+mar.

Leukemic T-cells from two patients (DW and JM) were also fused with mouse leukemic BW5147 T cells deficient in hypoxanthine phosphoribosyltransferase using polyethylene glycol. Hybrids were selected in hypoxanthine/aminopterine/thymidine (HAT) medium according to standard procedure (5, 13). Additional T-cell lines used in the present study were PEER,which expresses γ/δ chains, and MOLT-4.

Chromosomal Analysis. Chromosome preparations were made as described (12); at least 25 metaphases were examined for each hybrid. Selected metaphases were studied by the G11 banding technique (13) to confirm the human origin of the relevant chromosomes.

Isozyme Analysis. The expression of nucleotide phosphorylase, the gene for which is proximal to TCRα on chromosome 14, was studied by starch gel electrophoresis (5, 13).

Isolation and Analysis of Nucleic Acids. High molecular weight DNA was prepared and digested with restriction endonucleases for 5 hr, and 10–20 μg of samples was fractionated on 0.8% agarose gel (Sigma). Transfer of DNA from gel to nitrocellulose sheet (Millipore) was performed essentially as described by Southern (14). Hybridization probes were prepared by nick-translation to specific activities of >5 × 10^8 cpm/μg. DNA probes are as follows: (*i*) for $V_α$, an *Ava* I–*Pst* I fragment isolated from pHαT3 cDNA clone (12); (*ii*) for the immunoglobulin heavy chain joining region, pHj was used (12); and (*iii*) for the enzyme terminal deoxynucleotidyltransferase (TdT), pt106 was used (15).

DNA on nitrocellulose sheets was hybridized to ^{32}P-labeled probe DNAs in 0.6 M NaCl/60 mM sodium citrate, pH 7.0/50% (vol/vol) formamide at 37°C for 18 hr. Final washes with 15 mM NaCl/1.5 mM sodium citrate were followed by air drying and exposure to XAR-5 film (Kodak).

Isolation and Analysis of Recombinant Clones. Complete genomic libraries were constructed in the phage vector λ EMBL3 from partially *Sau*3A-digested DW leukemic T-cell DNA, hybrid 648-BD4 (derived from a fusion of JM's leukemic T cells with mouse BW5147 cell line), and human placenta DNA. Restriction maps were prepared by single and double digests of λ DNA and subclones were prepared in pUC19, PIBI25, and M13 vectors. Nucleotide sequencing was conducted in M13 or from dS plasmids using the dideoxynucleotide chain-termination method (16).

RESULTS

The TCR Rearrangements Are Due to Chromosome Translocation. This laboratory has described (3) the cloning of the entire δ locus of TCR. Using probe pjk3.0S, we have detected

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Abbreviations: TCR, T-cell receptor; TCRα and -δ, α and δ chains, respectively, of the TCR; $V_α$, $TCR_α$ variable region; $J_δ$, TCRδ joining region; TdT, terminal deoxynucleotidyltransferase.

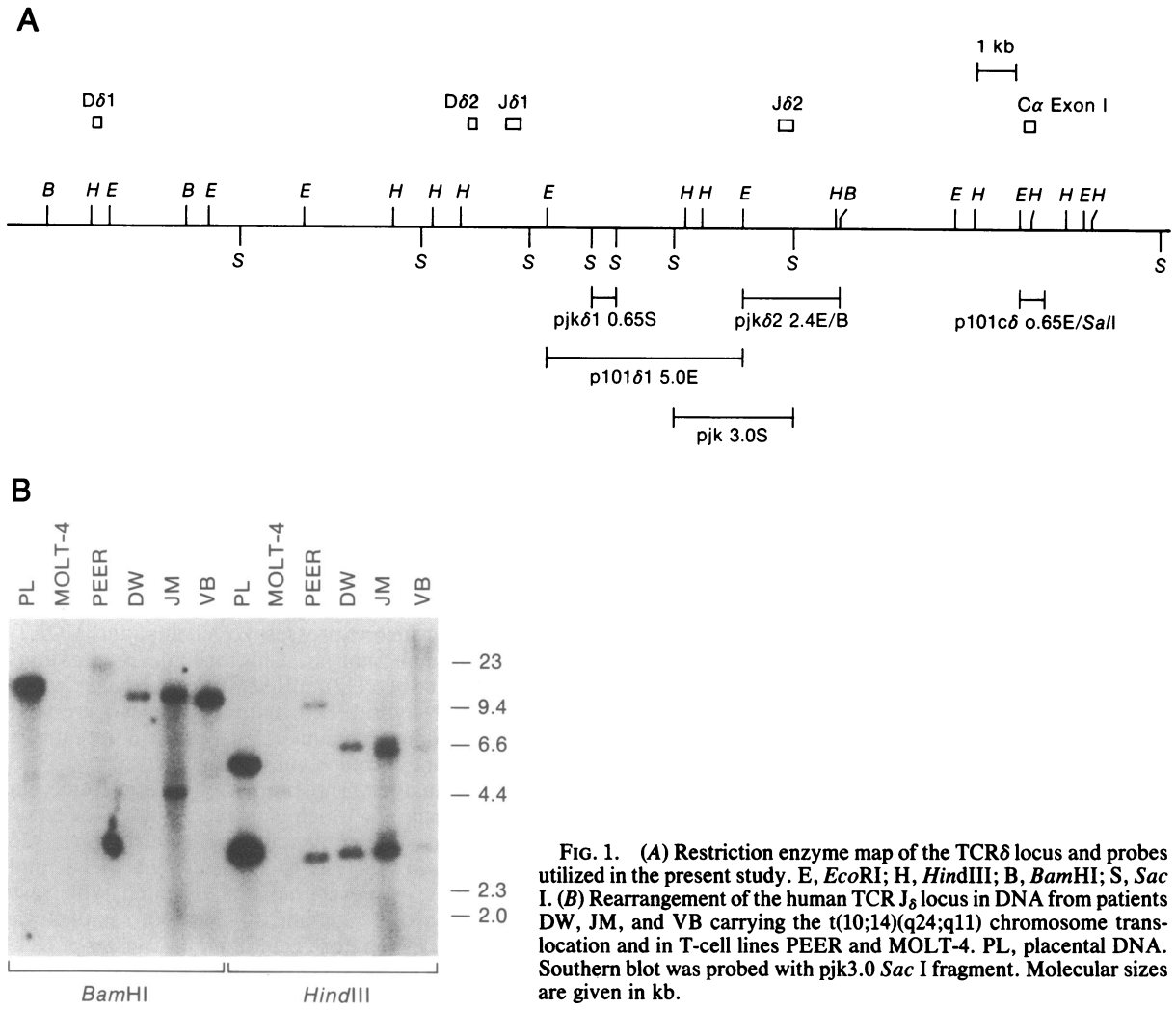

FIG. 1. (*A*) Restriction enzyme map of the TCRδ locus and probes utilized in the present study. E, *Eco*RI; H, *Hin*dIII; B, *Bam*HI; S, *Sac* I. (*B*) Rearrangement of the human TCR J_δ locus in DNA from patients DW, JM, and VB carrying the t(10;14)(q24;q11) chromosome translocation and in T-cell lines PEER and MOLT-4. PL, placental DNA. Southern blot was probed with pjk3.0 *Sac* I fragment. Molecular sizes are given in kb.

genomic rearrangements in J_δ on Southern blots of T-cell leukemia and lymphoma DNAs (Fig. 1). The first type of rearranged fragments was detected in a control T-cell line, PEER, that expresses the γ/δ chain TCR but does not have the t(10;14)(q24;q11) chromosomal translocation (17). These fragments correspond to 20-kilobase (kb) *Bam*HI and 12-kb *Hin*dIII, as compared to 16.5-kb *Bam*HI, 5.8-kb *Hin*dIII, and 3.2-kb *Hin*dIII germ-line fragments. Another control T-cell line, MOLT-4, shows no hybridization with this probe and is deleted in the J_δ region (two rearrangements were found in the α chain joining region; data not shown). A 4.7-kb *Bam*HI rearranged fragment that was detected in the DNA of one patient (JM) corresponds to physiological rearrangement at the $J_\delta 2$ segment of the TCRδ (data not shown). A third type of rearrangement was detected in DNA samples derived from all three patients with the t(10;14)(q24;q11) chromosome translocation in their leukemic cells: identically rearranged 13.9-kb *Bam*HI and 6.8-kb *Hin*dIII fragments (Fig. 1). These latter results strongly suggest that if the rearranged fragments are due to the chromosome translocation, then all translocation breakpoints are clustered in a very narrow region. To prove the direct involvement of the TCRδ locus in these translocations, we have employed somatic cell hybrids and molecular cloning techniques.

Table 1. Segregation of human genes in JM–BW5147 hybrids

	Status of human gene					Human chromosome, frequency*			
Cell line	V_α	$J_\delta 1$	NP	J_H	TdT	10	10q+	14	14q−
639-AD4	−	R	−	+	+	−	++	−	−
648-8E10	−	R	−	+	+	−	++	−	−
648-BD4	+	R	+	+	+	±	++	+	+
648-CC6	+	D	+	+	+	++	−	++	−
648-AC4	+	+	+	+	−	−	−	++	−
648-28F4	+	D	+	+	−	−	±	+	++
648-AA3	+	D	+	+	−	±	±	+	±

Hybrid 639 was derived from DW leukemic T cells (12), and hybrids 648 were derived from JM leukemic T cells. Hybrids 648-8E10 and 648-28F4 were subclones of 648-BD4. NP, nucleotide phosphorylase; J_H, immunoglobulin heavy chain joining region; $J_\delta 1$, TCRδ joining region 1 (p101δ5.0E in Fig. 1); R, rearranged; D, deleted. −, Absence of gene; +, presence of gene.

*Frequency of metaphases with the relevant human chromosome: −, none; ±, <10%; +, 10–30%; ++, >30%.

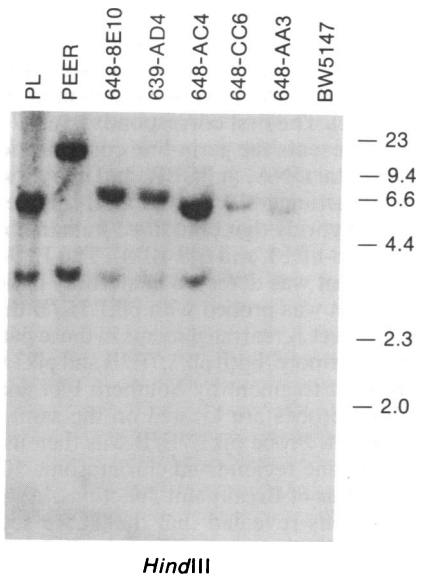

FIG. 2. Southern blot hybridization of *Hin*dIII-digested hybrid clone DNAs with $J_\delta 1$ probe p101Jδ15.0E. PL, placental DNA. Molecular sizes are given in kb.

JM and DW leukemic T cells carrying the t(10;14)(q24;q11) chromosome translocation were fused with mouse BW5147 leukemic T cells, and hybrids were examined for genetic markers of human chromosome 10 and 14. Hybrid 648 was derived from JM leukemic T cells, while hybrid 639 was derived from DW leukemic T cells, as described (12). Cytogenetic and Southern blot analyses of hybrids employed in the present study are summarized in Table 1. Hybrids 648-8E10 and 639-AD4 contained only the 10q+ chromosome, having lost the other three relevant human chromosomes (chromosomes 10, 14, and 14q−). Hybrid 648-BD4 retained all the relevant chromosomes, whereas hybrid 648-CC6 retained chromosomes 10 and 14. Hybrid 648-AA3 retained chromosome 14 and a low frequency of chromosomes 10, 10q+, and 14q−. Hybrid 648-AC4 contained chromosome 14 only. Hybrid 648-28F4 retained chromosomes 14 and 14q− and a low frequency of chromosome 10q+, which was not detected on Southern blot by the relevant genetic marker (TdT gene).

The breakpoint on chromosome 10 is distal to the TdT gene since hybrids 648-8E10 and 639-AD4, which contained only human chromosome 10q+ in the absence of other relevant human chromosomes, were positive for TdT. The human immunoglobulin heavy chain joining region located at band 14q32 was detected in all hybrids containing the normal human chromosome 14 or chromosome 10q+ (Table 1). On chromosome 14, the translocation splits the TCR J_δ locus between $J_\delta 1$ and V_α, since Southern blot analysis using probe p101Jδ15.0E on hybrid 648-8E10 and 639-AD4 DNA detected a rearranged 6.8-kb *Hin*dIII fragment and 3.2-kb *Hin*dIII germ-line fragment while hybrids 648-AC4, 648-CC6, and 648-AA3 contained only the 5.8-kb *Hin*dIII and 3.2-kb *Hin*dIII germ-line fragments (Fig. 2 and Table 1). Rehybridization of the same blot with the TCR V_α probe revealed that the V_α region segment was deleted from hybrids 648-8E10 and 639-AD4 (Table 1), providing further indication that the breakpoint splits the J_δ locus between the $J_\delta 1$ and V_α segments.

Cloning of the t(10;14)(q24;q11) Breakpoints. Additional evidence was obtained by molecular cloning of the chromosome breakpoints from genomic libraries constructed from DW leukemic T cell, JM hybrid 648-BD4, and human placental DNAs. Using probes pjk3.0S and p101δ15.0E specific for the TCR j_δ region, we were able to isolate eight overlapping phage clones that, by restriction enzyme map analysis, proved to have the rearranged fragments. Restriction enzyme maps of λBD4-14 and λW-2, two representative clones derived from two of the three patients with a t(10;14) chromosome translocation in their leukemic cells, had identical rearranged fragments and matched the germ-line TCR J_δ region at their 3′ ends but diverged at their 5′ ends (Fig. 3).

To determine the origin of the λW-2 and λBD4-14 DNA that differed from the J_δ germ-line map, we subcloned a 1.7E/B fragment into plasmid vector pIBI25 and used it to probe a panel of *Bam*HI-digested hybrids that were used to demonstrate the split in the TCR J_δ locus; the results are presented in Fig. 4*A*. Several features are apparent. (*i*) The pjk1.7E/B probe is derived from chromosome 10, since only hybrids that contained chromosome 10q+ (639-AD4, 648-8E10, and 648-BD4) or chromosome 10 (648-BD4 and 648-

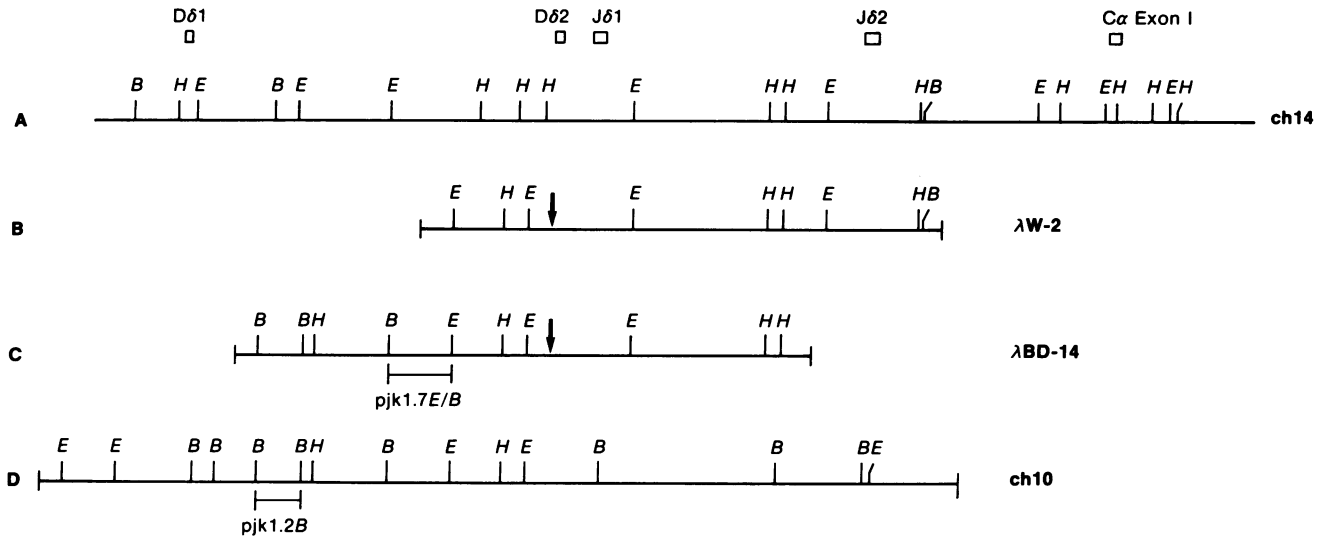

FIG. 3. Restriction maps of the regions surrounding the breakpoint in two t(10;14)(q24;q11) chromosome translocations. (*A*) The germ-line TCRδ locus on chromosome 14. (*B*) Restriction map of λW-2, a rearranged λ phage clone covering the translocation point isolated from DW's genomic library. (*C*) Restriction map of λBD4-14, a rearranged λ phage clone covering the translocation breakpoint, isolated from JM's hybrid 648-BD4 genomic library. (*D*) Germ-line chromosome 10 restriction map around the translocation point. Restriction maps of the germ-line chromosomes 10 and 14 were deduced from overlapping λ phage clones isolated from placental DNA genomic library.

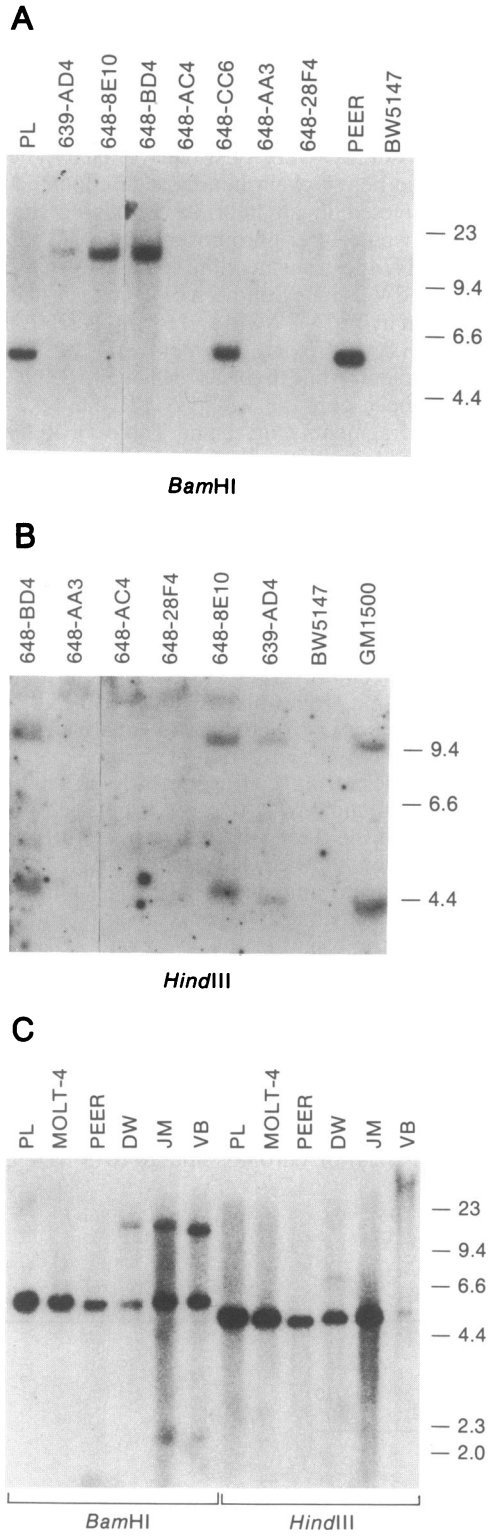

FIG. 4. Detection of the rearranged fragment with a chromosome 10 probe. (*A*) Hybridization of *Hin*dIII-digested hybrid DNA panels with pjk1.7E/B. (*B*) Hybridization of *Hin*dIII-digested hybrid DNAs with TdT (pt106), a chromosome 10 probe. (*C*) Detection of the rearranged fragment in DNA from three different patients with t(10;14)(q24;q11) chromosome translocation with chromosome 10 probe. pjk1.7E/B, a chromosome 10 probe, was hybridized with the same Southern blot that was used to detect rearrangement in J_δ region (*A*). PL, placental DNA. DW, JM, VB, patients with t(10;14)(q24;q11) chromosome translocation. Molecular sizes are given in kb.

CC6) hybridized to the probe; hybrid 648-AC4, which contains only human chromosome 14 of the relevant chromosomes, did not hybridize. (*ii*) The pjk1.7E/B probe segregates in the same manner as another chromosome 10 gene, TdT, when hybridized to a hybrid panel (Fig. 4*B*). (*iii*) Using pjk1.7E/B as a probe we have detected two *Bam*HI fragments (Fig. 4*A*). The first corresponds to a 5.7-kb *Bam*HI fragment that represents the germ-line configuration and was detected in placental DNA, in PEER, and in hybrid 648-CC6. The second is a rearranged 13.9-kb *Bam*HI fragment that was detected only in hybrids that contained human chromosome $10q^+$(639-AD4, 648-8E10, and 648-BD4). The 13.9-kb *Bam*HI rearranged fragment was detected in all three patients' DNA when the same blot was probed with pjk1.7E/B that was previously used to detect J_δ rearrangements in these patients (Figs. 1*A* and 4*C*). Furthermore, both pjk1.7E/B and pjk3.0S detected the 13.9-kb *Bam*HI fragment by Southern blot analysis, confirming that both probes are located on the same rearranged restriction fragment. Probe pjk1.7E/B was then used to clone the relevant germ-line region from chromosome 10.

Sequence Analysis of Breakpoint Junctions. Analysis at the breakpoint junctions revealed that the TCRδ sequences involved in the breakpoint are made of the $D_\delta 2$ segment (Fig. 5). The rearranged sequences upstream of $D_\delta 2$ in JM's breakpoint sequence (CCCCACA) probably correspond to N region diversity, indicating that the translocation occurred during an attempt to rearrange D_δ to $D_\delta 2$ segments or V to $D_\delta 2$ segments. Although the precise mechanism involved in the t(10;14)(q24;q11) chromosome translocation is still unclear, a heptamer-like sequence 8 base pairs (bp) downstream of JM's translocation point on chromosome 10 (Fig. 5) supports our hypothesis that these sequences may serve as signals for the recombinase system in joining DNA-specific sequences from two different chromosomes instead of joining TCR or immunoglobulin segments on the same chromosome (15, 16).

Comparison of DW breakpoint sequences with chromosome 10 germ-line sequences and with JM breakpoint sequences revealed that DW's breakpoint sequence diverged exactly 4 bp on the 5′ side of JM's breakpoint. DW's breakpoint sequence is further extended 263 bp on the 3′ side, where it joins the $D_\delta 2$ segment on chromosome 14 (Fig. 5). Since we did not detect these 263 bp on the corresponding chromosome 10 germ-line region, we speculate that this sequence stems either from a more 3′ chromosome 10 segment that was brought adjacent to the *TCL3* breakpoint region through deletion or from an insertion.

DISCUSSION

From the data presented, it appears that in all of the three cases with the t(10;14)(q24;q11) chromosome translocation we examined, the breakpoints on chromosome 14 involve the $D_\delta 2$ segment of the TCRδ locus, whereas the breakpoints on chromosome 10 cluster within a small region of 263 bp.

Sequence analysis of the breakpoint cluster regions revealed heptamer-like recombination signal adjacent to the breakpoint segment on chromosome 10 and a potential N region diversity sequence at the translocation joints (Fig. 5). These results support our previous suggestion (17, 18) that the translocation mechanism in these cases as well is mediated through the recombinase system.

The molecular cloning of a small cluster region on chromosome 10q24 that is involved in translocations in ≈10% of acute T-cell leukemias (11) strongly suggests that the tcl-3 locus is involved in the pathogenesis of specific subgroups of acute T-lymphoblastic leukemia. The relatively small clustering region should prove useful in clinical monitoring of this acute T-cell-leukemia subgroup. The TCL3 probe (pjk1.7E/B) or $J_\delta 1$ probes can detect rearrangements specific for the neoplastic clone with a single restriction enzyme digest (*Bam*HI). Furthermore, the small breakpoint clustering region is suitable for the polymerase chain reaction amplifica-

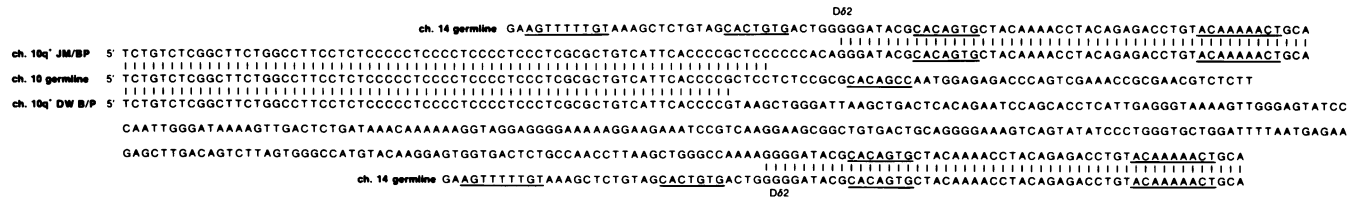

FIG. 5. Nucleotide sequence of the joining site between chromosomes 10 and 14 in JM and DW leukemic T cells and the corresponding chromosome 10 germ-line region. Vertical lines indicate identical nucleotides in the $D_\delta 2$ diversity segment of the TCRδ. The underlined regions are conserved heptamer–nonamer signal sequences.

tion and so should be applicable to the detection of minimal residual disease during remission (19).

Finally, although we have not detected a gene near the breakpoint cluster region, it is very likely that *TCL3* is further upstream, as demonstrated in some cases of Burkitt lymphoma where the translocation breakpoints occurred far away from the presumably involved *MYC* protooncogene (2, 20–24).

We thank Dr. Masaharu Isobe and Mr. Richard Harvey for providing several probes for the TCRα/δ locus and Ms. Charlotte Long for preparation of the manuscript. This work was supported by a grant from the National Institutes of Health (CA 39860) and an Outstanding Investigator Grant to C.M.C.

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