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Elizabeth A. McGuire

Washington University School of Medicine - St. Louis

Richard D. Hockett

Washington University School of Medicine - St. Louis

Karen M. Pollock

Washington University School of Medicine - St. Louis

Marty F. Bartholdi

Los Alamos National Laboratory

Stephen J. O'Brien

National Cancer Institute at Frederick, sobrien1@nova.edu

See next page for additional authors

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Authors

Elizabeth A. McGuire, Richard D. Hockett, Karen M. Pollock, Marty F. Bartholdi, Stephen J. O'Brien, and Stanley J. Korsmeyer

The t(11;14)(p15;q11) in a T-Cell Acute Lymphoblastic Leukemia Cell Line Activates Multiple Transcripts, Including *Ttg-1*, a Gene Encoding a Potential Zinc Finger Protein

ELIZABETH A. MCGUIRE,^{1*} RICHARD D. HOCKETT,¹ KAREN M. POLLOCK,¹ MARTY F. BARTHOLDI,² STEPHEN J. O'BRIEN,³ AND STANLEY J. KORSMEYER¹

Departments of Medicine, Microbiology, and Immunology, Howard Hughes Medical Institute, Washington University, St. Louis, Missouri 63110¹; Cell Biology Group, Los Alamos National Laboratory, Los Alamos, New Mexico 87545²; and Laboratory of Viral Carcinogenesis, National Cancer Institute, Frederick, Maryland 21701³

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Interchromosomal translocations within lymphoid neoplasms frequently involve the antigen receptor genes. We cloned the breakpoints of the t(11;14)(p15;q11) in a CD3-negative T-cell acute lymphoblastic leukemia cell line (RPMI 8402) in order to identify new genes potentially involved in T-cell neoplasia. An extensive comparison of both breakpoints and their germ line counterparts indicated that an inadvertent recombinase-mediated break at chromosome segment 11p15 recombined with the δ T-cell receptor at 14q11. The derivative 11 breakpoint resembles a coding joint in which 11p15 rather than a variable region was introduced 5' to a D₈₁D₈₂J₈₁ intermediate rearrangement. Conversely, the derivative 14 breakpoint corresponds to a signal joint between the 5' heptamer-spacer-nonamer recombinational signal of D₈₁ and an isolated heptamer at 11p15. Multiple, apparently distinct transcripts were found flanking both breakpoints of 8402. RNAs of 3.5, 4.4, 1.4, and 8.0 kilobases originating from either side of the derivative 14 breakpoint were highly expressed in 8402 compared with other cells. This suggests that this translocation deregulated multiple genes and provides the opportunity to assess any multifactorial contribution they may have to malignancy. We cloned and sequenced several cDNAs representing the 1.4-kilobase transcript (termed *Ttg-1* [T-cell translocation gene 1]) from an 8402 library. The predicted protein of 156 amino acids contained two internal repeats which could potentially form zinc fingers.

Chromosomal translocations are frequently found in malignant cells but not in their normal cellular counterparts. Moreover, unique translocations are associated with histologically and phenotypically distinct neoplasms (43, 56, 57). Lymphoid malignancies often possess translocations at the chromosomal sites of the antigen receptor genes, immunoglobulin genes in B-cell neoplasms, or T-cell receptor (TCR) genes in T-cell neoplasms (for a review, see reference 31). These genes normally rearrange during development to assemble separate variable (V), joining (J), and, at times, diversity (D) segments into a contiguous V(D)J coding joint. A site-specific recombinase cleaves at conserved heptamer-spacer-nonamer signal sequences, which flank the coding regions of these gene segments (33, 53), removing the intervening DNA segments and ligating the signal sequences to each other to generate extrachromosomal circles (22, 41). These genes prove to be the most frequent sites for illegitimate recombinations with nonhomologous chromosomes in lymphoid neoplasms (31).

Genes located at the sites of such translocations appear to directly participate in the development or maintenance of the malignant phenotype (29). Interchromosomal translocations can deregulate these candidate oncogenes by a variety of mechanisms. Transcriptional activation due to the introduction of enhancer elements has been shown for *c-myc* in a transgenic mouse model (1). Disruption of normal transcription by acquired somatic mutation has been noted for *c-myc* in the t(8;14)(q24;q32) of Burkitt's lymphoma (11). Translocations can also result in the production of abnormal mRNAs. Truncated RNAs from the *lyl-1* gene were seen in

the case of a T-cell acute lymphoblastic leukemia (T-ALL) bearing a t(7;19)(q34;p13) (13). Fusion transcripts containing sequences derived from both genes involved in the translocation can be found, such as the *Bcl-2-Ig* transcripts seen in follicular lymphomas bearing a t(14;18)(q32;q21) (14, 46), the *Ig- α TCR* transcripts seen in T-cell leukemias and lymphomas bearing a inv(14)(q11.2;q32.3) (16), and the *bcr-abl* transcripts seen in chronic myeloid leukemia cells bearing a t(9;22)(q34;q11) (23, 48). In the latter case, a chimeric protein containing coding sequences derived from both the *bcr* and *abl* genes is translated (4).

In order to identify new genes that might participate in T-cell growth and neoplasia, we cloned the breakpoints of both derivative chromosomes of the t(11;14)(p15;q11) found in the well-characterized T-ALL cell line, RPMI 8402 (26). This translocation involves the δ TCR locus at 14q11. One derivative breakpoint corresponds to an attempted coding joint, while the other is analogous to a signal joint. We have identified several distinct transcripts arising from sequences found on the derivative 14 [der (14)] chromosome. These mRNAs are increased in the 8402 cell line, suggesting that translocation may have activated more than one gene. We have cloned and sequenced the cDNA corresponding to one of these messages. We postulate that the protein encoded by this cDNA (termed *Ttg-1* for T-cell translocation gene 1) may form two zinc fingers similar in structure to those described for several families of DNA-binding proteins (for reviews, see references 5 and 30).

MATERIALS AND METHODS

Cells and cell lines. The RPMI 8402 cell line established from a patient with T-ALL and possessing a t(11;14)(p15;

* Corresponding author.

q11) (26) was used in this study. This phenotypically immature cell lacks CD3, CD1, CD4, and CD8 but displays CD7 and CD2 (21). While it expresses a 1.3-kilobase (kb) β TCR transcript, it does not express complete transcripts for the α , γ , or δ TCR (21; data not shown). Other cells and cell lines used for Northern (RNA) blot analysis included the following: RPMI 8432, an Epstein-Barr virus-transformed lymphoblastoid cell line established from the same patient as RPMI 8402 (26); Hut 102 and Hut 78 (17) and Jurkat (JM) (38), $\alpha\beta$ TCR-expressing T-cell lines; Peer (17), a $\gamma\delta$ -expressing T-ALL line; $\gamma\delta$ CBL and $\gamma\delta$ PBL, human T-cell lymphotropic virus type 1-transformed cord blood and peripheral blood lymphocytes, respectively (kindly provided by D. Cohen, National Institutes of Health); HSB-2 (21), CD3⁻ T-ALL; SUDHL-6 and SUDHL-4 (46), mature B-cell lines which bear a t(14;18)(q32;q21); and U937 (51), a monoblastic leukemia cell line.

Southern blot analysis. High-molecular-weight DNA was extracted from 8402 cells and human placenta. Genomic DNA (10 μ g) was digested to completion with the indicated restriction endonuclease, size fractionated in 0.7% agarose gels, and transferred to reinforced nitrocellulose (Nitroplus 2000; MSI, Westborough, Mass.) by using ammonium acetate (15). Blots were hybridized to labeled probes prepared by the random-priming method (20) and then washed and autoradiographed as previously described (25).

Northern blot analysis. Total cellular RNA was prepared by a guanidine thiocyanate lysis procedure (15). Samples consisting of 15 to 20 μ g of total RNA or 5 μ g of poly(A) RNA [selected by oligo(dT)-cellulose chromatography] were denatured in formamide, electrophoresed in agarose-formaldehyde gels, and transferred to Nitroplus 2000 (15). Hybridizations were carried out as described for Southern blot analysis except that the formamide concentration was increased from 40 to 50% (vol/vol).

Somatic cell hybrids. Genomic DNA from a previously characterized panel of hamster-human and mouse-human somatic cell hybrids (39, 40) was examined by Southern blot analysis as described above.

Flow-sorted chromosomes. 8402 cells were grown to a density of 4×10^5 to 6×10^5 cells per ml, and then 0.05 μ g of colcemid was added per ml. After a 14-h colcemid block, the mitotic index was 15 to 30%. Cells were harvested without mitotic cell enrichment by centrifuging them at $250 \times g$ for 10 min. The pellet was suspended in 75 mM KCl for 30 min to swell the cells. After a second centrifugation, the cells were suspended in Buffer III (polyamine) (3). The metaphase chromosomes were dispersed into suspension by vortexing them at high speed for 30 s. The chromosome preparation was stained with 124 μ M chromomycin A₃ and 4.8 μ M Hoechst 33258 (Hoechst-Roussel Pharmaceuticals, Inc., Somerville, N.J.). The interphase nuclei were removed by centrifugation at $100 \times g$ for 2 min. The chromosomes were sorted on an EPICS V flow cytometer as previously described (3). The peaks identified as der (11) and der (14) were not present in karyotypically normal cells, and the peak positions agreed well with the relative sizes of the derivative chromosomes present in the 8402 karyotype. Thirty thousand copies each of the normal chromosome 14 and of the der (11) and der (14) chromosomes and two hundred and ten thousand copies from the peak containing chromosomes 9, 10, 11, and 12 (of which 30,000 were normal chromosome 11) were sorted directly onto nitrocellulose filters as spots of less than 1-mm diameter. The filters were denatured in 0.5 M NaOH–1.5 M NaCl, neutralized in 3 M NaCl–0.5 M Tris hydrochloride (pH 7.5), and then baked under vacuum for 90

FIG. 1. Southern blot analysis of the δ TCR locus in the RPMI 8402 cell line. 8402 (t) or placental (G) DNA were digested with the indicated restriction endonuclease and hybridized to the ³²P-labeled (*) probes. JC δ , Partial JC δ cDNA (25); J δ , 1.6-kb *Xba*I J δ 1 fragment (25); C δ , 1.4-kb *Sph*I-*Pst*I fragment (25). Sizes (in kilobases) of detected fragments are indicated.

min at 80°C. Filters were hybridized under the same conditions described above for Southern blot analysis.

Phage cloning. Phage clones containing the der (11) and der (14) breakpoints were isolated from a RPMI 8402 completely *Xba*I-digested genomic library constructed in λ FIX (Stratagene, La Jolla, Calif.). Normal homologs of these clones were isolated from a human lung fibroblast genomic library consisting of partially *Mbo*I-digested DNA cloned in λ Fix (obtained from Stratagene). Phage DNA was transferred to nitrocellulose filters (Schleicher & Schuell, Inc., Keene, N.H.) and denatured (15) before hybridization with ³²P-labeled probes. Hybridization was carried out under the conditions for Southern blot analysis described above.

cDNA cloning. cDNA libraries were constructed with total cellular oligo(dT)-selected poly(A)⁺ RNA from RPMI 8402 cells. First- and second-strand synthesis was carried out as previously described (15). After the addition of *Eco*RI linkers (Pharmacia, Inc., Piscataway, N.J.), the cDNA was ligated into λ ZAP (Stratagene). Transfer of phage DNA and hybridization was performed as described above, except that the concentration of formamide was increased to 50% (vol/vol).

DNA sequencing. Areas of interest in the genomic phage clones and the entire 11B1 cDNA (both strands) were sequenced by the dideoxy method with modified T7 polymerase (Sequenase; USB, Cleveland, Ohio) in either M13 (mp10 and 11) phage vectors or Bluescript (Stratagene) plasmid vectors (15). The cDNA nucleotide sequence and predicted protein sequence were compared with those listed in GenBank (release 56) and National Biomedical Research Foundation Protein Sequence Database (release 16), respectively. Alignments were generated by using the FASTN (55) and FASTP (37) programs in the Washington University Biomedical Research Computing Facility VAX System.

A.

φ11-1 der (11) Chromosomal BP

C.

FIG. 2. Isolation and identification of the der (11) breakpoint. (A) Restriction map of 11.5-kb *XbaI* genomic clone, φ11-1, containing the der (11) breakpoint isolated from an 8402 genomic library. Portions arising from chromosomes 11 and 14 are denoted on either side of the breakpoint. The region which contained a DDJ₆ intermediate (see Fig. 4) and hybridized to a partial J_δ cDNA used to isolate the clone is indicated. Restriction sites are indicated as follows: X, *XbaI*; P, *PstI*; B, *BamHI*; S, *SacI*; H, *HindIII*; Sp, *SphI*; R, *EcoRI*. (B) Somatic cell hybrids. Southern blot analysis of *HindIII*-digested DNA from a human cell line (CEM) and from mouse-human (M) or hamster-human (H) hybrids probed with the 1.0-kb *SacI* probe (■ in panel A). The predetermined chromo-

RESULTS

t(11;14) in 8402 involves the δ TCR. The immature phenotype of the 8402 T-cell line, lacking the CD3 molecule and α TCR mRNA, supported the hypothesis that the t(11;14) (p15;q11) might involve the δ TCR locus, which our laboratory and others have placed within the α TCR locus at 14q11 (25, 27, 45, 52). Initially, we constructed a long-range restriction map surrounding the α/δ TCR locus in 8402 by pulsed-field gel electrophoresis (data not shown). One allele had undergone an attempted α TCR rearrangement such that V α and J α probes (44) could be placed on the same rearranged restriction fragment. The other allele was also rearranged. But in this case, V α and J α never hybridized to the same restriction fragment, suggesting that this rearrangement resulted from the chromosomal translocation.

Southern blot analysis of 8402 DNA digested with *XbaI* and probed with an incomplete J_δC_δ cDNA (containing only J_{δ1} and C_δ sequences) showed that there were no germ line alleles (Fig. 1). Instead, two rearranged fragments of 11.5 and 12.5 kb were seen. Further hybridization studies indicated that the 11.5-kb fragment hybridized only to a J_{δ1} probe, while the 12.5-kb fragment hybridized only to the C_δ probe (Fig. 1).

Isolation of the der (11) breakpoint. To determine whether either of the rearranged δ TCR fragments noted on Southern blots contained the interchromosomal breakpoint, both the 11.5- and 12.5-kb *XbaI* rearrangements were cloned from an *XbaI* genomic library of 8402. The 12.5-kb *XbaI* clone contained the C_δ region, and sequences introduced 3' to it proved to be of chromosome 14 origin and may be related to the inversion-deletion events at the 5' end of the J α noted by Baer et al. (2) (data not shown). Consequently, we focused our attention on φ11-1, which contained the 11.5-kb *XbaI* fragment that hybridized to the J_{δ1} probe. Restriction mapping of φ11-1 (Fig. 2A) showed that the J_{δ1} probe hybridized to a 0.4-kb *SacI* fragment close to one end of the phage clone. The restriction map 3' to this *SacI* fragment matched that of the germ line δ TCR, but the restriction map 5' to this 0.4-kb *SacI* fragment diverged from that of the δ TCR.

A 1.0-kb *SacI* fragment (adjacent to the 0.4-kb *SacI* fragment containing J_{δ1}) was used as a probe against a panel of well-characterized somatic cell hybrids. Figure 2B is a Southern blot of *HindIII*-digested human DNA and representative somatic cell hybrid DNA. Twenty-eight hybrids demonstrated complete concordance for the presence of human chromosome 11 when probed with the 1.0-kb *SacI* fragment, strongly suggesting that φ11-1 contains sequences from chromosome 11 juxtaposed to J_{δ1} from chromosome 14. To confirm this result and to formally determine whether

some 11 and 14 content of each hybrid is indicated. A summary of the segregation of chromosome 11 and hybridization to the human *HindIII* fragment (Hum) by the 1.0-kb *SacI* probe is shown at the bottom. Cross-hybridizing fragments in the mouse (Mou) and hamster (Ham) genomes are seen. The band hybridizing to hamster DNA in hybrids H₉, H₁₀, and H₁₂ was seen on a longer exposure (data not shown). (C) Flow-sorted chromosomes from the 8402 cell line. Histograms obtained upon separation of 8402 chromosomes by fluorescence-activated sorting are shown. Positions of der (11) and normal 11 and 14 chromosomes are shown on the left. On the right is an enlargement of the lower portion of the histogram to better indicate the location of der (14). The two derivative chromosomes and their normal partners were sorted onto nitrocellulose filters and hybridized with the 1.0-kb *SacI* probe. The autoradiograph is shown below the histograms.

FIG. 3. Restriction maps of phage clones containing the normal homolog of 11p15 and the der (14) breakpoint. (A) Restriction map of the normal counterpart of the 11p15 breakpoint region, φHG2-2, obtained from a human fibroblast genomic library. The 1.0-kb *SacI* fragment [identical to the 1.0-kb *SacI* probe from der (11) used to isolate this clone] is indicated (▨). The location of the heptamer sequence (see Fig. 4) is indicated. Restriction sites are labeled as in Fig. 2A. Additional restriction sites include K (*KpnI*) and Bs (*BssHII*). (B) Restriction map of the der (14) breakpoint-containing clone, φ14-1, isolated from the 8402 genomic library. Portions of the clone arising from chromosomes 11 and 14 are indicated. Not all *PstI* sites are shown in panels A and B. Probes which detected mRNAs of the indicated sizes in 8402 are shown (■) (see Results and Fig. 5).

this juncture was the der (11) or der (14) breakpoint, we turned to flow-sorted chromosomes. Chromosomes 11, 14, der (11), and der (14) from 8402 were sufficiently different in size that they could be separated from each other by fluorescence-activated chromosome sorting, as shown in the histograms in Fig. 2C. The sorted chromosomes were spotted onto nitrocellulose filters and hybridized to the 1.0-kb *SacI* probe. This probe hybridized to the normal chromosome 11 and to der (11) as shown. On the basis of the restriction map, somatic cell hybrids, and flow-sorted 8402 chromosomes, we concluded that φ11-1 contains the der (11) breakpoint.

Isolation of the corresponding germ line genomic region of 11p15. A phage clone containing the normal 11p15 region corresponding to chromosome 11 sequences adjacent to this breakpoint was isolated by screening a human lung fibroblast genomic library with the 1.0-kb *SacI* probe. The restriction map of this clone, φHG2-2, revealed that the 1.0-kb *SacI* probe hybridized to one end of the 13.5-kb insert (Fig. 3A). The restriction map and orientation of φHG2-2 indicated that it spans the site at 11p15 involved in the 8402 breakpoint and contains a substantial region of DNA expected to be translocated to der (14) of 8402.

Isolation of the der (14) breakpoint. We generated a unique 1.6-kb *BamHI* probe derived from φHG2-2 (Fig. 3A) to screen the 8402 *XbaI* genomic library. Duplicate filters were screened with the 1.0-kb *SacI* probe to eliminate isolates from the normal chromosome 11. A 13.5-kb phage clone, φ14-1, which was positive with the 1.6-kb *BamHI* probe but negative with the 1.0-kb *SacI* probe, was isolated. A restriction map of this clone (Fig. 3B) matched that of φHG2-2 to the right of the 1.0-kb *SacI* fragment. A D_{81} 5'-flanking region probe (25) hybridized to the left end of φ14-1 as

FIG. 4. Sequence analysis of the reciprocal breakpoints and their corresponding germ line regions. Sequences of the germ line 11, der (11), and der (14) were determined on subclones of the phage clones shown in Fig. 2 and 3. We have previously isolated and sequenced germ line clones for the D_{81} , D_{82} , and J_{81} regions of the δTCR on chromosome 14 (25). The breakpoints are indicated by arrows. The isolated heptamer sequence in the germ line 11 is underlined. The der (11) breakpoint (BP) resembles a coding joint in which chromosome 11 sequence was introduced in front of a $D_{81}D_{82}J_{81}$ intermediate as shown. Areas representing N segment additions are marked. The der (14) breakpoint contains the reciprocal signal joint. The nonamer, 12-base-pair spacer, and heptamer located 5' to D_{81} are fused flush to the isolated heptamer from germ line 11. Heptamer and nonamer sequences are boxed.

indicated in Fig. 3B, further confirming its identity as the der (14) breakpoint.

The der (11) and der (14) breakpoints resemble coding and signal joints, respectively. The breakpoint regions of both derivative chromosomes and their normal germ line chromosomal counterparts were sequenced (Fig. 4). Our sequence of the germ line 11 matched that published previously by Boehm et al. (9), with the exception of an additional cytidine 21 bases from the breakpoint, which may represent a normal polymorphism. Comparison of our detailed sequence data of the germ line diversity and joining segments of the δTCR (24) with the der (11) and der (14) breakpoints provided further information about this translocation. The der (11) breakpoint area revealed that the 8402 T cell had initiated δTCR recombination, possessing an intermediate rearrangement of the $D_{81}/D_{82}/J_{81}$ segments. However, instead of recombining a V_8 region with D_{81} , chromosome 11 sequences were inserted. In addition, extensive N segment addition (nucleotides not found in the germ line) was found at the $D_{82}-J_{81}$, $D_{81}-D_{82}$, and chromosome 11- D_{81} junctures (Fig. 4).

The der (14) breakpoint recombined the exact heptamer, 12-base-pair spacer, and nonamer cleaved from the 5' flank of the D_{81} gene segment with an isolated heptamer sequence, CACAGTG, found precisely at the breakpoint on the germ line 11. Thus, the der (14) breakpoint is analogous to a signal joint (see Discussion).

Identification of transcriptional units. In order to identify transcripts that might arise from either derivative chromosome, we identified unique fragments that spanned chromosome 11 and 14 sequences and used them as probes against Northern blots containing 8402 RNA. A 0.7-kb *SacI-BamHI* probe of chromosome 11 origin that remained on der (11) (Fig. 2A) hybridized to an approximately 0.3-kb transcript present in 8402 and in a wide spectrum of other lymphoid and hematopoietic cell lines tested (data not shown). The

FIG. 5. Northern blots demonstrating the presence of transcriptional units on either side of the der (14) breakpoint. Probes representing portions of der (14) recognized several transcripts in the 8402 cell line (probes were obtained from the phage clones shown in Fig. 3A and B). (a) 0.8-kb *HindIII-EcoRI* probe containing D_{81} (25). (b) 1.8-kb *BamHI-KpnI* probe. (c) 1.5-kb *HindIII-SacI* probe. (d) 1.6-kb *BamHI* probe. Two other probes located adjacent to the 1.6-kb *BamHI* fragment in the germ line 11 clone (ϕ HG2-2) hybridize to the same approximately 8.0-kb transcript (data not shown). See Materials and Methods for description of cell lines (indicated above the lanes). A total of 5 μ g of poly(A) RNA was used in each lane, except for DHL-6 (20 μ g of total RNA) and U937, 8402, and Jurkat RNAs in the right half of panel c (15 μ g of total RNA). Some cross-hybridization with 28S RNA can be seen in panel c.

presence of this transcript in multiple cell types makes it uncertain whether it originates from der (11) or normal chromosome 11 in 8402.

Evidence for deregulated transcriptional units in 8402 was obtained when fragments surrounding the der (14) breakpoint (Fig. 3) were used as probes on Northern blots. Multiple, apparently independent transcripts were seen in 8402 (Fig. 5). A 3.5-kb transcript was seen when a fragment containing D_{81} and its 5'- and 3'-flanking regions was used as a probe (Fig. 5a). Since only 5 base pairs of D_{81} remained on der (11) and the 3' flank had been excised during recombination to form the $D_{81}D_{82}J_{81}$ intermediate, the transcript must contain D_{81} 5'-flanking sequences located on der (14). No transcripts were seen with J_8 or C_8 probes (data not shown). It is interesting that none of the probes of chromosome 11 origin cross-hybridized with this 3.5-kb RNA.

A 1.8-kb *BamHI-KpnI* probe of chromosome 11 origin hybridized faintly to a 4.4-kb transcript in 8402 (Fig. 5b), which was similar in size to that noted by Boehm (9). This transcript appeared to be identical in size to the minor transcript seen with a 1.5-kb *HindIII-SacI* probe which recognized a major transcript of 1.4 kb. This *HindIII-SacI* genomic fragment was located less than 2 kb further downstream from the *BamHI-KpnI* fragment (Fig. 3). Thus, the

4.4-kb mRNA appeared to share some sequences present in the 1.4-kb mRNA. Much smaller amounts of the 1.4-kb transcript were seen in the monoblastic leukemia cell line U937 and in an $\alpha\beta$ T-cell line (Jurkat) but not in another $\alpha\beta$ T-cell line (Hut 102) (Fig. 5c). We examined a wider variety of cell types for the presence of the 1.4-kb transcript. The transcript was not seen in another $\alpha\beta$ T-cell line (Hut 78), $\gamma\delta$ T-cell lines (PEER, $\gamma\delta$ CBL, and $\gamma\delta$ PBL), CD3⁺ T-cell lines (HSB-2 and LC23), or 22-week fetal thymus (data not shown). The presence of intact RNA on these blots was confirmed with a β -actin cDNA control. Moreover, this transcript was not seen in a variety of other lymphoid or hematopoietic cell lines (data not shown). Hybridizations with any of three probes of chromosome 11 origin that are more distal to the breakpoint (Fig. 3) detected a large, approximately 8.0-kb mRNA found only in 8402 among the cells examined thus far (Fig. 5d).

Isolation of cDNA clones encoding the 1.4-kb transcript. We isolated several cDNAs corresponding to the 1.4-kb transcript identified by the 1.5-kb *HindIII-SacI* probe from two oligo(dT)-primed 8402 cDNA libraries. Figure 6A indicates the location of the probe used in relation to the breakpoint on der (14). Note the conservation of the rare cutting restriction site, *Bss*HII, within the genomic and cDNA clones. DNA sequencing of this site in the genomic and cDNA clones established the transcriptional orientation of this gene (genomic sequence not shown). This transcriptional unit, originating from chromosome 11 sequences, was oriented in a centromere-to-telomere fashion in which the 5' end of the message is closest to the der (14) breakpoint.

The longest clone, 11B1 (Fig. 6A), was 1.35 kb in length, which is in agreement with the apparent size of the transcript on Northern blots (Fig. 5C). The cDNA hybridized to several *HindIII* fragments in genomic DNA, indicating that the message contains several exons (data not shown).

The sequence of the 11B1 cDNA is shown in Fig. 6B. It contains a 5' untranslated region of close to 500 base pairs. We identified the two ATG codons back to back at positions 498 and 501 as the only reasonable start sites resulting in an open reading frame of 470 nucleotides. The second ATG of the pair fit best with the consensus sequence described by Kozak, with an A at position -3 and a G at position +4 (32). However, we cannot be certain which ATG is the true initiation codon. An in vitro translation production made from the 11B1 cDNA was compatible with the calculated size of 17.8 kilodaltons (data not shown). The predicted protein is rich in leucine (11.5%) and cysteine (9.6%). We propose the name *Ttg-1* (T-cell translocation gene 1).

Sequence analysis revealed two regions within the predicted protein which contain arrays of cysteine and histidine residues similar to those seen in zinc finger proteins (Fig. 7A) (see Discussion). These two regions share substantial homology with one another, as indicated by the number of identical amino acids or conservative amino acid changes seen when their sequences are compared. These two regions share a similar degree of homology with a portion of a cysteine-rich intestinal protein of rat (7), as demonstrated in Fig. 7B.

The 3' untranslated region of the 1.35-kb cDNA contained a presumed polyadenylation signal, ATTAAA (8), located 18 nucleotides upstream from a poly(A) tail. Two areas in the 3' untranslated region resembled the sequences which have been described as mediating a short mRNA half-life (10, 47) (Fig. 6B).

FIG. 6. Cloning and sequence of a cDNA corresponding to the 1.4-kb transcript of *Trg-1* from the 8402 cell line. (A) The der (14) breakpoint clone, $\phi 14-1$, is shown with the location of the 1.5-kb *HindIII-SacI* probe used to screen the cDNA libraries. A restriction map of the longest cDNA, 11B1, is shown. On the basis of the presence of a rare-cutting restriction endonuclease (*BssHII*) site in both the genomic and cDNA clones, we were able to determine the orientation of transcription. Restriction sites are indicated as follows: Bs, *BssHII*; R, *RsaI*; A, *ApaI*. (B) Entire sequence of 11B1 cDNA encoding *Trg-1*. The two possible initiating ATG codons are boxed. The predicted protein sequence for the open reading frame is shown below the nucleotide sequence (using the one-letter amino acid code). The likely polyadenylation signal is underlined (—). Sequences that could potentially mediate short mRNA half-life are also indicated (— —).

DISCUSSION

The human δ TCR is a gene within a gene located within the α TCR locus at 14q11 (25, 27, 45, 52). The δ TCR gene is the first of the four TCR genes to rearrange during early thymic development (12). While the α TCR locus is enormous, the δ TCR repertoire and geography are limited (25). These observations facilitate the molecular cloning of interchromosomal translocations that involve 14q11 in immature T-cell neoplasms such as the RPMI 8402 T-ALL line. The breakpoint in 8402 has also been studied by Boehm et al. (9), who noted that sequences at the breakpoint were analogous to the murine δ TCR and correctly concluded that the breakpoint most probably involved an intermediate rearrangement of the δ TCR locus. We extend and refine these observations by comparing the breakpoint region with our sequences for the germ line δ TCR gene segments.

The der (11) chromosome in 8402 possesses a $D_{\delta 1}D_{\delta 2}J_{\delta 1}$ intermediate which probably recombined before the translocation. Moreover, extensive N segments are present at all

sites of recombination on this allele, as typically occurs at the coding joints of normally rearranged δ TCR genes (24, 25). However, 8402 introduces chromosome segment 11p15 instead of a V_{δ} segment. In contrast, der (14) resembles a signal joint possessing the juncture of the exact heptamer-spacer-nonamer cleaved from the 5' flank of $D_{\delta 1}$ with a perfect heptamer (CACAGTG) from 11p15. Such signal joints are normally found on looped out extrachromosomal circles created during antigen receptor assemblage (22, 41). Isolated heptamers are sufficient for antigen receptor gene rearrangement, as evidenced by the recombining sequence 18 and κ -deleting element sequences of immunoglobulin genes (49). Thus, errant recognition of an apparently isolated heptamer at 11p15 by recombinase appears to be involved in the generation of this translocation.

The foreign sequences introduced 5' to the DDJ_{δ} intermediate in $\phi 11-1$ were proven to be of chromosome 11 origin by somatic cell hybrids and flow-sorted chromosomes. The assignment of $\phi 11-1$ as the der (11) breakpoint was con-

FIG. 7. Repeated domains within *Ttg-1* may form zinc fingers and are homologous to a domain in rat cysteine-rich intestinal protein. (A) The program PRTALN was used to align two domains within the 11B1 protein sequence. Amino acid identities are indicated (|), as are conservative substitutions (37) (|). *, Cysteine-cysteine or histidine-cysteine pairs that could potentially coordinate to bind a single Zn^{2+} atom. Numbers indicate amino acid positions in the predicted open reading frame. (B) Comparison of domains shown in panel A with a homologous region of rat cysteine-rich intestinal protein (CRIP) (7). Symbols are as described for panel A.

firmed by hybridization to flow-sorted chromosomes from 8402 but could also be inferred from the known chromosomal orientation of the $\alpha\delta$ TCR locus (19, 34). However, the flow-sorted chromosomes provided an important reagent for localizing other genes telomeric or centromeric to these breakpoints at 14q11 and 11p15.

Multiple, apparently distinct transcripts surround the breakpoint in 8402. A small, approximately 0.3-kb mRNA was detected by a chromosome 11 origin probe which is found on der (11). However, the expression of this gene appears to be rather constitutive, being present in all hematopoietic cell lines examined. In contrast, three distinct transcripts were recognized by sequences found on der (14). A probe from the 5'-flanking region of $D_{\delta 1}$ recognized a 3.5-kb transcript only in 8402 (Fig. 5a). However, no transcripts were detected with J_{δ} or C_{δ} probes (data not shown). No cDNA of this transcript has been characterized, so its orientation is unknown. Furthermore, we do not know if it is exclusively of chromosome 14 origin, although it does not cross-hybridize to the contiguous chromosome 11 probes. Incomplete β TCR cDNAs bearing $DJ_{C_{\beta}}$ but no V_{β} segment have been noted, implying that cryptic promoters may exist 5' to D segments (28, 50). Since the δ TCR gene on the normal chromosome 14 has been deleted, the 3.5-kb mRNA must originate from der (14) (Fig. 3). The probes derived from chromosome 11 located closest to the der (14) breakpoint recognized 4.4- and 1.4-kb mRNAs that may be related. The *Bam*HI-*Kpn*I probe nearest the breakpoint recognized a 4.4-kb transcript corresponding to the transcript reported by Boehm et al. (9) (Fig. 5b). However, the *Hind*III-*Sac*I probe also recognized a 4.4-kb mRNA as a minor transcript while revealing a major 1.4-kb species (Fig. 5c). We speculate that the 4.4-kb species is an alternative or incompletely processed form of the predominant 1.4-kb mRNA. The genomic

region most distal to the der (14) breakpoint in ϕ 14-1 recognized an approximately 8.0-kb mRNA in 8402 (Fig. 5d).

The presence of higher levels of this set of mRNAs in 8402 than other cells examined supports the hypothesis that they are derived from der (14) and are deregulated by a common event, most likely the chromosomal translocation. The activation of more than one gene at the site of chromosomal breakage is distinctly unusual. The best-characterized translocations include the t(8;14) of Burkitt's lymphoma, the t(9;22) of chronic myelogenous leukemia, and the t(14;18) of follicular lymphoma; all result in the deregulation of a single gene or a single fusion gene. The t(11;14)(p15;q11) provides a model to test whether a single translocation event can produce multigenic transformation by deregulating multiple genes.

We have isolated several cDNA clones representing the 1.4-kb transcript of *Ttg-1*. The longest of these, 11B1 (Fig. 6A), is 1,356 nucleotides. We believe that this encodes the entire transcript which has an apparent size of 1.4 kb by Northern blot. The apparent open reading frame (Fig. 6B), which initiates at one of two adjacent ATG codons located at nucleotides 498 and 501, predicts a very basic protein (estimated pI, 10.08). It has no apparent amino-terminal hydrophobic signal peptide. There are two repeated domains in the predicted protein (Fig. 7A). The arrangement of cysteine-cysteine or histidine-cysteine pairs separated by a stretch of predominantly basic and polar amino acids resembles regions in a family of DNA-binding proteins (termed zinc finger proteins) in which two such pairs coordinately bind one Zn^{2+} atom. The region between these pairs, containing mostly basic and polar amino acids, forms a loop or finger which binds to DNA (for reviews, see references 5 and 30).

The prototype for this family of zinc finger proteins is TFIIIA, a *Xenopus* transcription factor, which binds to the 5S RNA gene to activate transcription and binds to the 5S RNA molecule itself (42). It contains nine imperfect repeats of a domain with the general structure CX_2 to $5CX_{12}HX_2$ to $3H$ which binds zinc (for reviews, see references 5 and 30). Comparison of the repeated domains in TFIIIA and several other homologous DNA-binding proteins led to the description of a consensus sequence for these loops. However, it now appears that the zinc finger is a more broadly distributed and more varied motif for a variety of proteins whose common feature is DNA binding. The glucocorticoid receptor family contains similar cysteine-cysteine pairs which bind zinc and DNA (30). The retinoblastoma gene product, a nuclear phosphoprotein which binds to DNA in vitro, has been proposed to contain two zinc fingers with larger loops of 27 and 31 amino acids (35).

Zinc-stabilized loops may also be important in protein-protein interactions. The larger protein product of the adenovirus *E1A* gene contains a potential zinc finger and interacts with cellular factors to stimulate transcription from early promoters (6, 36). It is interesting that the *E1A* gene product interacts with the retinoblastoma gene product described above (54). Aside from the arrangement of cysteine-cysteine or histidine-cysteine pairs, the sequences of the finger regions in the proteins described above are quite variable. It is possible that the two repeated domains in *Ttg-1* may well form zinc fingers, given the spacing of the cysteine-cysteine or histidine-cysteine pairs and the amino acid content of the region between these pairs. The two potential zinc finger domains in *Ttg-1* share homology with a region in cysteine-rich intestinal protein of rat that is only slightly less than their self homology (Fig. 7). The mRNA for cysteine-

rich intestinal protein of rat is highly expressed in the large and small intestines of rats which are being weaned and is highly conserved throughout evolution (7). It has not been determined whether this protein binds zinc or DNA (J. Gordon, personal communication). Cloning the t(11;14)(p15;q11) breakpoint enabled us to isolate this gene and provides the opportunity to assess its function and contribution to T-cell growth and neoplasia.

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