A Third tal-1 Promoter Is Specifically Used in Human T Cell Leukemias

By Olivier Bernard, Orly Azogui,* Nathalie Lecointe, Francine Mugneret,‡ Roland Berger, Christian J. Larsen, and Danièle Mathieu-Mahul

Summary

A common feature of T cell acute lymphoblastic leukemias (T-ALLs) is the presence of structural alteration of the 5' part of the tal-1 locus, localized on chromosomal band 1p32. These alterations consist of either a t(1;14)(p32;q11) chromosomal translocation (3% of T-ALLs) or tal^d submicroscopic deletion (12–25% additional T-ALLs). We have characterized a case of T-ALL with t(1;14)(p32;q11) in which, unlike the majority of t(1;14), the recombination with the T cell receptor δ elements affected the 3' side of the tal-1 locus. In this case, tal-1 transcription is initiated from a promoter located within the fourth exon similarly to the DU 528 cell line. In a T-ALL bearing a t(1;14) affecting the 5' part of tal-1, two types of tal-1 transcripts were observed, namely those probably initiated from the Dδ region juxtaposed to tal-1 by the translocation, and those from the exon 4 promoter. It is interesting that this exon 4 promotion was also found in leukemic T cell lines and T-ALL samples without apparent tal-1 genomic alteration. In contrast, no transcript initiated from the exon 4 promoter was found in T-ALL with tal^{d1} or tal^{d2} deletion. In these cells, tal-1 is expressed via SIL-tal-1 fused transcripts. Finally, this exon 4 initiation was detected neither in normal bone marrow, nor in malignant cells from the erythroid/megakaryocytic lineages. Taken as a whole, these data suggest that the exon 4 promoter is specifically active in T cell lineage.

The tal-1 gene (also called TCL-5 or SCL) was identified because of its involvement in the t(1;14)(p32;q11) translocation in T cell acute lymphoblastic leukemia (T-ALL)[1] (1–4). It belongs to a family of genes, the products of which are defined by a primary amino acid motif referred to as the basic region-helix-loop-helix (bHLH) motif. This motif is a dimerization and DNA binding motif common to a number of proteins involved in the control of cell growth and differentiation (5, 6). Some members of this family such as c-myc are also implicated in human leukemogenesis. Beside the strong association of tal-1 with T-ALL, two closely related genes, designated as tal-2 and lyl-1, have been characterized in t(7;9)(q35;q32) and t(7;19)(q35;p13), respectively (7, 8). Because these chromosomal abnormalities are found in T-ALL, these results are highly suggestive of a specific involvement of this gene family in T-ALL leukemogenesis.

Genomic rearrangements of the tal-1 locus frequently occur in T-ALLs in two different ways. The most common alteration (12–25% of T-ALL patients) is a submicroscopic deletional event, tal^d, that leads to a 90-kb deletion of 1p32 chromosomal sequences, centromeric to the tal-1 gene. The telomeric endpoint of the deletion lies within the 5' part of the tal-1 locus, either between the two first exons (tal^{d1}) or 2-kb upstream (tal^{d2}), outside of the transcribed region (9–12). In both tal^d situations, the 5' untranslated region of another gene, named SIL, lies at the centromeric end of the deletion. As a result, a fused SIL-tal-1 transcript has been isolated in the tal^{d1}-bearing HSB-2 T cell line (11, 13). Similar fusion transcripts have been recently reported from varying tal^d patients (12).

An additional 3% of T-ALL patients present a t(1;14)(p32; q11) translocation (14). In six of the seven cases studied at the molecular level, the recombination, which is likely to be due to lymphoid recombinase activity, was shown to occur between TCR-δ and the tal-1 locus. The 1p32 breakpoints are clustered within 1 kb, surrounding the third exon of tal-1 (9, 15, 16). In the DU 528 cell line, derived from an acute

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leukemia, the translocation breakpoint falls in the 3' untranslated part of the gene (17). When tested, t(1;14) translocations are associated with a high level of *tal*-1 gene expression (1, 2). Since, up to now, *tal*-1 expression has not been detected in normal T lymphocytes, the transcriptional deregulation of *tal*-1 by deletion or translocation is likely to be a critical factor in T cell leukemogenesis (9, 10, 11, 16).

Recently, Xia et al. (18) described a case of t(1;14) in which the 1p32 breakpoint differed from the previous ones since it was localized 25 kb downstream of the *tal*-1 gene. A more distal breakpoint (35 kb) was characterized by Fitzgerald et al. (19) during the study of a t(1;7)(p32;q35) involving TCR-β on 7q35. In these two cases, the consequences of these 1p32 rearrangements on *tal*-1 expression were not investigated.

We report thereafter the molecular analysis of a new case of t(1;14) in a T-ALL. Recombination occurred between the TCR-δ gene on chromosome 14, and a 1p32 segment located 5.5 kb downstream of the *tal*-1 transcription unit. In this case, *tal*-1 transcription was shown to be initiated only within the fourth exon of the gene as observed in DU 528 cells (9, 16). This initiation was also evident in another t(1;14) T-ALL (in which the breakpoint is located in the third exon of *tal*-1 [9]) and in T-ALLs without evident genomic alteration of the *tal*-1 locus. In contrast, this peculiar exon 4 promotion was not detected either in normal bone marrow, nor in malignant cells from the erythroid/megakaryocytic lineages, suggesting that it might be T cell specific. This initiation was not detected either in tal^{d1} and tal^{d2} samples. In those cases, our investigations confirm that both types of tal^{d} similarly result in *SIL-tal-1* fusion transcripts (11).

Materials and Methods

Cells and Cell Lines. The patient was a 23-year-old male with T-ALL. At diagnosis, the white blood count was 380 × 10^9/liter with 92% lymphoblasts. Bone marrow was invaded by 95% lymphoblasts. Surface markers were: CD2$^+$, CD5$^+$, CD7$^+$, CD4$^+$, CD8$^+$, CD3$^-$, and intracytoplasmic CD3$^+$. Cytogenetic analysis revealed an abnormal karyotype: 46,XY,t(1;14)(p32;q11), inv(7)-(p15q35) in 11 mitoses, and a normal 46,XY in 28. The patient entered complete remission after intensive chemotherapy. Patient material analyzed in this report is a leukemic blood sample obtained just before treatment.

Patients samples were provided by the Hematology Department of Hôpital Saint-Louis (Paris, France). Cell lines were cultivated in RPMI 1640 10% FCS as described (2, 9).

Southern Blots. DNA was extracted as described (2). Southern blot hybridization was carried out in 0.5 M Na$_2$HPO$_4$, pH 7.2, 7% SDS, 1 mM EDTA, with radiolabeled probes at 68°C. Washes were carried out in 40 mM NaH$_2$PO$_4$/Na$_2$HPO$_4$, 1% SDS at 68°C (20).

Probes were isolated as described (2, 21) and were as follows: the Dδ1/2 probe is a 1.8-kb BamHI-EcoRI fragment; the Jδ1 probe is a 1.5-kb XbaI-EcoRI fragment, and Jδ3 is a 0.56-kb EcoRV-XbaI fragment.

RNase Protection Assay. RNA was extracted by guanidium isothiocyanate disruption of the cells followed by cesium chloride selection or phenol extraction (2, 22).

Uniformly labeled ^{32}P-antisense RNA was synthesized from relevant fragments subcloned in pGEM or Bluescript by using

Promega Corp. (Madison, WI) reagents. 2 × 10^5 cpm of antisense RNA was hybridized to 5–20 μg of test RNA samples in 80% formamide for 16 h at 65°C. After RNase A digestion, samples were phenol/chloroform extracted, ethanol precipitated, and analyzed by electrophoresis on a 5% acrylamide-7 M urea denaturing gel.

PCR Experiments. Bi-specific PCR was performed on genomic DNA or on random-primed cDNA as previously described (9). Oligonucleotides, provided by Genset (Paris, France) were JU5': ATGGATACAGAGCCCTTCCAC; JU3': CTGCAGGGGCTG-GTCCAAAG; Dδ2: AGCATTGGTGAAAGGAGTTC; Jδ3': AGAGTTTGATGCCAGTTCCG; SIL: TGCAAACAGACCTCA-GCTC; Z2: TTCGCTGAGAGGCCTGCAGT; and Z8: CTCCCG-GCTGTTGGTGAA. Z2 and Z8 correspond to *tal*-1 exons 3 and 6, respectively.

Anchored PCR was performed as described (23, 24) using successively nested oligonucleotides, Z8, Z7 (GGGACCATCAG-TAATCTCC), and Z3 (AAGGCATCCGGCTCCCCAAA).

Results

t(1;14) in JU Cells Affects the 3' Part of the tal-1 Gene. Leukemic cells obtained at diagnosis from patient JU showed a typical t(1;14)(p32;q11) translocation. To determine whether the chromosome 1 breakpoint occurred within the *tal*-1 locus, we performed Southern blot experiments with probes encompassing 35 kb of the *tal*-1 locus. No rearranged fragments were detected with probes derived from the 5' part of the gene. On the other hand, probes derived from the 3' part of *tal*-1 revealed an abnormal pattern in addition to the germline pattern. More precisely, a 0.6-kb PstI fragment was able to detect two rearranged fragments and a germline, one in each digest (Fig. 1). These rearrangements were likely to correspond to both chromosomal translocation junctions and allowed us to map the putative breakpoint at 5.5 kb downstream of the *tal*-1 polyadenylation signal.

Much as we had with the previously reported t(1;14) cases, we reasoned that the chromosome 14 breakpoint could involve the TCR-δ gene. Genomic probes covering either the Dδ1/2 (Fig. 1) or the Jδ1 (data not shown) regions detected only one rearranged fragment in each digestion. The same blot hybridized with a Jδ3 probe, revealed rearranged fragments in addition to the germline fragments. The Dδ2 rearranged fragments did not comigrate with those detected by the Jδ1 or Jδ3 probes, which suggests that they were not a result of an incomplete D-J assembly. The presence of three different rearrangements within TCR-δ locus suggested that some of them may correspond to the translocated gene segment. Actually, the Dδ1/2 rearranged fragment was identical in size to the *tal*-1 abnormal one. Similarly, the size of the Jδ3 additional fragment was identical to the other *tal*-1 rearranged fragment. These data were consistent with a V-Jδ1 assembly on one allele and the translocation-associated rearrangement affecting Dδ1/2 and Jδ3 on the other allele.

To confirm the data deducted from the Southern blot analysis, we cloned the potential chromosomal breakpoints by PCR. The nucleotide (nt) sequence of the 0.6 PstI fragment was determined to select two oligonucleotides on each side
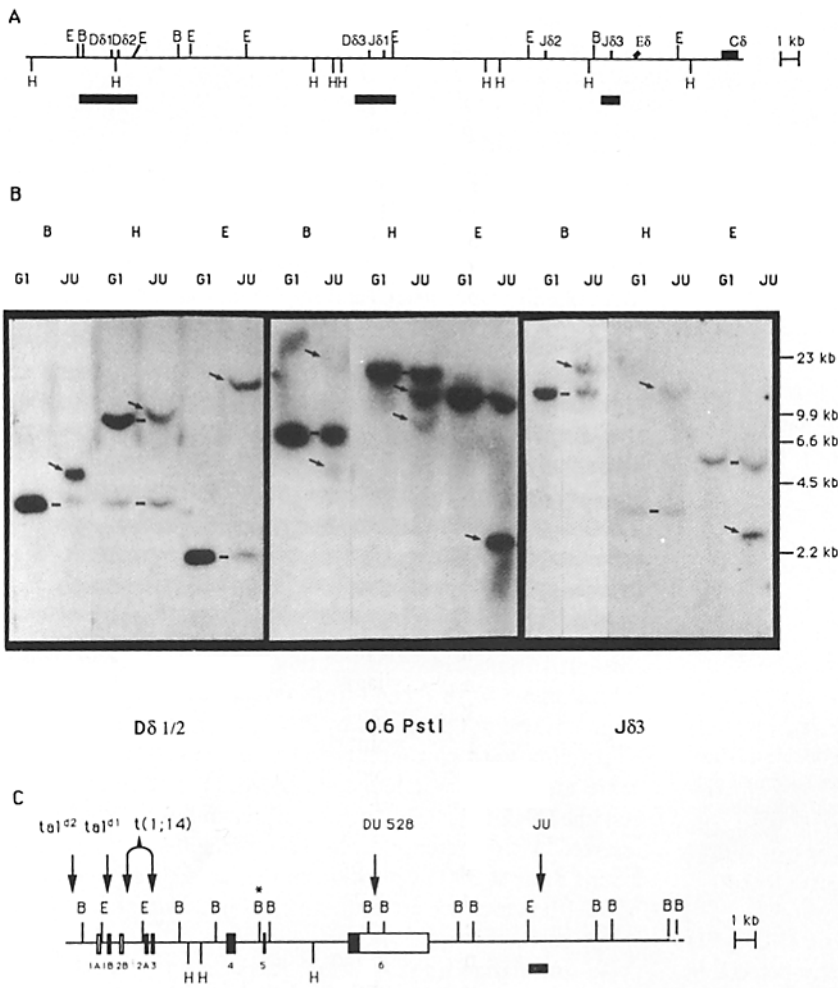
Figure 1. Characterization of the t(1;14) translocation in JU T-ALL. (*A*) Schematic map of the TCR-α/δ locus at 14q11. The location of the probes is underlined. (*B*) Southern blot analysis of JU blast DNA and germline control (HEL cell line DNA) digested by EcoRI (*E*), HindIII (*H*), BamHI (*B*), and PstI (*Ps*) hybridized with indicated probe. (▬) Germline fragments. (→) Rearranged fragments. (*Left*) Hybridization with Dδ1/2 genomic probe. This probe detects abnormal fragments in JU leukemic cell DNA. Submolar germline fragments are certainly derived from nonleukemic cells present in the blood sample. The Dδ1/2 probe detects two germline fragments because it possesses an HindIII internal site. (*Middle*) Hybridization with the 0.6-kb PstI probe. In each digest, this probe detected two abnormal fragments in addition to germline chromosome 1 fragments. Note that hybridization intensity of the rearranged fragments corresponding to the 14q⁻ junction is very weak because the breakage occurred at 60 nucleotides from the edge of the probe. This fragment is not detectable in the EcoRI digest. (*Right*) Hybridization with the Jδ3 probe. Germline fragments are revealed in each lane. Rearranged fragments were also detected in DNA from JU leukemic cells. (*C*) Restriction map of the *tal*-1 locus. (↓) Location of tal^d and t(1;14) breakpoints. The location of the 0.6 PstI probe is underlined.

of the putative breakpoint (JU5′ and JU3′). They were used in PCR experiments together with specific Dδ2 or Jδ3 oligonucleotides. Amplified fragments were size selected, cloned in T vectors (25, 26), and sequenced. Nucleotide sequences of both t(1;14) junctions were compared with their germline counterparts (Fig. 2).

The alignments indicated that the breaks on chromosome 14 had occurred at the recombination signals, respectively 3′ of the Dδ2 segment and 5′ of the Jδ3 segment. They also revealed the loss of one nucleotide from chromosome 1 and the presence of nucleotide stretches at the translocation junctions (30 nucleotides at the der(1) junction and 2 nucleotides at the der(14) junction), which were derived from neither chromosome 1 nor 14. This situation is reminiscent of N insertion associated with normal V(D)J assembly (27). Altogether, these data implicated the recombinase complex in the mechanism of the translocation.

Initiation of tal-1 Transcription within Exon 4 Specifically Occurs in Leukemic T Cells. Under normal conditions, at least five *tal*-1 mRNA species result from two distinct promoters (IA and IB), differential splicing, and alternative exon usage. Two main transcripts can be distinguished with respect to their coding capacity, depending upon the presence or absence of exon 4. These two types encode either a 40- or a 25-kD pro-

tein, which differ from each other by their NH₂ terminus (9, 15, 28).

The expression of *tal*-1 in leukemic T cells bearing a t(1;14) translocation has been documented in only two cases: patient Kd (2) and the DU 528 cell line (1, 2). In the case of Kd, in which the breakpoint was mapped at the 5′ part of exon 3, Northern blot analysis revealed a 5-kb species, but was not informative with respect to transcription start site. In DU 528 cells, in which the translocation breakpoint is located within the last exon, a transcript is initiated within exon 4 and contains TCR-δ sequences in its 3′ part, thus leading to a truncated hybrid mRNA (17). We wondered whether the use of this cryptic promoter internal to exon 4 was found in other malignant cells. For that purpose, we designed a RNase protection assay that discriminates between transcripts containing the totality of the fourth exon, and transcripts initiated within exon 4.

RNase assay was performed using a series of RNAs extracted from T-ALL patients and established T cell lines (*a*) associated with t(1;14) translocation (patients: Kd, JU, and DU 528 cell line); (*b*) associated with tal^d (patients Leb, 7, and 414, and cell lines HSB-2, RPMI 8402, and CCRF-CEM); and (*c*) devoid of apparent *tal*-1 genomic alteration (patients Buf., 109, and Vil., and cell lines Rex and Jurkat). The
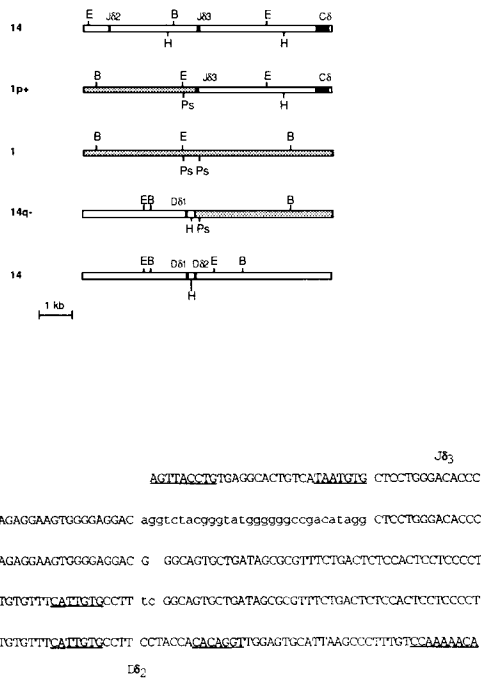
Figure 2. Molecular cloning of t(1;14) in JU leukemic cells. (*Top*) Partial restriction map of germline and translocated alleles of JU. Not all sites are shown. (*Bottom*) Comparison of nucleotide sequences encompassing translocation breakpoints and normal counterparts. 14q11 germline sequences are from references 35 and 36. Recombination signals are underlined. N nucleotides are in lowercase letters. These sequence data are available from EMBL/GenBank/DDBJ under accession number X67500.

erythroid (K562 and HEL) and megakaryocytic (MEG-01) leukemic cell lines, which are also devoid of any detectable *tal*-1 genomic abnormality, were included as controls, since we have previously shown that they express high levels of

tal-1 transcript (Mouthon, M.-A., O. Bernard, M.-T. Mitjavila, P.-H. Romeo, W. Vainchenker, and D. Mathieu-Mahul, manuscript submitted for publication).

From the data presented on Fig. 3, we distinguish three different situations: first, cases where a unique fragment of 413 nt is protected, which corresponds to transcription of the entire exon 4 (plus exons 5 and 6). This situation is found in the three cell lines MEG-01, HEL, and K562, and in tal^d-bearing cells. Second, cases where a faster migrating fragment of 380 nt is protected and the 413-nt fragment is missing, as in DU 528 cells and in JU sample. Finally, both 413- and 380-nt fragments may be present simultaneously, as observed in the case of T-ALL patients Buf, 109, and Kd, and in Rex and Jurkat T cell lines. According to previous studies on DU 528 cells (9, 16), we interpret the protection of the 380-nt fragment as reflecting the initiation of transcription within exon 4, whereas the protection of the 413 nt fragment, is considered to reflect regular transcriptional initiation of un-rearranged *tal*-1 gene, since it is observed in normal bone marrow. It should be noted that the 380-nt fragment was not detected in the control myeloid cell lines. To test whether this initiation could occur in other cell types, we performed RNase assays with RNAs from several origins. The 380-nt fragment was not protected by RNAs from leukemic samples of patients with chronic myelogenous leukemia (CML) or acute megakaryocytic leukemia (AML-7), nor from a preB cell line (Nalm 6). Regular *tal*-1 expression in these cells was asserted by the presence of the 413-nt fragment. We noticed a faint band at 380 nt position in the lane corresponding to MEG-01, which we attribute to the high level of *tal*-1 transcription in this cell line.

To summarize, the initiation of transcription within the fourth exon was detected only in leukemic T cells. In addition, this initiation is the only one detected in those DU
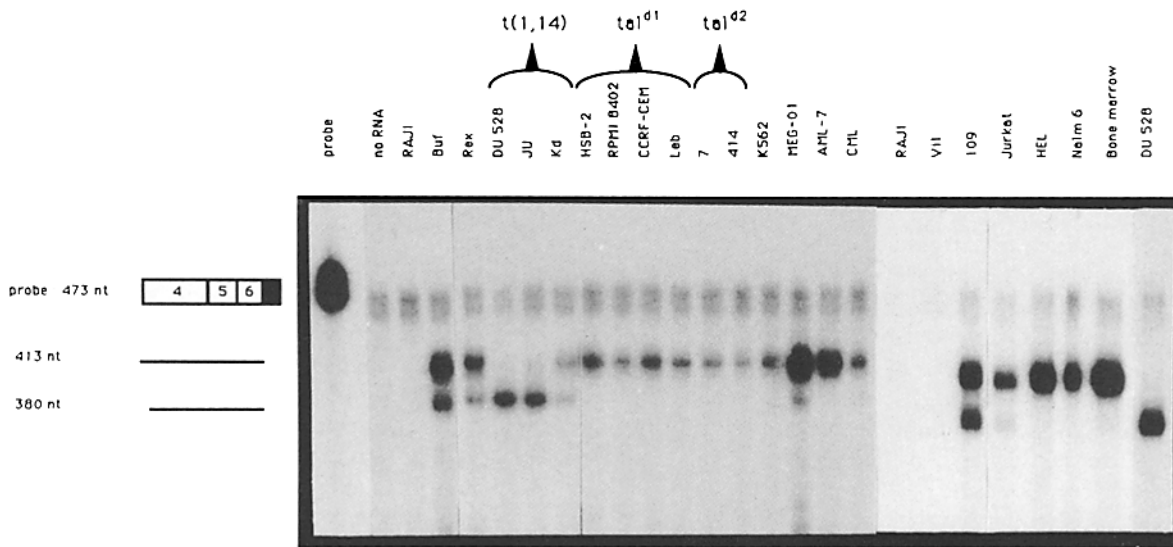

Figure 3. RNase protection experiments. The probe is a 413-nt cDNA fragment obtained after ExoIII digestion and contains a part of exons 4, 5, and 6 up to SacI site. (*Black box*) Vector polylinker sequences. A fragment of 413 nt, representing a full-length protection is seen in most samples. With the exception of tal^d-bearing cells, all lanes corresponding to T cell lines or T-ALL samples show a ∼380-nt fragment representing protection of ∼200 nt of the 3' part of exon 4.

528 and JU cells that both harbor t(1;14) chromosomal breakpoints 3' of *tal*-1.

In contrast, no transcript starting from this promoter was detected in any of tal[d] leukemic cells tested. Aplan et al. (12) isolated in the tal[d1]- bearing HSB-2 cell line a fusion transcript initiated at *SIL* exon 1 and spliced to *tal*-1 exon 3. To compare the nature of *tal*-1 transcripts between tal[d1]- and tal[d2]-bearing cells, anchored PCR technique was performed on tal[d1] CCRF-CEM RNA and tal[d2] T-ALL RNA (Fig. 4). By using oligonucleotides corresponding to *tal*-1 exons 5 and 6 (Fig. 4), two types of cDNAs were identified in both samples. The first type contained *SIL* exon 1 and *tal*-1 exons 3, 5, and 6. The second type also contained *SIL* exon 1 plus a part of *SIL* intron 1. In this case, a downstream donor site was used to directly splice *SIL* sequences to *tal*-1 exon 5, then spliced to exon 6. No cDNA containing exon 4 was isolated. The discrepancy between these data and those of RNase assays presented above is presumably due to the difficulty of reverse-transcribing and amplifying the CG-rich region appearing within exon 4.

To confirm the presence of fusion messages in other tal[d]-bearing cells, bi-specific PCR experiments were performed using primers corresponding to *SIL* exon 1 and *tal*-1 exon 3 or 6. One tal[d1] and two tal[d2] patients were found to express both types of transcripts.
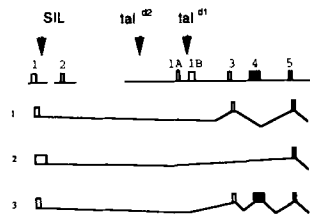
SIL genomic sequence
TGCAAACAGACCTCAGCTCCGCGGAAGTTGCG/GTAAGTGGAGCTTTGTGC
TCCTGGTTTGTGCTCCTGGCCCCTTGAGAGGCGGCAGCGGGGCCCTGGGA
AGGTTGGTGGGTAACATTCAAAGCCCTGTAGTGGGTTCCGCCCTCCAGGA
GCCTGGAGCAGATGACGAGAAGGGGAGCTAGTGGGAGAAATTAAGCAGTC
CATGAAATCCTTGGGTATCTGAGCTAAG/GTATGTGAAAGAGGTTTGCAGT
CGATAACGTGCCATTTAAA

transcript 1 : SIL ex. 1 splice 5'/tal-1 ex. 3/ Ex. 5
TGCAAACAGACCTCAGCTCCGCGGAAGTTGCG/ATCGCCCAGGACCACACCG
CAGCGTAACTGCAGGCCTCTCAGCGAAAAAGGGGGAAAGCAAAGACCCGGGT
GTGCATCCTCTTCCTCGGCTTCCGCCCCTTTCCGGCGGAGTGGAGATCCTAT
TCAGAGGGGCCGGTCTCTCTAAATATGCCCCAG/ Ex. 4

transcript 2 : SIL ex. 1 splice 3'/tal-1 Ex. 5
TGCAAACAGACCTCAGCTCCGCGGAAGTTGCGGTAAGTGGAGCTTTGTGC
TCCTGGTTTGTGCTCCTGGCCCCTTGAGAGGCGGCAGCGGGGCCCTGGGA
AGGTTGGTGGGTAACATTCAAAGCCCTGTAGTGGGTTCCGCCCTCCAGGA
GCCTGGAGCAGATGACGAGAAGGGGAGCTAGTGGGAGAAATTAAGCAGTC
CATGAAATCCTTGGGTATCTGAGCTAAG/CGGGTTCTTTGGGGAGCCGGAT
GCCTTCCCTATGTTCACCACCAACAATCGAGTGAAGAGGAGACCTTCCCC
CTATGAGATGGAGATTACTGATGGT/

Figure 4. *tal*-1 transcription in tal[d]-bearing cells. (*Top*) Schematic drawing (not to scale) of *SIL·tal-1* fusion transcripts. (▼) Deletion breakpoints. (*Bottom*) Nucleotide sequence of genomic *SIL* exon I/intron I (10) and of representative cDNA amplified from tal[d]-bearing cells. The *SIL* oligonucleotide is underlined. Splice sites are marked.

Discussion

We have analyzed a new case of t(1;14)(p32;q11) in a T-ALL patient (JU). In agreement with cytogenetic observation, the translocation breakpoints were localized within the TCR-δ gene, on band 14q11, and in the vicinity of the *tal*-1 gene on band 1p32. Involvement of Dδ2 and Jδ3 segments and nucleotide sequence comparison of chromosomal junctions to their germline counterparts argued in favor of a recombinase mediated translocation process.

To date, most of t(1;14) breakpoints affect the 5' side of *tal*-1 and are clustered within 1 kb. In the JU case, the translocation breakpoint was located 5.5 kb downstream of the *tal*-1 polyadenylation site. In the four cases currently described which affect the 3' side of *tal*-1 (three t(1;14) and one t(1;7) translocations), the sites of breakage appear to be quite dispersed since they are spread over 40 kb, from within the *tal*-1 transcription unit (DU 528) to 35 kb downstream of the gene (19).

Since the translocation breakpoints are located either upstream or downstream of the *tal*-1 gene, it was of evident interest to examine the possible consequences of both situations on its transcription. Previous studies indicated that in the DU 528 cell line, the *tal*-1 transcription start site was located within exon 4, whereas no transcript was found to be initiated at the regular promoters 1A or 1B (9, 15). It is interesting that an identical situation was observed in the case of JU leukemic cells. On the other hand, in Kd leukemic cells in which the t(1;14) break is located in the 5' part of the gene (9), our data indicated that transcription initiation can occur simultaneously within the fourth exon of *tal*-1 and from an upstream promoter. In view of the position of the translocation breakpoint, the promotion may occur either in the *tal*-1 exon 3/intron 3, or more probably, within the Dδ region.

Transposition of TCR-δ regulatory elements in the vicinity of the *tal*-1 gene by the t(1;14) translocation, is supposed to influence *tal*-1 transcription, as it is initiated at the peculiar promoter located in exon 4. When breakage occurs 3' of *tal*-1, the direct juxtaposition of the TCR-δ enhancer (29, 30) to *tal*-1 sequences appears to dictate the transcription to start exclusively at this internal promoter. When breakage affects the 5' part of *tal*-1, a dual promotion (within exon 4 and upstream) is observed and is probably due to the presence of as yet uncharacterized Dδ regulation elements. By analogy with the situation described for the TCR-β and IgH genes (31, 32), the upstream initiated transcript may start within the Dδ region and go on through the *tal*-1 sequences. In this respect, it is worth emphasizing that most chromosomal translocations involving the TCR-δ gene, result in the relocation of the Dδ region to the 5' side of putative oncogenes frequently associated with truncation of the gene (33).

Transcriptional initiation within exon 4 is also observed in T-ALL samples without evident *tal*-1 gene alteration. Thus, this peculiar initiation is not linked to the translocation itself, but rather reflects the activation of a functional promoter. Two observations suggest that the use of this exon 4 promoter is restricted to T cell lineage. First, in a survey of he-

matopoietic cell lines and leukemic cell samples, this promotion within exon 4 was observed only in malignant T cells. Second, we did not detect the utilization of this promoter in normal bone marrow. The most 5' AUGs (corresponding to no. 3 and no. 4 in the large open reading frame (ORF) [9, 16]) present in this mRNA species were found to be weak translation initiation codons in in vitro experiments (9). In this respect, the translation should start at the third AUG (corresponding to no. 5 in the large ORF) and produce the 25-kD protein (9, 16, 28).

It is notable that the exon 4 promoter activity was not found in any tal^d leukemic samples. Indeed, in both types of deletions (tal^{d1} and tal^{d2}), tal-1 sequences are expressed through fusion transcripts initiated at the SIL gene promoter. To explain the lack of exon 4 promoter usage in tal^d leukemic cells, one can speculate that the tal-1 protein translated from SIL-tal-1 mRNA acts negatively onto this promoter by a feedback mechanism, and that SIL regulatory elements are unable to cis-activate this tal-1 promoter.

In the course of the analysis of the mRNA species associated with both tal^d, we identified at least two types of fusion transcripts. The first one fuses SIL exon 1 sequences to tal-1 exon 3, and the second one splices the first intron of SIL directly to tal-1 exon 5. In no case, is an in-frame AUG codon introduced upstream of the tal-1 reading frame, which rules

out translation of a fusion protein. Therefore, although tal^{d2} does not directly affect the tal-1 transcriptional unit as does tal^{d1}, both have similar effects on tal-1 expression. These results confirm those recently published by Aplan et al. (11).

Throughout this study, we characterized tal-1 expression in some T cell lines and T-ALL samples without any obvious tal-1 genomic rearrangement. In these cases, transcriptional initiation was shown to involve both exon 4 promoter (this paper) and promoter 1B, and, in one case of T-ALL, also promoter 1A (data not shown).

In conclusion, it appears that the exon 4 promoter is specifically used in T-ALLs with t(1;14) translocation and T-ALLs without evident tal-1 genomic rearrangements. In tal^d T-ALLs, the transcription of tal-1 is under the dependence of the modified SIL gene.

Up to now, tal-1 expression has not been detected in normal T cells. However, the possible involvement of the lymphoid recombinase in the genesis of t(1;14) and tal^d in T-ALLs, suggests that these illegitimate recombinations occur during early T cell development. The association of tal^d occurrence in T-ALLs with the commitment to the TCR-α/β lineage supports this hypothesis (34). In that respect, one can speculate that tal-1 expression occurs at some specific stages during thymic ontogeny.

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