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Genetics

Chromosome walking on the *TCL1* locus involved in T-cell neoplasia

(chromosome translocations/oncogene activation/human chromosome 14 band q32.1)

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ABSTRACT **The *TCL1* locus on chromosome 14 band q32.1 is frequently involved in the chromosomal translocations and inversions with the T-cell receptor genes observed in several T-cell tumors, including T-prolymphocytic leukemias, acute and chronic leukemias associated with the immunodeficiency syndrome ataxia–telangiectasia, and adult T-cell leukemia. All breakpoints cloned in this area have been mapped to 14q32.1, an area distant ≈10,000 kb from the immunoglobulin heavy-chain gene locus on chromosome 14q band 32.3. Except for two cases of inversion, no physical linkage of the cloned breakpoints has been reported, nor has a gene been identified in this region. Taking advantage of chromosome-walking techniques and of the P1 phage, we cloned and characterized 450 kb of the germ-line *TCL1* locus, starting from the breakpoints of two independent T-cell leukemias. We show that all molecular rearrangements characterized so far map to these clones, indicating not only that this region is the target of chromosomal rearrangements occurring in this area but also that both inversion and translocations occur within a 300-kb region in the T-cell leukemias. In the attempt to identify a candidate oncogene responsible for the malignant transformation, a CpG island centromeric to the inversions and to the translocations has been identified. Two probes near the CpG island have detected sequences conserved among species, as well as two transcripts in the K562 human erythroleukemia cell line. On the basis of these data, a model of activation of the putative *TCL1* oncogene is suggested.**

Specific chromosomal translocations or inversions are associated with several types of human tumors, especially of the hematopoietic lineage. These chromosomal rearrangements can lead to gene fusion resulting in chimeric oncoproteins, as is observed in the majority of the tumors involving the myeloid lineage, and sometimes lymphocytes: good examples of these are the t(9;22) translocation of chronic myelogenous leukemia and Philadelphia chromosome-positive (Ph^+) acute lymphocytic leukemia (ALL) and the t(1;19), t(15;17), t(6;9), and t(4;11) translocations observed in pre-B, myeloid, and mixed-lineage leukemias, all of which generate chimeric transcripts (1–4). Alternatively, chromosomal rearrangements can lead to deregulation of protooncogenes by their juxtaposition to a regulatory element active in the hematopoietic cells. This is the case observed in many types of translocations occurring in the lymphocytic lineage and involving the immunoglobulin (Ig) or T-cell antigen receptor (TCR) loci translocated to the vicinity of genes such as *MYC*, *BCL2*, *TCL2*, *BCL1*, or *TCL3* (for a review see refs. 5 and 6). In this latter case the gene juxtaposed to the Ig or TCR locus can be quite close to (within 10–30 kb) or rather far from (a few hundred kilobases) the genomic breakpoint. *BCL2* on chromosome 18q21, *BCL3* on chromosome 19q13.1, *MYC* on 8q24 in sporadic cases of Burkitt lymphoma and *TCL5*/SCL/TAL on chromosome 1p32 are examples of protooncogenes translocated to within 30 kb of the Ig or TCR activating locus. *BCL1* on chromosome 11q13 and *MYC*, in the endemic cases of Burkitt lymphoma, are examples for which the protooncogene could be separated by few hundred kilobases from the genomic breakpoint. In either case the deregulation of the (onco)gene involved is probably due to the translocation of control elements of the Ig or TCR genes near the new sequences.

In T-cell tumors, these chromosomal translocations are usually a consequence of the faulty joining of genes during the physiological process leading to VDJ recombination of the TCR genes located on chromosomes 14q11 (*TCRA/D*), 7q35 (*TCRB*), and 7p15 (*TCRG*). This aberrant joining leads to rearrangements with chromosomes 8q24 (*MYC*), 14q32.1 (*TCL1*), 11p15 (*TCL2*/Rhombotin-1/Ttg-1), and 10q23 (*TCL3*/Hox11). Chromosome region 14q32.1 is commonly involved in chromosomal rearrangements in several T-cell neoplasms. These rearrangements take the form of inv(14)(q11q32) inversion or t(14;14)(q11;q32) or t(7;14)(q35;q32) translocations and are observed in T-prolymphocytic leukemia (T-PLL), in chronic and acute T-cell leukemias arising in patients with the immunodeficiency syndrome ataxia–telangiectasia (AT), and in nonmalignant clonal expansion of T cells of patients with AT (for review, see ref. 7). Similar chromosomal alterations have been reported in 28% of adult T-cell leukemia (ATL) associated with infection by human T-lymphotropic virus type I (8). We and others have cloned and mapped several chromosomal translocations in the *TCL1* locus (9–14). However, only two of these had been physically linked (15) and no gene has been identified at this locus. Difficulty in finding the affected gene could be due to the complexity of the chromosomal rearrangements, which include inversions, translocations, and, in some well-documented cases, inversions with duplications of the end of chromosome 14q32, all involving the same loci on 14q11 and on 14q32.1. We have undertaken chromosome walking within the 14q32.1 region with the aim of defining the organization and the physical linkage of the reported breakpoints, a necessary step in the identification of the candidate *TCL1* gene responsible for malignant transformation in the specific neoplasia.

MATERIALS AND METHODS

Construction and Screening of Human P1 and Cosmid Libraries. The P1 bacteriophage library was constructed with a commercial kit (DuPont/NEN). Human placental DNA was partially digested with *Sau*3A1 to an average size of

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Abbreviations: ALL, acute lymphocytic leukemia; CLL, chronic lymphocytic leukemia; PLL, prolymphocytic leukemia; AT, ataxia–telangiectasia; TCR, T-cell antigen receptor.

80–100 kb and ligated into the *Bam*HI restriction site of the tetracycline-resistance gene in the vector pNS582tet14Ad10 (16). The DNA was then packaged and plated (≈5 × 10^3 colonies per plate) on *Escherichia coli* NS3145 on Luria–Bertani agar with kanamycin (50 μg/ml). Approximately 7–10 × 10^4 colonies were obtained and amplified by individual plate (17). Screening was performed either by standard colony hybridization with the probes of interest or by PCR amplification with the intent to minimize the manipulations and the long-term storage problems of recombinant libraries. Briefly, each primary plate was separately amplified and stored in 20% (vol/vol) glycerol at −80°C. An aliquot of each stock was later inoculated and grown in 5 ml of Luria–Bertani broth supplemented with kanamycin. DNA was extracted and used as template for PCR-based screening. At each screening PCR was carried out on the 20 different DNA pools with the appropriate primers. After identification of positive pools, only the sublibraries of interest were plated and screened by colony hybridization with the required probe. This approach allowed us to overcome some of the shortcomings engendered by the low copy number of the P1 phage and by repeat screening of amplified libraries. The cosmid library was constructed from human placental DNA and screened according to Isobe *et al.* (18).

Standard DNA Methods. DNA and RNA from various sources were prepared and processed for restriction digestion, Southern blotting, Northern blotting, sequencing, and PCR (17, 19). Sequences were analyzed with the Wisconsin Genetics Computer Group software (20) and with the commercial software DNASIS (Hitachi).

Oligonucleotide Primers and Probes. Oligodeoxynucleotide primers used for PCR on the P1 library were MT5′ and MT3′ (5′-CTGCCTCATTGGCCTTCCAA-3′ and 5′-GACAAGAAGTGCTGGGATTC-3′), from sequence adjacent to the breakpoint in patient MP (13); 21-2HH1/5′ and 21-2HH1/3′ (5′-TTGACATGATATAATGGCGG-3′ and 5′-CAGTTCCTAGGTACGTGAC-3′), from the left-end sequence of clone 21-2; 5-2/5′ and 5-2/3′ (5′-TGTAGGGATTGTGCTTCAGC-3′ and 5′-TACTATGGGTGCTGCCCATC-3′), from the right-end sequence of clone 5-2; 9-1KK/5′ and 9-1KK/3′ (5′-CTACACCAGGGAGGGGCA-3′ and 5′-TGCCACCTCCTACACCAT-3′), from internal sequences of clone 9-1; pLC1/5′ and pLC1/3′ (5′-AACTCTGAAGTTCTCCTT-3′ and 5′-GGGCAGAGGCTGAGACTT-3′), from the left-end sequence of cosmid clone pLC1; and 25-5/5′ and 25-5/3′ (5′-GCAGGACAAGGCGGACTA-3′ and 5′-AGATTCACTCCCGCCAAG-3′) from the left-end sequence of clone 25-5.

Oligonucleotides used from previously cloned sequences of T-cell malignancies involving the 14q32.1 area were IKImer (5′-ATTTACAAATTGCATCTGAG-3′), from a T-cell chronic lymphocytic leukemia (T-CLL) of the AT patient PH (12); PT5mer (5′-TGCAGAAAGGAAACTGGGTA-3′), from a T-PLL of the patient Pt (9); AT5B1mer (5′-TCTCTTACACCAAACAGTCATCA-3′), from a T-CLL of the patient AT5B1 (15); and IKIImer (5′-AGGTCACCTGAACTAAGGAGGGA-3′), from the cell line NL of a patient with T-CLL (14). Probe B0.9 and pE/S have been described (11, 13) and are adjacent respectively to the t(14;14) of MP and to the t(7;14) of ALL320 on chromosome 14q32.1. The *Sal* I–*Nru* I (325 bp) and *Bam*HI–*Eco*RV (188 bp) fragments of pBR322, flanking the bacteriophage P1 *Bam*HI cloning site, were used to detect left or right ends of newly isolated P1 human clones.

RESULTS

Cloning of the Genomic *TCL1* locus. To obtain a complete physical map of the 14q32.1 area, chromosome walking was started from two sites (Fig. 1) previously characterized by us (11, 13): the breakpoint of a t(7;14)(q35;q32) translocation of a T-ALL patient with AT, ALL320, and the breakpoint of a t(14;14)(q11;q32) translocation of another T-cell leukemia patient with AT, MP. Primers and probes (see *Materials and Methods* and Fig. 1) derived from sequences adjacent to the two breakpoints were used to screen the human P1 genomic library. Two positive clones were isolated in the first round of walking, 21-2 (insert size, 75 kb) and 5-2 (83 kb), containing germ-line sequence on 14q32.1. Restriction enzyme maps of the two clones are shown in Fig. 1.

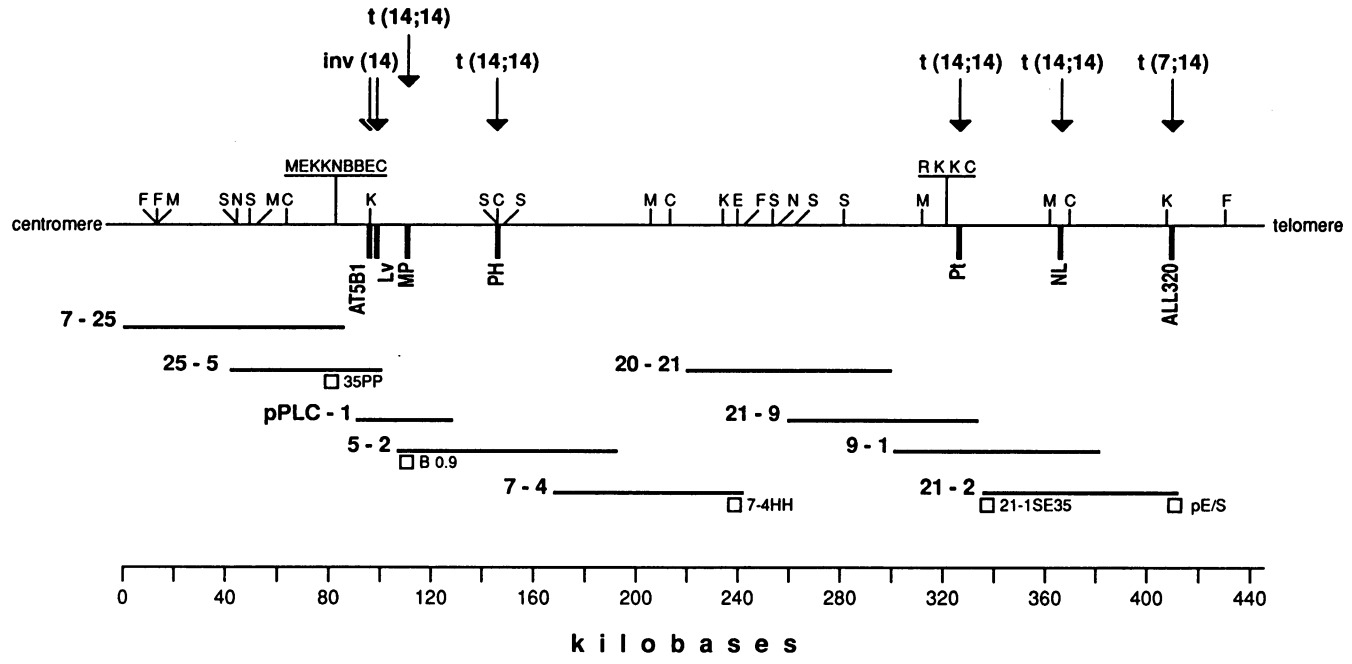

FIG. 1. Genomic organization of the *TCL1* locus on chromosome 14q32.1. Restriction sites are given for *Bss*HII (B), *Cla* I (C), *Eag* I (E), *Sfi* I (F), *Ksp* I (K), *Mlu* I (M), *Not* I (N), *Nru* I (R), and *Sal* I (S). Ranges covered by isolated P1 clones or cosmid clone pLC1 shown by horizontal bars. Arrows and vertical bars illustrate the position of chromosome breakpoints described in the text. Open boxes represent probes (described in the text) used for isolation of P1 clones and for hybridization.

Probes 21-2SE35, and pE/S, corresponding to the left and right ends of 21-2, respectively, failed to hybridize to clone 5-2 (Fig. 1), indicating that the two P1 clones were not overlapping (data not shown). *In situ* hybridization, performed with the P1 clone 21-2 on the somatic cell hybrid 513AC3B10, which contains a 14q+ from patient MP, showed three separate hybridization signals, two close to the translocation breakpoint and one at a very telomeric site (data not shown). This result is consistent with the presence of an inversion with duplication of the telomere of the 14q+ chromosome, as already observed in this and similar cases (13, 21, 22), suggesting that the orientation of the two probes on the chromosome is the following: centromere–B0.9–pE/S–telomere.

To join the gap between the two clones, a chromosome walk was performed. To generate new probes for the next round of screening, the sequences at the ends of the two human inserts were identified by hybridization with probes flanking the cloning site. For this purpose we used on one side the pBR322 *Sal* I–*Nru* I fragment and on the opposite side of the pBR322 *Bam*HI–*Eco*RV fragment. The end fragments, containing vector and human sequences, were then subcloned, mapped, and sequenced to generate new germ-line 14q32.1 primers and probes. The cloning proceeded until two overlapping P1 clones coming from opposite directions (7-4 and 20-21) were found. These results were confirmed by hybridization of the probe 7-4HH (Fig. 1) to DNA from clone 20-21, indicating that the gap between the two breakpoints had been covered (data not shown). Since no overlapping clone was found for one region by using a probe from clone 5-2, an additional cosmid library was screened. Clone pLC1 was isolated from this library (Fig. 1).

A total of 12 clones were isolated that cover an area of 420 kb (Fig. 1). The resulting physical map demonstrated that the two breakpoints used as starting sites are 300 kb apart, confirming the involvement of a large area in the chromosome 14q32.1 translocations.

We had reported (13) that the two probes pE/S and B0.9 might be contained within a 250-kb *Sfi* I restriction fragment. However, cloning data indicate that these two probes are on different *Sfi* I fragments of ≈240 kb (B0.9) and ≈215 kb (pE/S).

To ascertain that no interchromosomal rearrangements had taken place during the cloning process, the primers employed for chromosome walking were also tested for amplification on DNA derived from a rodent–human somatic cell hybrid, 52-63C17, which contains a single human chromosome 14q+ (14pter–14q32.1::Xq13–xqter) (11). Every primer pair tested was found to amplify the expected fragment from this DNA, indicating all the clones were isolated from the correct region of chromosome 14.

Mapping of Four Additional Breakpoints. To determine whether the cloned area was the target of other breakpoints observed in similar T-cell neoplasms, sequences from other cloned 14q32.1 rearrangements, whose relationship to our MP and ALL320 breakpoint had not been studied, were hybridized to the germ-line P1 and cosmid clones. Oligonucleotides derived from sequences adjacent to the breakpoints of three t(14;14) translocations, Pt, PH, and NL, were synthesized and hybridized to the P1 clones. Pt mapped to clone 9-1 about 80 kb centromeric to pE/S, whereas PH mapped to clone 5-2 about 25 kb telomeric to B0.9 and NL on clone 21-2 about 45 kb centromeric to pE/S. The results of the mapping and hybridizations are summarized in Figs. 1 and 2.

Further, it was of interest to ascertain whether the breakpoints of reported cases of inv(14)(q11;q32) physically mapped to the same genomic region. In fact, inv(14) chromosomal rearrangements have been observed in several patients with T-CLL, T-PLL, and T-cell lymphoma and their

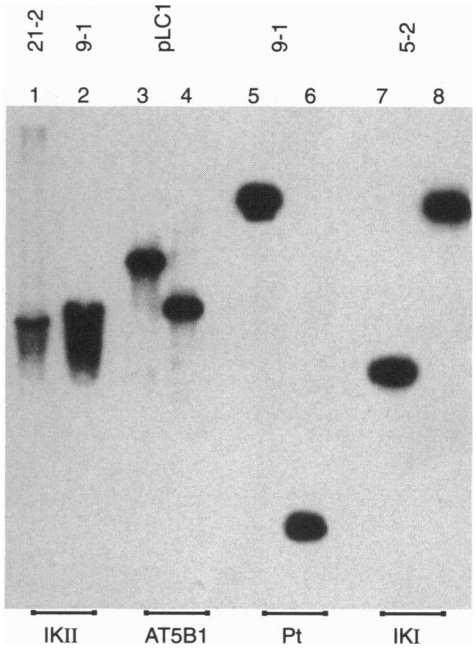

FIG. 2. Localization by Southern blotting of chromosomal breakpoints on the P1 and cosmid clones. P1 or cosmid clone used is indicated at the top, and the oligonucleotide used as probe (see *Materials and Methods*) is indicated at the bottom.

breakpoints cytogenetically and molecularly mapped to band 14q32.1 (7, 9, 15, 23, 24). For this purpose the oligonucleotide AT5B1, originating from the inv(14) AT5B1, was hybridized to a filter containing DNA of the P1 clones and of the cosmid clone pLC1. No hybridization was observed with DNA from the P1 clones, but the probe hybridized to the cosmid clone pLC1 (Fig. 2). In fact, restriction enzyme fine mapping allowed us to locate this sequence 7 kb centromeric to B0.9. This inversion had also been reported to be only 2.1 kb from a second one, Lv (15). These results indicate that the chromosomal rearrangements t(14;14) and inv(14) involve the relatively small (500-kb) region of 14q32.1, which we have now cloned in its entirety and for which we have determined the relationship of six tumor-specific breakpoints.

Identification of a CpG Island and Two Transcripts. A restriction map of all the clones was established with rare-cutter enzymes (Fig. 1) and revealed the presence of a CpG island. The area adjacent to the CpG island was investigated for the presence of transcribed sequences, since C+G-rich regions occur at the 5′ end of genes (25). A *Pst* I–*Pst* I segment of 5.5 kb (35PP, Fig. 1), spanning the CpG island, was subcloned and two probes were isolated: EN1.6 (*Eco*RI–*Not* I fragment of 1.6 kb) and EE2.2 (*Pst* I–*Eco*RI fragment of 2.2 kb). To check for the presence of conserved sequences, the two probes were hybridized to Southern blots of DNA from various species. The EN1.6 probe showed strong cross hybridization with chicken and pig DNA (Fig. 3*a*), whereas the EE2.2 probe did not. However, upon hybridization of the two probes to Northern blots, the EE2.2 probe gave two bands, at 1.8 kb and 0.5 kb (Fig. 3*b*), but no hybridization was observed with EN1.6. The signals with the EE2.2 probe were most evident with K562 erythroleukemia cell RNA, which showed strong hybridization of both bands. The lower band was evident almost ubiquitously in the RNAs tested, including samples from various human cell lines (data not shown), whereas the upper band was not readily visible in RNA from other sources.

The CpG island is centromeric to all the breakpoints. If the mechanism of activation of a putative *TCL1* gene is the same as observed in similar rearrangements observed in leukemia

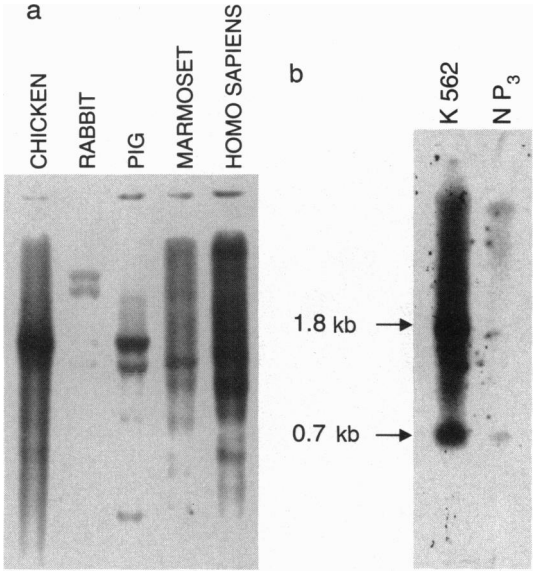

FIG. 3. (*a*) Southern blot hybridization of the probe EN1.6 flanking the CpG island to *Eco*RI-cleaved genomic DNAs (10 μg per lane) of various species. (*b*) Hybridization of probe EE2.2 flanking the CpG island to poly(A)$^+$ RNA (5 μg per lane) of the human leukemic cell line K562 and of the mouse myeloma cell line NP3.

and lymphoma, the affected gene would be expected to be telomeric to the breaks in the cases of inverted chromosomes 14. Further molecular investigation is needed to ascertain the role and significance of these transcripts.

DISCUSSION

Molecular cloning of several hundred kilobases of germ-line sequences in the 14q32.1 area has allowed further characterization of the *TCL1* locus involved in various T-cell leukemias. Starting from two previously cloned breakpoints and using chromosome-walking techniques with a P1 cloning system, we have cloned and defined a germ-line area of 450 kb on chromosome 14q32.1 that encompasses the two starting clones adjacent to t(14;14)(q11;q32) and t(7;14)(q35;q32) breakpoints occurring in two independent T-cell leukemias of patients with AT. Further, we have shown that several other previously cloned breakpoints also lie within this 450-kb region, indicating that a region of ≈300 kb is the target of the chromosome 14 rearrangements involved in hematopoietic malignancies such as T-PLL, T-CLL, and T-ALL of patients with or without AT. These chromosome rearrangements can take form of inv(14)(q11q32) inversion, or t(14;14)(q11q32) and t(7;14)(q35;q32) translocations, with the common involvement of band 14q32.1. The two chromosome inversions Lv and AT5B1 were reported by Mengle-Gaw *et al.* (15) to lie 2 kb apart. It is evident from our results that the translocations and inversions occur in the same relatively small area, since the two inversions are located only 10 kb from a t(14;14) translocation which occurred in a malignant T cell from an AT patient. This finding strongly suggests that the inversions and translocations occur by the same mechanism and affect the same target gene on 14q32.1, the putative oncogene *TCL1*.

To explain how both the inversions and translocations could activate this candidate oncogene, some considerations must be made with respect to the orientation and activation of the genes involved in these leukemias. The situation could, in our opinion, be similar to the one observed in the chromosomal translocations of Burkitt lymphomas, where activation of the *MYC* locus is due either to a translocation of this oncogene to within the Ig heavy-chain locus on chromosome 14, 5′ of the Ig constant segments (centromere–3′ Ig heavy chain 5′–5′ *MYC* 3′), or to the translocation of the Ig κ or light-chain gene λ on chromosome 8, 3′ to *MYC* (centromere–5′ *MYC* 3′–5′ Ig κ 3′) (26). As a result of these rearrangements, a deregulation of *MYC* ensues consequent to its proximity to control elements (enhancers) that are located 3′ to the constant regions of the Ig loci (5).

An analogous situation can be expected for the chromosomal abnormalities involving the *TCL1* locus. Enhancer elements have been described 4.5 kb and 5.5 kb, respectively, 3′ of the TCR C_α (27) and $C_{\beta 2}$ (28) gene segments. As a consequence of the rearrangements in the *TCL1* locus, either in the inversion or in the translocations, both of these elements are moved to the 14q32.1 area, hence suggesting the activation of the candidate *TCL1* oncogene by their juxtaposition at 14q32.1, resembling the *MYC* model of activation. In fact, all of the rearrangements analyzed in this report involve chromosome segments at 14q11 and 14q32.1. In the 14q11 area they affect the TCR α-chain gene (*TCRA*), which spans an area of ≈80 kb; usually J_α joining segments or pseudosegments (orientation on 14q11 is centromere–V_α–J_α–C_α–telomere; Fig. 4*a*) are directly involved in the chromosome rearrangements. In the 14q32 area the rearrangements involve the 300-kb area described in this work, with an orien-

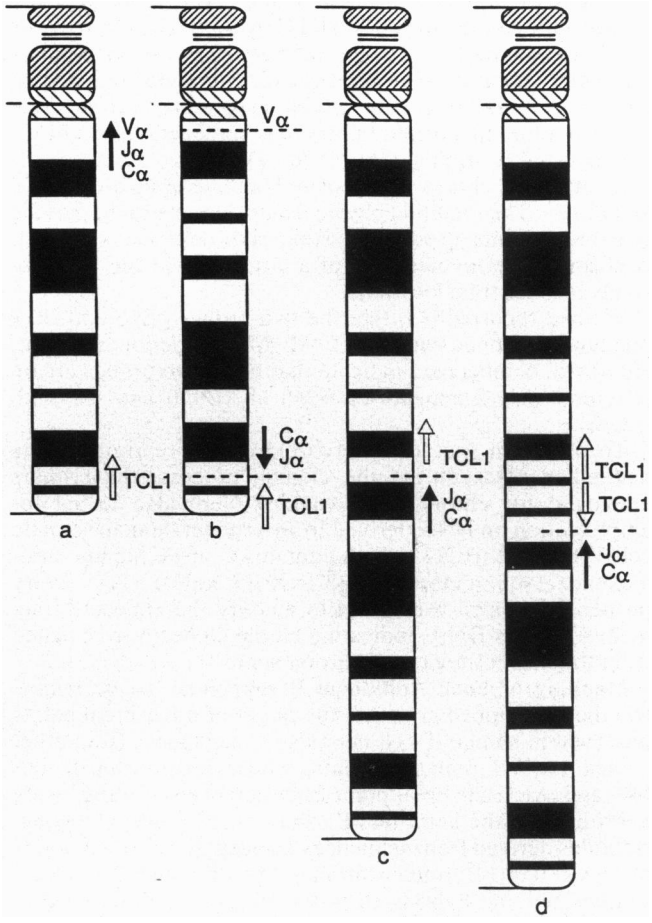

FIG. 4. Schematic representation of the chromosomal rearrangements involving the *TCL1* locus on chromosome 14q32.1 and the TCR α-chain locus (*TCRA*) chromosome 14q11.2. The filled arrow represents the orientation of *TCRA*, and the open arrow represents an arbitrary orientation of *TCL1*. (*a*) Normal chromosome 14. (*b*) An inv(14) chromosomal inversion. (*c*) A t(14;14)(q11;q32) classical chromosomal translocation. (*d*) A t(14;14)(q11;q32) chromosomal translocation with an inverted duplication of 14q32.1–14qter. V_α, J_α, and C_α represent variable, joining, and constant gene segments of *TCRA*.

tation centromere–AT5B1–B0.9–pE/S–telomere (this paper; M.I. and G.R. unpublished work). By taking the physical map and gene orientation into account, the following model of activation can be proposed: an inversion (Fig. 4*b*), as in AT5B1 and Lv, will bring the J_α–C_α region 5′ to the *TCL1* locus, with an orientation of the newly formed locus at 14q32.1 that will be centromere–C_α–J_α–*TCL1*–telomere. A translocation (probably like the one observed in patient Pt) will instead bring the incoming 14q11 sequences 3′ to the *TCL1* locus, in the orientation centromere–*TCL1*–J_α–C_α–telomere (Fig. 4*c*). The situation is complicated by the observation that some of the translocations in this region are accompanied by an inversion with duplication involving the tip of chromosome 14q32, as in the cases of the AT malignant T-cell clones MP and ALL320 (refs. 11, 13, 21; also described in ref. 22 and 29). The duplication event, whose significance, limits, and time of occurrence are not understood, does not affect the position of the break on chromosome 14q32.1. This is demonstrated by the proximity of the two inversions (AT5B1 and Lv) to the MP translocation. Structurally, an inverted duplication (Fig. 4*d*) will behave, in terms of juxtaposition of TCR elements and *TCL1*, as observed for the chromosome inversion cases, suggesting a similar mechanism of activation.

Inverted duplications of the telomeric region of chromosome 14, which may be underestimated due to the difficulty of molecular and cytogenetic demonstration, have been observed uniquely in translocations involving T-cell clones derived from AT and never in primary tumors (22); if the duplication represents a specific feature of AT rearrangements, as suggested by Stern *et al.* (22), perhaps this results from the chromosomal instability underlying the primary defect of AT; alternatively they may be underdetected characteristics of the t(14;14) translocations of non-AT-associated tumors. Clarification of this issue deserves attention, since it has implications for the expected position of the affected *TCL1* gene.

The frequency of these chromosome 14 rearrangements seems to be different in T malignancies associated with AT and T-PLL. Matutes *et al.* (30) reported that in T-PLL the 14 inversions are predominant (19 out of 23 cases examined), whereas in AT malignant cases a much higher percentage (G.R., unpublished observation) are translocations. Also, the clonal rearrangements involving the *TCL1* locus are present in AT patients for a long time before the onset of overt leukemia, whereas T-PLL is a very aggressive disease; however, other cytogenetic abnormalities, such as a trisomy 8, have been reported (30) in T-PLL as well as in the progression of the T-cell clones from AT patients. It is possible, as already suggested by us (13) and others (7), that the *TCL1* rearrangement is the initiating event that could confer a proliferative advantage to the T-cell, and that an additional event(s) is necessary for the development of a frank leukemia.

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