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Identification of a gene, *MLL*, that spans the breakpoint in 11q23 translocations associated with human leukemias

(lymphoid/myeloid leukemias/gene mapping/*in situ* hybridization/transcript analysis/phorbol ester induction)

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ABSTRACT **Recurring chromosomal translocations involving chromosome 11, band q23, have been observed in acute lymphoid leukemias and especially in acute myeloid leukemias. We recently showed that breakpoints in four 11q23 translocations, t(4;11)(q21;q23), t(6;11)(q27;q23), t(9;11)(p22;q23), and t(11;19)(q23;p13.3), were contained within a yeast artificial chromosome clone bearing the *CD3D* and *CD3G* gene loci. We have identified within the *CD3* yeast artificial chromosome a transcription unit that spans the breakpoint junctions of the 4;11, 9;11, and 11;19 translocations, and we describe two other, related transcripts that are upregulated in the RS4;11 cell line. We have named this gene *MLL* (*m*yeloid/*l*ymphoid, or *m*ixed-*l*ineage, *l*eukemia).**

Recurring rearrangements involving chromosome 11, band q23, are frequently observed in both acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML), especially acute monoblastic leukemia (AML-M5) and acute myelomonocytic leukemia (AMML-M4) (1, 2). The hypothesis that the rearrangement of 11q23 may affect an early progenitor cell capable of both myeloid and lymphoid differentiation (3) has received further support from the association of aberrations of band 11q23 with biphenotypic or acute mixed-lineage leukemias (4, 5). Recently, we reported that the breakpoints of four reciprocal translocations involving band 11q23 were within a 330-kilobase-pair (kb) yeast artificial chromosome (YAC) that contained the CD3 δ and γ (*CD3D* and *CD3G*) genes (6). By use of genomic DNA subclones generated from the *CD3* YAC, a more detailed map of the breakpoint region was constructed. By Southern blot analysis and fluorescence *in situ* hybridization we show that these clones bracket the breakpoints. Unique sequences from these clones detect transcripts that are present in cells with 11q23 abnormalities and in samples from both hematopoietic and nonhematopoietic tissue. These data demonstrate that a transcription unit spans the 11q23 breakpoints and that it is split as a result of the translocations.

MATERIALS AND METHODS

Cell Lines and Patient Material. The establishment and characterization of the RS4;11, RCH-ACV, RCH-ADD, RC-K8, BV173, SUP-T13, and SUP-T19 cell lines have been described (7–11). The clinical and cytogenetic characteristics of the patients and the cell lines with 11q23 abnormalities are listed in Table 1. Methods for preparation of metaphase cells and for fluorescence *in situ* hybridization are described in ref. 6.

Preparation of DNA, Gel Electrophoresis, and Southern Transfers. High molecular weight DNA in solution or embedded in agarose was isolated from recombinant yeast cells (12), cell lines, and peripheral blood and bone marrow samples from patients with leukemia. These DNA samples were digested with restriction enzymes, and the fragments were separated by either pulsed-field gel electrophoresis or by conventional methods in agarose gel slabs and transferred to nylon membranes (13).

Molecular Subcloning. High molecular weight DNA from the yeast clone containing the YAC was digested with the restriction enzyme *Bam*HI, ligated to the arms of the λ bacteriophage Lambda Dash II, and packaged with the Gigapack system (Stratagene). Resultant recombinant phage plaques were screened, and clones containing DNA of human origin were selected using human placental DNA as a probe. Subclones were generated from the phage clones, in Bluescript or Bluescribe vectors (Stratagene).

Preparation of RNA, Gel Electrophoresis, and Northern Transfers. Poly(A)$^+$ RNA from cell lines and from cultured primary leukemia cells was extracted with the Fast Track Isolation Kit (Invitrogen). Five micrograms of formamide/formaldehyde-denatured mRNA was electrophoresed in the presence of formaldehyde and transferred to nylon membranes (13).

Preparation of DNA and RNA Probes and Hybridization Protocols. DNA fragments were purified by electrophoresis and labeled with ^{32}P by random oligonucleotide priming and extension with Klenow fragment of DNA polymerase I (Pharmacia). RNAs were generated for sense and antisense probes by using the T3 and T7 promoters in the Bluescript and Bluescribe plasmid vectors of the subclones (Stratagene). Hybridization protocols and washing conditions were as described previously (14) unless otherwise noted.

Phorbol Ester Induction Experiments. The tumor promoter phorbol 12-myristate-13-acetate (PMA, 10 ng/ml) was added to exponentially growing cells. At intervals from 30 min to 48 hr, mRNA was extracted from aliquots of 50–100 million cells of the cultures (as described above).

RESULTS

Southern Blots and Fluorescence *in Situ* Hybridization Analysis. Probes for genes (*ETS1*, *CBL2*, *THY1*, *CD3D*, and *CD3E*) from 11q23 were hybridized to pulsed-field-gel and conventional-gel Southern blots of human DNA isolated from samples that contained 11q23 translocations. With the exception of the *CD3D* gene probe, all of these probes detected

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Abbreviations: YAC, yeast artificial chromosome; PMA, phorbol 12-myristate-13-acetate; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia.

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Table 1. Clinical and cytogenetic features of patients or cell lines involving 11q23

Patient or cell line	Age, yr/sex	Hematologic disease*	Stage	Karyotype(s)†
1	64/M	t-MDS	Relapse	46,XY,t(9;11)(p22;q23) (100%)
2	2/M	AML-M5	Diagnosis	46,XY (5%)/46,XY,inv(11)(p13q23),t(9;11)(p22;q23) (95%)
3	53/F	t-AML-M5a	Diagnosis	46,XX,t(6;19)(p23;p11),t(9;11)(p22;q23) (100%)
4	22/F	T-cell ALL	Diagnosis	45,−X,t(X;?)(q2?8;?),del(7)(q21q36),t(10;14)(q24;q32), t(11;14)(q23;q11) (66%)
RS4;11	32/F	B-cell ALL with monocytoid features	Relapse	46,XX,i(7q),t(4;11)(q21;q23) (100%)
RC-K8	55/M	Histiocytic lymphoma	Relapse	46,X,t(Y;7)(q12;q32),−8,−14,t(2;2)(p25;p23), t(3;4)(q29;q31), t(10;15)(p11;p13), t(11;14)(q23;q32), t(13;20)(q12;q13),+der(8)t(8;8)(p22;q11),+mar
SUP-T13	2/F	T-cell ALL	Relapse	46,XX,t(1;8)(q32;q24)/46,XX,del(9)(q22q34),t(1;8), t(1;5)(q41;p11),t(1;8),t(11;19)(q23;p13)

*t-, therapy-related; MDS, myelodysplastic syndrome.
†Karyotypes were determined by M.M.L.B. (patients 1–4) or were reported in ref. 7 (RS4;11), ref. 9 (RC-K8), or ref. 10 (SUP-T13).

only the germ-line genomic DNA fragments with all restriction enzymes tested. The single exception, the *CD3D* probe, detected rearranged fragments in DNA from samples with the t(4;11), t(9;11), or t(11;19) that were digested with *Sfi* I (Fig. 1*A* and data not shown). In the *Sfi* I digestions, the size of the germ-line fragment detected by *CD3D* is 220 kb (Fig. 1*A*, lanes 1–5). The size of the rearranged *Sfi* I fragments varied from 170 to 675 kb in the DNA samples from cells with 11q23 translocations (Fig. 1*A*, lanes 2–5, and data not shown). The *CD3E* probe, which is just centromeric to the *CD3D* gene, recognized only a 50-kb germ-line *Sfi* I fragment (data not shown). Both the *CD3E* and *CD3D* probes recognize only a 300-kb germ-line *Not* I fragment (Fig. 1*B*). Therefore, the breakpoint is telomeric to the *CD3* gene cluster.

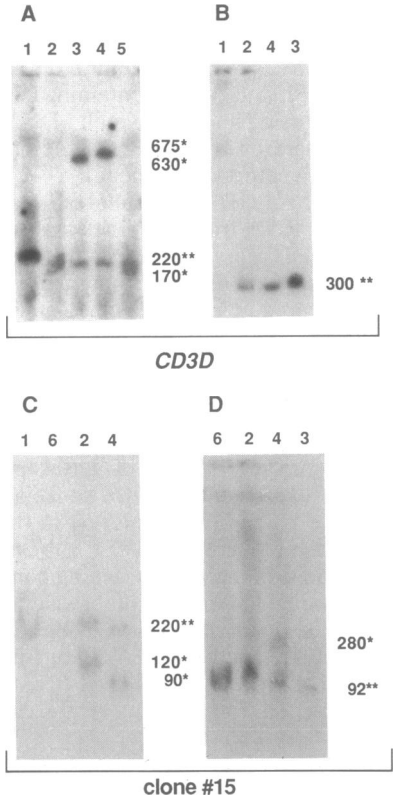

FIG. 1. Southern blot analysis of genomic DNA digested with *Sfi* I or *Not* I and separated by pulsed-field electrophoresis. Lanes: 1, BV173 control cell line; 2, RS4;11 cell line; 3, patient 3; 4, patient 2; 5, patient 1; 6, SUP-T19 control cell line. (*A*) *Sfi* I digest of DNA hybridized to the *CD3D* gene probe. (*B*) *Not* I digest of DNA hybridized to the *CD3D* gene probe. (*C*) *Sfi* I digest of DNA hybridized to clone 15. (*D*) *Not* I digest of DNA hybridized to clone 15. In *A* lane 1, overloading of DNA caused a slight retardation of the 220-kb band, and in *B* lane 1, the presence of an extra band in the control sample is due to partial digestion due to methylation. DNA fragment sizes in kilobase pairs are shown to the right of each panel. Single star, rearranged band; double star, germ-line band.

To determine whether the breakpoints at 11q23 in other translocations involved the sequences in the *CD3* YAC, we examined cells with two other 11q23 rearrangements by fluorescence *in situ* hybridization. Analysis of cells from a patient with T-cell ALL and a t(11;14)(q23;q11) (Table 1, patient 4) and of the cell line RC-K8, derived from a patient with a histiocytic lymphoma, containing a t(11;14)(q23;q32), revealed that the complete *CD3* YAC remained on the der(11) (data not shown). DNA from the RC-K8 cell line displayed only the germ-line *Sfi* I fragment after hybridization to the *CD3D* probe (data not shown). Therefore, not all 11q23 translocation breakpoints are within the *CD3* YAC.

Southern blot analysis of placental DNA using the *CD3* probes and YAC vector end probes revealed the location of several rare restriction enzyme sites (Fig. 2*B*). Some of the rare restriction enzyme sites that we mapped within 11q23 matched recently published maps (15, 16), with a few exceptions (16). Comparison of the maps shows that the majority of the human insert in the 330-kb YAC lies telomeric to the *CD3* locus and centromeric to the *CBL2/THY1* region (Fig. 2 *A* and *B*). An *Sfi* I site is located 207 kb telomeric to the *CD3D* gene, and two *Not* I sites are located 110 kb and 202 kb telomeric to the *CD3D* gene within the *CD3* YAC. Therefore, the combined data from the pulsed-field gel analysis and mapping define the region of the breakpoint junctions to be within a 92-kb *Not* I fragment in the center of the *CD3* YAC (Fig. 2*C*).

Phage λ human genomic subclones generated from a complete *Bam*HI digestion of yeast DNA containing the *CD3* YAC were mapped, and only those subclones which were present within the 92-kb *Not* I region were analyzed further. The locations of three of these *Bam*HI inserts, designated clone 1 (a 7.4-kb fragment), clone 14 (a 14.5-kb fragment), and clone 15 (a 6.8-kb fragment), are shown in Fig. 2*C*. These subclones were biotin-labeled and were hybridized to metaphase cells characterized by the t(4;11), t(9;11), or t(11;19). The *in situ* hybridization analysis demonstrated that clone 1 hybridizes to the derivative chromosome 11 [der(11)] and that clones 14 and 15 hybridize to the der(4), der(9), and der(19) in the different translocations (data not shown).

Unique, nonrepetitive subfragments were isolated from these clones and were used as probes on Southern blots of DNA from leukemia cells with abnormalities of 11q23. All three unique fragments from clones 1 (a 0.4-kb *Stu* I fragment), 14 (a 2.1-kb *Bam*HI–*Stu* I fragment), and 15 (a 0.5-kb

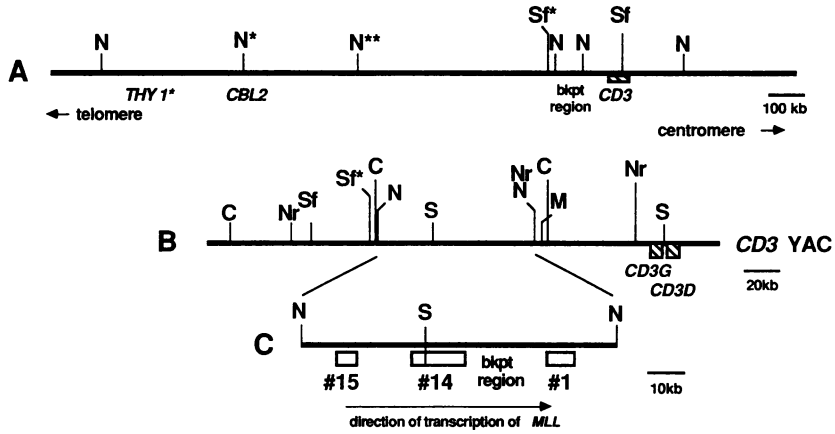

FIG. 2. Restriction map depicting rare-cutting enzymes: S, *Sal* I; *Sf*, *Sfi* I; N, *Not* I; C, *Cla* I; Nr, *Nru* I; M, *Mlu* I. (*A*) Long-range map of the 11q23 chromosomal region. We have confirmed the linkage of *THY1* and *CBL2*, which are separated by a *Not* I site (N*) that is methylated in some cell lines (data not shown). N** is a *Not* I site that was linked to the *CD3* loci by Tunnacliffe and McGuire (15). The 11q23 breakpoints are telomeric to the *CD3* loci and centromeric to the Sf* site. (*B*) Map of the *CD3* YAC. Not all restriction sites are indicated. Several rare enzyme sites are clustered with the two *Not* I sites. Tunnacliffe and McGuire (15) postulated that the more distal one was the one near the 11q23 breakpoint. (*C*) The 92-kb *Not* I restriction fragment that contains the 11q23 breakpoint region. Subclones 1, 14, and 15 from the YAC clone map within this *Not* I fragment. Arrow indicates the direction of the transcript hybridizing to the three subclones that spans the breakpoint region.

*Hin*dIII–*Bgl* II fragment) detected the 220-kb *Sfi* I germ-line band. Clone 1 detected the same rearranged *Sfi* I fragment as the *CD3D* probe representing the der(11) chromosome, whereas clones 14 and 15 detected the 220-kb germ-line band and other rearranged *Sfi* I fragments representing the der(4) or der(9) (Fig. 1*C* and data not shown). As previously mentioned, the *CD3D* gene probe detected only a 300-kb *Not* I germ-line band. Clones 1, 14, and 15 detected a 92-kb *Not* I germ-line fragment (Fig. 1 *B* and *D*; data not shown). However, as expected, clone 1 detected additional rearranged *Not* I fragments representing the der(11), and clones 14 and 15 detected rearranged *Not* I fragments from the der(4) and the der(9) (Fig. 1*D* and data not shown). Based on restriction mapping analysis, clone 14 and clone 15 appear to be within 25 kb of each other, and clone 1 and clone 14 are within 30 kb of each other (Fig. 2*C* and data not shown). Together, these data confirm that the 11q23 breakpoint junctions that we have analyzed lie between clone 1 and clone 14 and are within a 30-kb genomic region. Nevertheless, no rearrangements were detected when these probes were hybridized to Southern blots of DNA digested with *Eco*RI, *Bam*HI, and many other frequently cutting enzymes.

Northern Blot Analysis. To determine the presence of conserved sequences within the subclones generated from the *CD3* YAC, hybridization to a Southern blot of DNA from bovine, mouse, rat, and Chinese hamster DNA was carried out. Unique DNA fragments in all species were found to cross-hybridize to all three subclones (data not shown). Northern analysis was performed to determine whether these conserved sequences represent a functional transcription unit. The unique nonrepetitive subclones from clones 1, 14, and 15 detected a 12.5-kb transcript in mRNA from hematopoietic and nonhematopoietic cell lines and from primary leukemia cells with or without abnormalities of 11q23 (Fig. 3). This transcript appeared to be expressed at very low abundance in all cells tested. Since genomic DNA analysis showed that clone 1 and clones 14 and 15 bracket the breakpoints, this 12.5-kb transcription unit presumably spans the 11q23 breakpoints.

Clones 1 and 14 detected additional transcripts. In the RS4;11 cell line, cells in logarithmic or stationary growth phase expressed a more abundant 11.5-kb transcript and an 11.0-kb transcript (Fig. 3, lanes 1 and 2). Other cell lines without 11q23 abnormalities also displayed these two transcripts, but at a much lower level (Fig. 3, compare lanes 5 and 6, where *ACTB* is used as an internal control). Moreover, in logarithmic growth phase, the ratio of the 11.5-kb message to the 12.5-kb message in RS4;11 was greater than in stationary phase (Fig. 3, lanes 1 and 2). In nonhematopoietic cell lines, specifically human oligodendroglial and fibroblast cell lines, a 1.5-kb message was also detected with clone 14 (Fig. 3, lanes 7 and 8). The orientation of the transcripts was determined using sense and antisense RNA probes transcribed with T3 or T7 RNA polymerase from these subcloned fragments. The transcripts appear to have a telomere-to-centromere, 5′ → 3′, orientation (data not shown; orientation indicated by arrow on Fig. 2*C*). Also detected are 0.5- and 5.0-kb transcripts in the opposite orientation (data not shown).

Gene Expression After PMA Induction. Initially, an attempt was made to distinguish among the differently sized transcripts recognized by clone 14, based on their response to induction by PMA. The RS4;11 cell line is known to differentiate into a monocytoid phenotype upon induction with

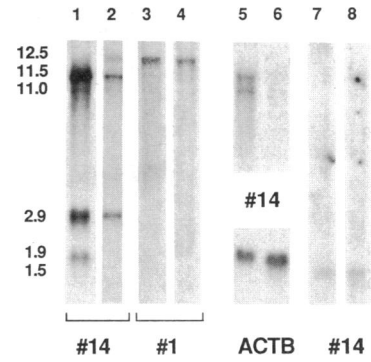

FIG. 3. Northern blot of poly(A)$^+$ RNA (lanes 1–6) or total cellular RNA (lanes 7 and 8) from various human cell lines. Lanes: 1–5, RS4;11 cell line; 6, RCH-ADD control cell line; 7, human fibroblast cell line 8, human oligodendroglial cell line. Lanes 1 and 3 represent cells in logarithmic growth phase; lanes 2 and 4 represent cells in stationary growth phase. Lanes 1, 2, 7, and 8 are hybridized to a unique probe from clone 14. Hybridization to the oncogene *vav* (2.9 kb) and the β-actin gene (*ACTB*, 1.9 kb) are also shown in lanes 1 and 2 to show accurate positioning of the blots. Lanes 3 and 4 are hybridized to a unique probe from clone 1, and lanes 5 and 6 are the same blot hybridized sequentially to clone 14 and to *ACTB*. RNA sizes in kilobases are shown at left.

PMA (17, 18). The RCH-ACV pre-B-cell line becomes adherent and appears to differentiate and to express lymphoid markers after PMA induction (flow cytometry data not shown). We performed a detailed analysis of PMA induction on the RS4;11 B ALL cell line and a control pre-B ALL cell line, RCH-ACV. Poly(A)$^+$ mRNA was isolated at the zero time point and after PMA induction, at 1, 8, 12, 24, and 48 hr. Northern blots of these mRNAs were probed with the unique fragments from all three phage clones (Figs. 4 and 5). The 12.5-kb transcript detected by clones 1, 14, and 15 displayed a distinct pattern of PMA-induced downregulation of expression in both cell lines (Figs. 4*A* and 5*A*; data not shown). A complete loss of this message was seen at the 1-hr time point and a moderate level of expression was resumed at 8 hr that continued through the 24-hr time point. The 11.5-kb and 11.0-kb messages detected by clone 14 were downregulated at 1 hr in the cell line RCH-ACV but returned to control levels of expression at 8 hr (Fig. 5*B*). However, the 11.5-kb and 11.0-kb transcripts in the RS4;11 cell line did not show this dramatic downregulation. Both transcripts remained within control levels throughout 24 hr of induction (Fig. 4*B*).

To confirm that the observed cellular morphological changes were indeed due to PMA induction, we studied the effect of PMA on *MYC* and *MYB*, whose increased levels of expression after phorbol ester induction are well documented (Figs. 4*D* and 5*D*). Further, we examined the expression of the B-cell-specific homeobox gene *OCT2* (Fig. 4*E*) (19) and the DNA-binding-protein gene *MBP1* (Fig. 4*F*) (20) after PMA induction in the RS4;11 cell line. A significant upregulation in expression of *OCT2* was detected at 8 hr, while *MBP1* displayed a constant low level of expression until 12 hr, when it began an increase that continued through the 24-hr time point.

DISCUSSION

We recently reported that the DNA sequences homologous to the *CD3* YAC are split in four of the recurring translocations in acute leukemia involving 11q23 (6). The human insert within the *CD3* YAC is telomeric to the *NCAM, CLG, PGR,*

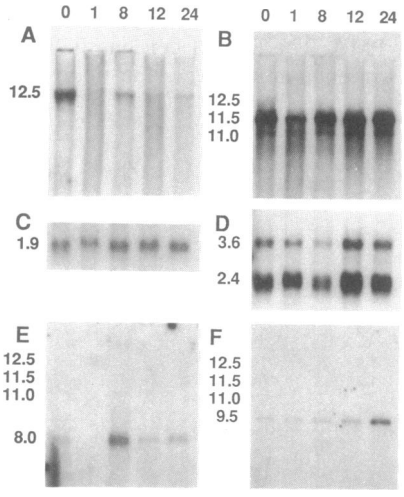

FIG. 4. Northern blot analysis of poly(A)$^+$ RNA from the RS4;11 cell line (5 μg per lane). Cells in logarithmic growth phase were cultured in the presence of PMA (10 ng/ml). Poly(A)$^+$ RNA was isolated after 0, 1, 8, 12, and 24 hr, separated in a formaldehyde/1% agarose gel, and blotted onto a nylon filter membrane (GeneScreen-*Plus*, NEN). The same Northern blot was hybridized successively to clone 1 (*A*), clone 14 (*B*), *ACTB* gene probe (*C*), *MYC* and *MYB* gene probes (*D*), *OCT2* gene probe (*E*), and *MBP1* gene probe (*F*). A large section of the Northern blot hybridized to *OCT2* and *MBP1* is displayed to show the lack of cross-hybridization in the >11-kb region. RNA sizes in kilobases are at left of each panel.

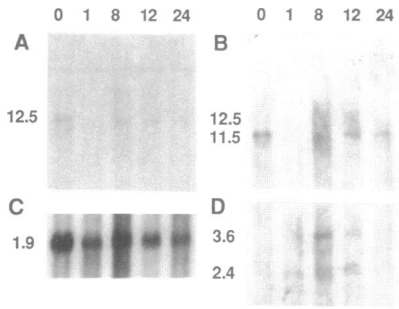

FIG. 5. Northern blot analysis of poly(A)$^+$ RNA from the RCH-ACV control cell line (3 μg per lane). Cells in logarithmic growth phase were cultured in the presence of PMA (10 ng/ml) and processed as in Fig. 4. The same Northern blot was hybridized successively to clone 1 (*A*), clone 14 (*B*), *ACTB* gene probe (*C*), and *MYC* and *MYB* gene probes simultaneously (*D*). RNA sizes in kilobases are at left of each panel.

and *AT1* loci and centromeric to the *PBGD, CBL2, THY1*, and *ETS1* genes (Fig. 2*A*) (6, 21, 22). The *CD3G* and *CD3D* genes are at the centromeric end of the insert. We now present additional analyses of these 11q23 breakpoints and an initial characterization of a transcription unit that spans the 11q23 breakpoint. We also show that not all 11q23 translocations associated with leukemia, particularly those that are associated with the antigen-receptor genes *TCRD* (at 14q11) and *IGH* (at 14q32), are within the 11q23 segment contained within the *CD3* YAC. Our detailed mapping and Southern blot analysis show that breakpoint junctions within the *CD3* YAC are present within a 92-kb *Not* I fragment (Fig. 2*B*). In addition to the *Not* I sites, this 92-kb fragment appears to be bracketed by recognition sites for other enzymes that also occur rarely within mammalian DNA and that are found mainly within undermethylated CpG-rich islands. These CpG islands are often associated with transcribed regions within the genome (23).

The *CD3D* probe and three subclones from the 92-kb *Not* I fragment detect the normal chromosome 11 by *in situ* hybridization analysis, and they all detect the same germ-line DNA fragments on Southern blot analysis of *Sfi* I digests. The *CD3D* probe and subclone 1 detect the same rearranged *Sfi* I DNA fragments, whereas subclones 14 and 15 detect differently sized rearranged fragments. This was confirmed by *in situ* hybridization analysis of cells with the t(4;11), which showed that *CD3D* and subclone 1 remained on the der(11) chromosome, whereas subclones 14 and 15 were translocated to the reciprocal chromosome. With the enzyme *Not* I, no rearrangements were detected with *CD3D* in cells with the 11q23 translocation; however, rearrangements were found on hybridization with each of the three subclones. As expected from the *Sfi* I data, the rearranged *Not* I band detected by subclone 1 differed in size from that detected by the subclones 14 and 15. These results and the *in situ* hybridization studies indicate that the rearranged bands detected by subclones 14 and 15 represent the breakpoint junction on the der(4), der(9), or der(19). Based on detailed molecular mapping (Fig. 2*C*), subclones 1 and 14 are $\approx$30 kb apart; thus we have identified a breakpoint region on chromosome 11 that is involved in recurrent chromosome translocations.

The estimate of Das *et al.* (24) of a breakpoint location within 200 kb of the *CD3G* gene is compatible with our results. Their failure to detect a rearranged *Sfi* I fragment may be attributed to the small difference in size between the rearranged and germ-line *Sfi* I fragments in RS4;11 (Fig. 2*A*), which may not have been resolved in their pulsed-field gel separations.

Northern blot analysis revealed a complex pattern of expression of mRNA transcripts recognized by all of the subclones. The largest transcript, a 12.5-kb mRNA recognized by all three subclones, is expressed in very low abundance in samples containing 11q23 abnormalities as well as in normal hematopoietic and nonhematopoietic cells (Fig. 3 and data not shown). Since subclones 1 and 14 bracket the breakpoint junction, these data indicate that this 12.5-kb transcript spans the 11q23 breakpoint junction. Subclone 14 recognizes other transcripts in addition to the 12.5-kb transcript, which suggests possible alternative splicing and different exon usage. In all hematopoietic cells tested so far, subclone 14 recognizes an 11.5- and an 11.0-kb transcript, whereas in nonhematopoietic cells, a 1.5-kb transcript is also detected. However, the 11.5- and 11.0-kb transcripts are more highly expressed in the RS4;11 cell line compared with cells that do not have the 11q23 translocation (Fig. 3). In addition these two transcripts in the RS4;11 cell line escape the marked PMA-induced downregulation observed in control cell lines (Figs. 4 and 5). Our data support the hypothesis that the 11.5- and 11.0-kb transcripts may be abnormally regulated due to the translocation event. Several mechanisms for this deregulation are possible, including disruption of the normal gene regulatory region and/or apposition of other gene regulatory sequences that lead to abnormally high gene expression. These abnormally regulated transcripts must come from either the normal chromosome 11 or from the der(4) chromosome.

The RS4;11 cell line displays both B-cell and monocytoid features (7) and, upon continuous exposure to PMA, slowly differentiates toward adherent monocytes (17). Our preliminary results indicate that *OCT2* and *MBP1*, which are known lymphoid markers, display a very distinctive pattern of regulation in the RS4;11 cell line after PMA induction.

The gene that we identified, which we have named *MLL*, appears to have a telomere-to-centromere, 5′ → 3′ transcriptional orientation. It is possible that the function of *MLL* is affected because 3′ regulatory sequences on the der(11) are consistently lost or altered by the translocation event or are replaced by material coming from the other chromosome, thus causing an upregulation of expression of *MLL*. Whether a *MLL* chimeric product is formed in these translocation events is unknown. Our data indicate that the translocation event on 11q23 involves the splitting of the *MLL* transcriptional unit, resulting in abnormally high expression of two *MLL* transcripts. The significance of this high expression in the RS4;11 cell line and its relation to the leukemia has yet to be resolved.

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