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Consistent intergenic splicing and production of multiple transcripts between *AML1* at 21q22 and unrelated genes at 3q26 in (3;21)(q26;q22) translocations

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ABSTRACT **Two genes have been implicated in leukemias of patients with abnormalities of chromosome 3, band q26: *EVI1*, which can be activated over long distances by chromosomal rearrangements involving 3q26, and *EAP*, a ribosomal gene that fuses with *AML1* in a therapy-related myelodysplasia patient with a t(3;21)(q26.2;q22). *AML1* was identified by its involvement in the t(8;21)(q22;q22) of acute myeloid leukemia. Here we report the consistent identification of fusion transcripts between *AML1* and *EAP* or between *AML1* and previously unidentified sequences that we named *MDS1* (MDS-associated sequences) in the leukemic cells of four patients with therapy-related myelodysplasia/acute myeloid leukemia and in one patient with chronic myelogenous leukemia in blast crisis, all of whom had a t(3;21). In addition, we have identified a third chimeric transcript, *AML1/EVI1*, in one of the therapy-related acute myeloid leukemia patients. Pulsed-field gel electrophoresis established the order of the genes as *EAP*, the most telomeric, and *EVI1*, the most centromeric, gene. The results indicate that translocations could involve multiple genes and affect gene expression over long distances.**

The molecular analysis of recurring chromosomal translocations in leukemias has led to the identification of protooncogenes located at the translocation breakpoint that are activated either by altered expression or by gene fusion. One of the most common translocations in acute myeloid leukemia is the t(8;21)(q22;q22), which has recently been shown to involve the *AML1* gene at 21q22 (1) fused to the *ETO* gene at 8q22 (2, 3). *AML1* is identical to the murine transcription factor *pebp2α* and the DNA-binding subunit of the human transcription core factor *CBF* (1, 4). The human and murine AML1 polypeptides are 99% homologous in their first 242 residues, but they differ in overall size. *pebp2α* encodes a predicted polypeptide of 452 residues containing the DNA-binding and dimerization region encoded by the *Drosophila melanogaster* runt (*run*) homology segment at the N terminus (2, 5), as well as a region rich in serine, threonine, and proline, suggestive of a transcription activation domain at the C terminus (4). The sequence of the human *AML1* cDNA is 250 residues shorter than that of *pebp2α* and lacks the serine-, threonine-, and proline-rich region. It probably represents a spliced isoform of the mRNA consisting mostly of the *run* homology segment.

Chromosome 21, band q22, is also involved in the t(3;21)(q26.2;q22) in therapy-related acute myeloid leukemia/myelodysplasia (t-AML/t-MDS) or chronic myelogenous leukemia in blast crisis (CML-BC) (6, 7). Recent studies have shown that this translocation involves the *AML1* gene and a gene at 3q26, which is *EAP* (8, 9). *EAP* codes for a small (129 amino acids) ribosomal protein, L22, and belongs to a large family of genes. Although *EAP* is highly conserved and abundantly expressed in all tissues (10, 11) and in hematopoietic cell lines (9), it is not known whether the allele at 3q26 is the one that is expressed. In t(3;21), the translocation results in the fusion of all of *AML1* except for the last 9 codons to the last 96 codons of *EAP*, but the fusion does not maintain the *EAP* reading frame, and a stop codon is introduced shortly after the junction (9). Thus, the *AML1/EAP* chimeric transcript expresses an AML1 protein that is similar in size to that encoded by the cloned human *AML1* cDNA (1, 9), which lacks the serine-, threonine-, and proline-rich region contained in the murine homolog *pebp2α*.

In some patients with a t(3;21), we have found that the translocation of the 5′ part of *AML1* to chromosome 3 affects other genes besides *EAP*. Here we report on the isolation of a second chimeric transcript between *AML1* and previously unreported sequences that we named *MDS1* (sequences identified in myelodysplasia syndrome). This transcript was isolated from the same patient's library from which we isolated the *AML1/EAP* fusion gene. In addition, we report on the consistent detection of the same two transcripts, *AML1/EAP* and *AML1/MDS1*, in one patient with CML-BC and in three additional patients with t-MDS/t-AML who had t(3;21). In one of the t-AML patients, we detected a third chimeric transcript between the same 5′ region of *AML1* and *EVI1*, a gene whose aberrant activation has also been implicated in leukemogenesis (12). By using pulsed-field gel electrophoresis (PFGE) analysis, we have determined that the three sequences are located within 400–750 kb of each other, and that *EAP* is the most telomeric and *EVI1* is the most centromeric of the genes.

MATERIALS AND METHODS

Cells and Cultures. Peripheral blood samples and bone marrow aspirates were obtained from one CML-BC and four t-AML/t-MDS patients with a t(3;21). Four different Chinese hamster ovary (CHO)–human somatic cell hybrids were maintained and propagated as described (9, 12): 6918, containing human chromosome band 21q22 spanning the *AML1* region; UCTP2A3, containing the entire human chromosome 3; and H10-c and H3-4, containing, respectively, the 3q+ and the 3q− chromosomes from a t(3;3)(q21;q26) leukemic sample. The parental CHO cell line UC2 was used as a control.

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Abbreviations: t-AML, therapy-related acute myeloid leukemia; t-MDS, therapy-related myelodysplasia; CML-BC, chronic myelogenous leukemia in blast crisis; PFGE, pulsed-field gel electrophoresis; RT, reverse transcription.

DNA and RNA Isolation, Reverse Transcription (RT)-PCR, and Standard and PFGE Southern Blots. DNA was extracted and manipulated as described (9). Polyadenylylated RNA was prepared from viably frozen cells from the t(3;21) patients by use of a Fast Track mRNA kit (Invitrogen) under conditions recommended by the manufacturer. RT-PCR and standard and PFGE Southern blots were performed as described (9, 12). The blot containing genomic DNA isolated from different animal species (Bios, New Haven, CT) and the blot containing poly(A) RNA from human tissues (Clonetech) were used according to the manufacturer's suggestions. The sequences of the PCR primers 1 (upstream primer) and 2–4 (downstream primers) are as follows: 1 (*AML1* primer), ACAAACCCACCGCAAGT; 2 (*EAP* primer), ACTTTGATCCTTTCTTG; 3 (*MDS1* primer), CATCTGCATCTGGCATT; 4 (*EVI1* primer), GCAGCGATATTGCCGTT.

Construction and Screening of the cDNA Library. Construction of the cDNA library in λ ZAP II (Stratagene) with peripheral blood samples from a t-MDS patient with a t(3;21) and the AML1 0.6-kb probe used to screen the library has been described (9). The plasmids were rescued from the positive phage clones by use of the *in vivo* excision protocol provided by the manufacturer.

cDNA Sequencing. PCR products, after cloning in pKSII-(+) (Stratagene), and plasmid cDNA clones were sequenced with the Sequenase kit (United States Biochemical) according to Sanger's method by use of vector- and sequence-specific primers.

RESULTS

Identification and Characterization of the Second Chimeric Transcript, *AML1/MDS*. The *AML1/MDS1* chimeric clone, pMDS1, was isolated from the t(3;21) peripheral blood cell library as described, with the AML1 0.6-kb probe (10). We found that pMDS1 hybridized to a 1.8-kb *Eco*RI restriction fragment of placental DNA and of human–CHO somatic cell hybrid UCTP2A3, containing only human chromosome 3, but not to 6918 DNA, the human–CHO somatic cell hybrid containing chromosome band 21q22, which spans the *AML1* gene (data not shown). pMDS1 had an insert of ≈2.5 kb. After sequencing, we determined that the 5′ part of the insert (≈1 kb) contained the same segment of *AML1* that was fused to *EAP* in the *AML1/EAP* fusion gene (9). However, the remaining part of pMDS1 (≈1.5 kb) diverged sharply from that of *AML1* or *EAP* after the breakpoint junction. The pattern obtained by Southern blot hybridization of the placental and somatic cell hybrid DNA with pMDS1 as a probe was different from that obtained with an EAP probe (data not shown).

Fig. 1 shows the alignment of the predicted amino acid sequence of pMDS1 with those of AML1 (1) and AML1/EAP (9). The three sequences were identical from residues 6 to 242 of AML1. Upstream of residue 6, the three sequences diverged, and each encoded a short stretch of residues representing three diverging amino ends from different spliced forms of the mRNA. After residue 242 of AML1, the AML1/EAP and the AML1/MDS1 sequences diverged from that of AML1. Whereas the predicted AML1/EAP polypeptide contained 17 residues unrelated to the AML1 sequence before a stop codon (9), the fusion between AML1 and MDS1 was in frame and added 127 residues containing a high percentage of prolines and serines and of acidic and basic residues, with a preponderance of acidic residues. The coding region of *AML1/MDS1* was followed by a stretch of 1.2 kb of A+T-rich sequence probably representing the 3′ untranslated part of the chimeric cDNA.

Northern Blot Analysis. Hybridization of the MDS1–1.5 probe to a panel of poly(A)$^+$ mRNAs isolated from adult human tissues and cell lines revealed transcripts of 5.1 and 6.4 kb were readily detected in lung, pancreas, and kidney

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AML1/EAP    1   M N P C R D V H D A S T S R R F T P P S T A L S P G K M S E A L P
AML1        1       M R I P V * * * * * * * * * * * * * * * * * * * * * * * * *
AML1/MDS1   1           M R * * * * * * * * * * * * * * * * * * * * * * * * * *

AML1/EAP   34       L G A P D A G A A L A G K L R S G D R S M V E V L A D H P G
AML1       31       * * * * * * * * * * * * * * * * * * * * * * * * * * * * *
AML1/MDS1  28       * * * * * * * * * * * * * * * * * * * * * * * * * * * * *

AML1/EAP   64       E L V R T D S P N F L C S V L P T H W R C N K T L P I A F K
AML1       61       * * * * * * * * * * * * * * * * * * * * * * * * * * * * *
AML1/MDS1  58       * * * * * * * * * * * * * * * * * * * * * * * * * * * * *

AML1/EAP   94       V V A L G D V P D G T L V T V M A G N D E N Y S A E L R N A
AML1       91       * * * * * * * * * * * * * * * * * * * * * * * * * * * * *
AML1/MDS1  88       * * * * * * * * * * * * * * * * * * * * * * * * * * * * *

AML1/EAP  124       T A A M K N Q V A R F N D L R F V G R S G R G K S F T L T I
AML1      121       * * * * * * * * * * * * * * * * * * * * * * * * * * * * *
AML1/MDS1 118       * * * * * * * * * * * * * * * * * * * * * * * * * * * * *
                                                                      ▼
AML1/EAP  154       T V F T N P P Q V A T Y H R A I K I T V D G P R E P R R H R
AML1      151       * * * * * * * * * * * * * * * * * * * * * * * * * * * * *
AML1/MDS1 148       * * * * * * * * * * * * * * * * * * * * * * * * * * * * *

AML1/EAP  184       Q K L D D Q T K P G S L S F S E R L S E L E Q L R R T A M R
AML1      181       * * * * * * * * * * * * * * * * * * * * * * * * * * * * *
AML1/MDS1 178       * * * * * * * * * * * * * * * * * * * * * * * * * * * * *

AML1/EAP  214       V S P H H P A P T P N P R A S L N H S T A F N P Q P Q S Q M
AML1      211       * * * * * * * * * * * * * * * * * * * * * * * * * * * * *
AML1/MDS1 208       * * * * * * * * * * * * * * * * * * * * * * * * * * * * *
                    ▼
                    ▼
AML1/EAP  244       Q E S W M L P I L S S F C K K G S K -
AML1      241       * D A R Q I Q P S P -
AML1/MDS1 238       * N E C V Y G N Y P E I P L E E M P D A D G V A S T P S L N

AML1/MDS1 268       I Q E P C S P A T S S E A F T P K E G S P Y K A P I Y I P D

AML1/MDS1 298       D I P I P A E F E L R E S N M P G A G L G I W T K R K I E V

AML1/MDS1 328       G E K F G P Y V G E Q R S N L K D P S Y G W E V R M Y F E L

AML1/MDS1 358       T H L Y I A A V -
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FIG. 1. Amino acid alignment of AML1 and of the two chimeric polypeptides AML1/EAP and AML1/MDS1. Asterisks indicate identities. The segment homologous to the *D. melanogaster* segmentation protein runt is indicated in italics. Single arrowhead indicates the consistent site of the fusion with the protein ETO in the 8;21 translocation. Double arrowhead indicates the beginning of the *AML1* intron containing the t(3;21) breakpoint. In our patients with a t(3;21), cDNA fusion junctions have been detected between *AML1* (at the site indicated with the single arrowhead and with the double arrowhead; see text) and *EAP*, *MDS1*, or *EVI1*. The region included between the single and double arrowheads corresponds to exon 6 of *AML1*, as shown in Fig. 7.

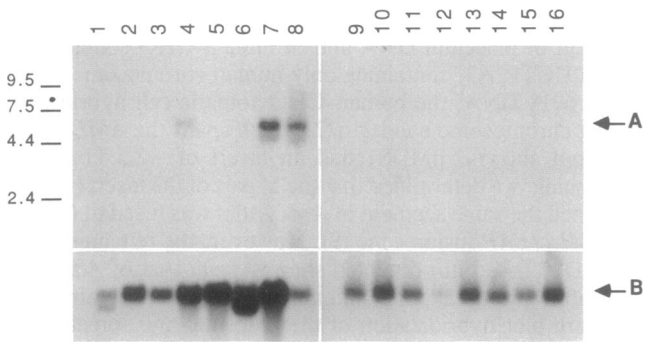

FIG. 2. Expression of *MDS1* in different human tissues. Lanes: 1, heart; 2, brain; 3, placenta; 4, lung; 5, liver; 6, skeletal muscle; 7, kidney; 8, pancreas; 9, spleen; 10, thymus; 11, prostate; 12, testis; 13, ovary; 14, small intestine; 15, colon; 16, peripheral blood leukocytes. A, MDS1–1.5 probe; B, β-Actin probe. Numbers on left are kb.

(Fig. 2, lanes 4, 7, and 8). The transcripts were weakly represented in heart, placenta, and skeletal muscle (lanes 1, 3, and 6) but not in most other tissues, including the spleen, thymus, peripheral blood, or leukocytes (lanes 9–16), or in myeloid and T- and B-cell lines (data not shown), implying that these messages are not normally expressed in hematopoietic cells. The distribution of tissue expression and the pattern of Northern blot hybridization observed with the MDS1 probe were completely different from those obtained when an EAP probe was used (9), thus further confirming that the two probes were derived from separate genes.

RT-PCR Analysis. To determine whether the formation of two chimeric transcripts was a frequent outcome of the 3;21 translocation, we examined leukemic cells from one CML-BC and three additional t-MDS/t-AML patients by RT-PCR. Primers were designed from the 5′ end of *AML1* and from the 3′ end of *EAP* and *MDS1*. Fig. 3 shows the ethidium bromide-stained gel of the PCRs. The pattern of amplification was identical for each patient and contained the expected size of the *AML1/EAP* junction (370 bp; lanes 1–5) or the expected size of the *AML1/MDS1* junction (386 bp; lanes 7–11). In addition, a smaller fragment was consistently amplified with primers from both *EAP* and *MDS1*. The difference between the larger and smaller bands in each pair of bands was ≈190 bp. By sequencing, we determined that the two bands were isoforms in which one exon of 190 bp of the *AML1* gene had been spliced out in the smaller fragments. This exon of *AML1* coded for the region shown between the single and the double arrowheads in Fig. 1, representing the breakpoint of *AML1* in t(8;21) (single arrowhead) or that in

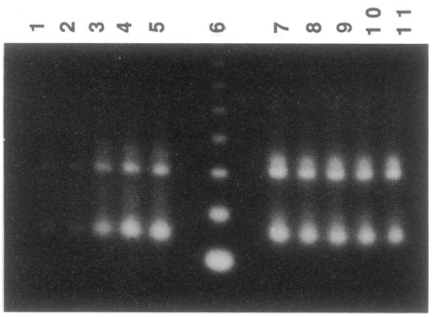

FIG. 3. Ethidium bromide stain of RT-PCR products after separation on an agarose gel. Lanes: 1 and 7, t-MDS patient; 2–4 and 8–10, three t-AML patients; 5 and 11, CML-BC patient; 6, 123-bp DNA marker. Lanes 1–5 show the RT-PCR products with *AML1/EAP* primers. The expected sizes of the bands are 370 bp for the larger band and 180 bp for the smaller band. Lanes 7–11 show the products of the RT-PCR with *AML1/MDS1* primers. The expected sizes of the bands are 386 bp for the larger band and 196 bp for the smaller band.

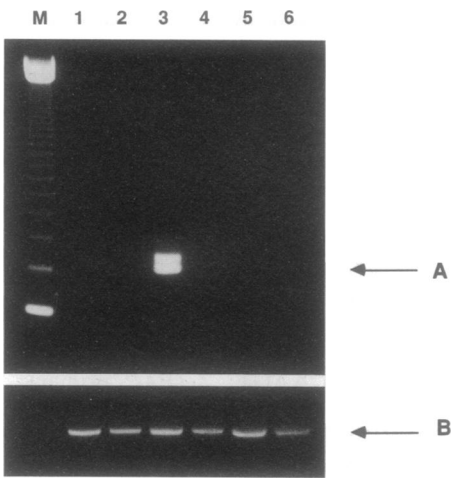

FIG. 4. Ethidium bromide stain of RT-PCR products with *EVI1* primers after separation on an agarose gel. The *EVI1*-specific primers used in these reactions and their sequence have been reported (12). Two major bands of 268 and 298 bp are expected. Lanes: 1, poly(A)RNA isolated from the CML-BC patient; 2, poly(A)RNA isolated from the t-MDS patient; 3–5, poly(A)RNA isolated from the three t-AML patients; 6, poly(A)RNA isolated from the peripheral blood samples of an AML patient with a normal karyotype; M, 123-bp DNA size marker. A, *EVI1* primers; B, β-actin primers.

t(3;21) (double arrowhead). The segments of either *MDS1* or *EAP* fused to *AML1* remained the same, indicating that the differential splicing affected only *AML1*. Interestingly, removal of this exon did not change the reading frame of the chimeric cDNAs.

Detection of Chimeric Transcripts Between *AML1* and *EVI1*. Chromosomal band 3q26 is also the location of *EVI1*, whose transcription can be activated by translocation 13–330 kb upstream of the 5′ end of the gene as well as by rearrangement 150 kb downstream of the 5′ end of the gene (12). *EVI1* activation has been implicated in the leukemogenesis of 7% of AML patients with abnormalities involving band 3q26 (12). Only one of our t-AML patients expressed this gene (Fig. 4), as determined by use of RT-PCR and *EVI1* primers for detecting the 3′ end of the mRNA (12). To determine whether, in this patient, *EVI1* was also expressed as a fusion gene with *AML1*, we designed primers from the published *EVI1* sequence (13). One of the *EVI1* primers amplified four major bands when used with the *AML1* primer. The four fragments (Fig. 5) were cloned and sequenced. The two smaller fragments contained the junction between *AML1* and either exon 2 of *EVI1* or exon 3 of *EVI1* (data not shown). The two larger fragments represented complex fusion clones, including *AML1* fused to *MDS1*, which was fused to exon 2 of *EVI1*, and the difference between them (190 bp) corre-

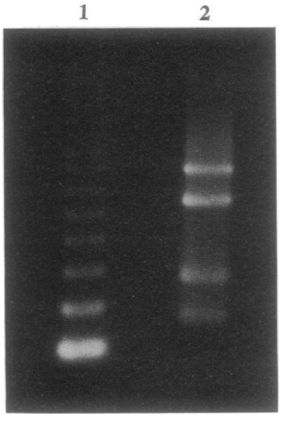

FIG. 5. Ethidium bromide stain of RT-PCR products amplified with *AML1* and *EVI1* primers (primers 1 and 4) after separation on an agarose gel. Lane 1, 123-bp DNA size markers; lane 2, t-AML patient's sample (patient in lane 3 in Fig. 4).

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AML1              151  T V F T N P P Q V A T Y H R A I K I T V D G P R E P R R H R
AML1              181  Q K L D D Q T K P G S L S F S E R L S E L E Q L R R T A M R
AML1              211  V S P H H P A P T P N P R A S L N H S T A F N P Q P Q S Q M
                            ▼
                            ▼
AML1/MDS1         241  Q N E C V Y G N Y P E I P L E E M P D A D G V A S T P S L N
AML1/MDS1         271  I Q E P C S P A T S S E A F T P K E G S P Y K A P I Y I P D
AML1/MDS1         301  D I P I P A E F E L R E S N M P G A G L G I W T K R K I E V
AML1/MDS1/EVI1    331  G E K F G P Y V G E Q R S N L K D P S Y G W E  I L D E F Y N
AML1/MDS1/EVI1    361  V K F C I D A S Q R D V G S W L K Y I R F A G C Y D Q H N L
AML1/MDS1/EVI1    391  V A C Q I N D Q I F Y R V V A D I A P G E E L L L F M K S E
AML1/MDS1/EVI1    421  D T P H E T M A P D I H E E R G
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FIG. 6. Predicted amino acid sequence of the triple junction of *AML1*, *MDS1*, and *EVI1*. Sequence shown is flanked by the *AML1* upstream primer and the *EVI1* downstream primer used for the RT-PCR. Numbering of amino acids is that of the AML1 polypeptide. Region between single and double arrowheads is spliced out in the shorter fragment containing the *AML1/MDS1/EVI1* junction. Underlined segment represents the MDS1 sequence, which is fused to AML1 at the N terminus and to EVI1 at the C terminus. Region in italics represents predicted translation of exon 2 of *EVI1*. Double underlined region starting with a methionine residue shows the published beginning of the *EVI1* sequence at exon 3.

sponded to the alternatively spliced isoforms of the *AML1* exon. The sequence of the AML1/MDS1/EVI1 fusion peptides predicted from the two larger junction fragments is shown in Fig. 6. In all of these junctions, the *AML1* reading frame was maintained, thus presumably leading to complex fusion peptides. A schematic representation of all of the different junctions detected by RT-PCR and sequencing in these patients is shown in Fig. 7.

Mapping of *EAP*, *MDS1*, and *EVI1* by PFGE and Southern Blot Analysis. To localize the genes, we probed filters from pulsed-field gels. Previous studies have shown that a CpG island exists 5′ of the *EVI1* gene, which contains sites for *Bss*HII, and that the next *Bss*HII site occurs ≈600 kb 5′ and telomeric to the gene (12). In addition, there is an *Sfi* I site ≈170 kb telomeric of the *Bss*HII site in the 5′ region of the *EVI1* gene (12). Both *EAP* and *MDS1* sequences were

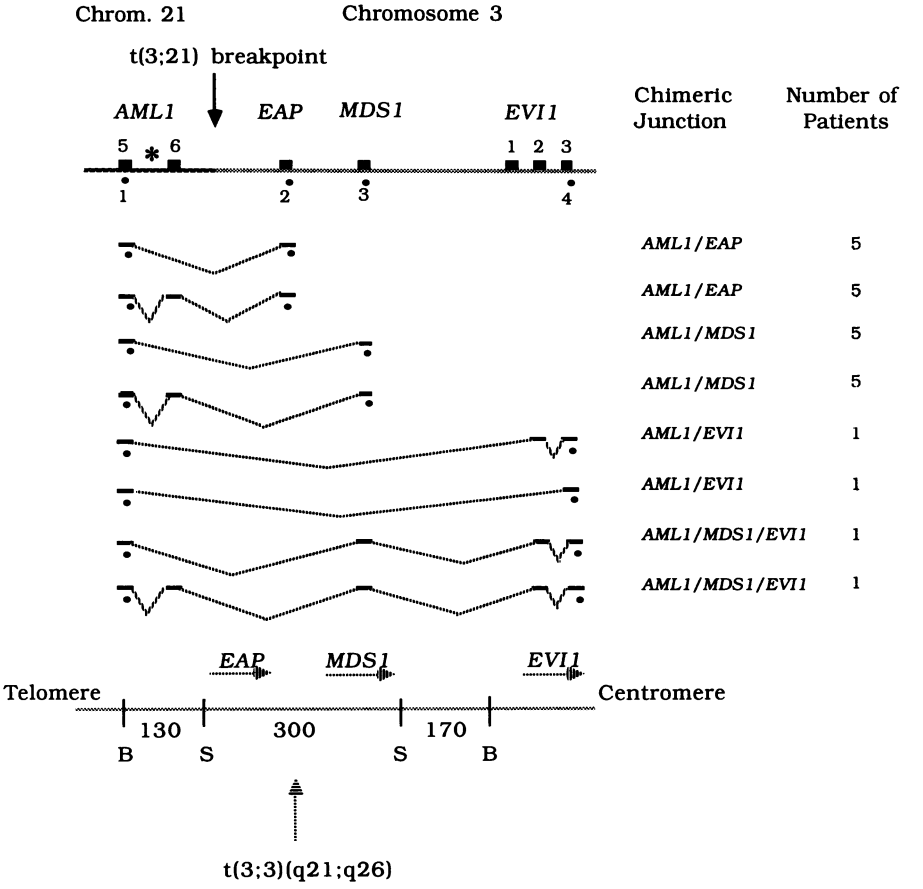

FIG. 7. (*Upper*) Chimeric junctions detected by RT-PCR and confirmed by nucleotide sequencing. Heavily shaded portion of the horizontal line represents translocated region of chromosome 21 containing the promoter and the 5′ region of *AML1*. Lightly shaded portion of the horizontal line represents the region of chromosome 3 containing the *EAP*, *MDS1*, and *EVI1* sequences. Vertical arrow indicates genomic junction. Solid boxes represent exons involved in chimeric junctions and identified by nucleotide sequencing; numbers above solid boxes indicate published numbering of *AML1* and *EVI1* exons. Asterisk between exons 5 and 6 of *AML1* indicates the intron where the breakpoints of the t(8;21) have been consistently detected. Solid dots below the horizontal line indicate position of the RT-PCR primers. Short solid horizontal segments joined by the V-shaped dotted lines represent exons amplified by PCR and sequenced. Two columns on the right indicate the type of junction and the number of patients who had the chimeric junction. (*Lower*) PFGE mapping results of genes on chromosome 3. Horizontal line represents the region on 3q26 where the genes are located. Horizontal arrows indicate the direction of transcription. Short vertical bars indicate restriction site for *Bss*HII (B) and *Sfi* I (S), and numbers below horizontal lines indicate size (kb) of restriction fragments. Dashed vertical arrow indicates approximate position of the t(3;3) breakpoint used for mapping the genes.

localized on the *Bss*HII fragment that is detected by a 5′ *EVI1* probe (Fig. 7). However, both probes detected the same *Sfi* I fragment of ≈300 kb. Therefore, both *EAP* and *MDS1* sequences are at least 170 kb telomeric of *EVI1*. To localize the genes further, we mapped them relative to the breakpoint found in a case of AML with t(3;3)(q21;q26) for which somatic cell hybrids existed that contained the derivative chromosomes (12, 14). Previous studies have shown that this breakpoint occurs within the region 400–170 kb 5′ of *EVI1* (12). By Southern blot analysis, only the *EAP*-specific probe hybridized with cells containing the 3q− derivative chromosome, demonstrating that *EAP* was telomeric of the breakpoint, whereas the *MDS1* probe was centromeric of the breakpoint (Fig. 7). Thus, the order of the sequences from the telomere is *EAP–MDS1–EVI1*. We also used fluorescence *in situ* hybridization to confirm that *EVI1* was centromeric to *MDS1* (data not shown).

DISCUSSION

It is generally accepted that, in most translocations associated with leukemia, the genes that are fused across the breakpoint are those directly modified in their expression or in their molecular nature and function and thus are critically involved in leukemogenesis. In fact, there are only a few examples of genes that are affected at a long distance by a translocation (15, 16), and the effect is limited to altering the expression of the involved gene. The cases of t(3;21) that we have investigated are unique in containing chimeric transcripts between *AML1* and unrelated genes located over a region of at least 400 kb at 3q26. Although we have no direct evidence that *AML1* and the partner genes at 3q26 can be transcribed as a single unprocessed nuclear message, the identification of chimeric junctions containing the same exons of *AML1* individually spliced to exons from the other genes would suggest that such nuclear transcripts exist and undergo differential splicing while 5′ RACE (rapid amplification of cDNA ends) and S1 mapping have shown that in the *EVI1* locus the transcriptional start sites of *EVI1* are within a few kilobases upstream of exon 1 (18), we cannot rule out the possibility that the *MDS1* sequences represent even more 5′ previously unreported exon(s) of *EVI1*.

Although the cases we have examined contain complex fusion transcripts, the roles of the individual transcripts in leukemia are not known. The common occurrence of carboxyl truncations in AML1 in t(3;21) and t(8;21) strongly implicates alterations in this gene as an important etiologic component in these cases of AML. Based on insights obtained from the analysis of other fusion transcripts, the sequences that are contributed by the other fusion partner are important in leukemogenesis. The fact that the fusions with EAP alter the reading frame and only contribute 17 amino acids is of unknown functional significance. Nevertheless, when studies are designed to establish the transforming activity of *AML1*, it will be important to consider the various fusions that have been identified.

It is possible that, in addition to producing fusion transcripts, the translocations also alter the chromosomal structure to allow the inappropriate expression of *EAP*, *MDS1*, and *EVI1*. This cannot easily be assessed for *EAP* because a number of highly related loci exist that cannot be easily distinguished. In the case of *MDS1*, we have found that this transcript is not normally expressed in myeloid cells, and therefore it will be important to determine whether *MDS1* is expressed in other cases of AML. In the one case in which we detected *EVI1* transcripts, levels of transcripts detected with internal probes were much higher than those detected with the probes for the fusion transcript. Therefore, in the case of the *EVI1* gene, transcriptional activation may occur at sites other than the *AML1* promoter.

That this complex fusion event is an important component of the leukemogenesis process is supported by the detection of multiple fusions in all of the patients studied. How these two (or three) genes interact to further leukemogenesis is unclear. All of these patients had prior chemotherapy either for a previous cancer or for CML in the chronic phase. The t(8;21) has also been observed in AML developing in patients previously treated for cancer. Therefore, *AML1* is susceptible to mutagen-induced translocations. The t(3;3)(q21;q26) has also been observed in patients who had an occupational exposure to mutagenic agents (17). The role of prior chemotherapy in leading to the 3;21 translocation remains to be determined.

Note Added in Proof. After submission of this work, Mitani *et al.* (19) reported that the t(3;21) generates an *AML1–EVI1* fusion gene in CML-BC patients. Multiple chimeric junctions were not detected in these patients.

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