Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 7784–7788, August 1993
Medical Sciences

The 3;21 translocation in myelodysplasia results in a fusion transcript between the *AML1* gene and the gene for EAP, a highly conserved protein associated with the Epstein–Barr virus small RNA EBER 1

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ABSTRACT **In the 8;21 translocation, the *AML1* gene, located at chromosome band 21q22, is translocated to chromosome 8 (q22), where it is fused to the *ETO* gene and transcribed as a chimeric gene. *AML1* is the human homolog of the recently cloned mouse gene *pebp2αB*, homologous to the DNA binding α subunit of the polyoma enhancer factor *pebp2*. *AML1* is also involved in a translocation with chromosome 3 that is seen in patients with therapy-related acute myeloid leukemia and myelodysplastic syndrome and in chronic myelogenous leukemia in blast crisis. We have isolated a fusion cDNA clone from a t(3;21) library derived from a patient with therapy-related myelodysplastic syndrome; this clone contains sequences from *AML1* and from *EAP*, which we have now localized to band 3q26. EAP has previously been characterized as a highly expressed small nuclear protein of 128 residues (EBER 1) associated with Epstein–Barr virus small RNA. The fusion clone contains the DNA binding 5′ part of *AML1* that is fused to *ETO* in the t(8;21) and, in addition, at least one other exon. The translocation replaces the last nine codons of *AML1* with the last 96 codons of *EAP*. The fusion does not maintain the correct reading frame of *EAP* and may not lead to a functional chimeric protein.**

Malignant cells from the majority of patients with therapy-related myelodysplastic syndrome (t-MDS) or acute myeloid leukemia (t-AML) have acquired clonal chromosomal abnormalities (1). An identical reciprocal translocation between the long arm of chromosomes 3 and 21, at bands 3q26 and 21q22, occurs in a small number of these patients (1) as well as in some patients with chronic myeloid leukemia in blast crisis (2). By analogy with other chromosomal translocations in human leukemias that have been studied at the molecular level, the consistency of the breakpoints in the t(3;21) implies that specific genes in bands 3q26 and 21q22 participate in this rearrangement. Band 21q22 is involved in a number of other chromosomal translocations in leukemic patients, and we (3) and others (4) have recently reported that the *AML1* gene, located at 21q22, was involved in all of the patients with the t(8;21) and AML-M2 whom we examined (3). Several *AML1* alternative messages between 2 and 8 kb in size have been identified by Northern blot analysis of lymphoid tissues and cell lines (4). Three of these messages have been cloned and sequenced as cDNAs. The first *AML1* cDNA reported coded for a 250-aa polypeptide, AML1, with striking similarity (69% amino acid identity over 118 residues) in the central part of the molecule to the *Drosophila melanogaster* segmentation gene *runt* (5, 6). The second cDNA, pF4-7b, was reported by our laboratory; this clone was different from *AML1* at the 3′ end, where the nine terminal codons of *AML1* were replaced by 16 different codons in pF4-7b (7). The third cDNA, *pebp2αB*, was cloned from a mouse B-cell library and is expressed in murine T and B cells and in most cell lines. pebp2αB is 99% homologous at the amino acid level to human AML1 encoded by pF4-7B but continues after the stop codon of pF4-7B with a long region coding for abundant serine, proline, and threonine residues. This additional region has 54% homology to the α subunit of the polyoma enhancer binding protein PEBP2 (8). All three alternative cDNAs share the same 5′-end region that includes the *runt* homology segment coding for a motif with DNA-binding properties (8).

AML1 is transcribed from telomere to centromere (4), and the 8;21 translocation produces a chimeric gene transcribed on the der(8) chromosome that contains the 5′ region of *AML1*, including the segment homologous to *runt*, fused to the 3′ region of *ETO* (5). The junction of the chimeric transcript has been detected consistently by the reverse transcription–polymerase chain reaction (RT–PCR) in AML-M2 patients with the t(8;21) (3), indicating that the breakpoints on the two chromosomes occur in the same introns of *AML1* and *ETO*. Recently, we reported (7) that the pF4-7b cDNA is split in t(3;21) patients who have t-MDS/t-AML or chronic myeloid leukemia in blast crisis. Two distinct clusters of breakpoints in *AML1* in the t(8;21) and the t(3;21) are separated by 40–60 kb and by at least one exon of *AML1* (7). By analogy with the t(8;21) in which the translocation produces a fusion gene transcribed on the der(8) chromosome, we hypothesized that the t(3;21) could also result in a chimeric gene. The *AML1* segment including the promoter and the homology to *runt* would be maintained in a putative chimeric transcript originating on the der(3) chromosome.

To investigate this possibility, we constructed and screened a cDNA library from the polyadenylylated RNA of a t(3;21) t-MDS patient. We report here on the isolation and characterization of a fusion transcript between the predicted region of *AML1* and a previously characterized gene, *EAP*, that we have located on chromosome 3.§ EAP is a protein that is highly expressed in all cell lines and tissues analyzed. It recognizes a conserved stem loop in the Epstein–Barr virus small RNA (EBER 1) (9, 10) and has recently been identified as the ribosomal protein L22 (David Toczyski, Greg Matera, David Ward, and Joan Steitz, personal communication).

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Abbreviations: AML, acute myeloid leukemia; t-MDS, therapy-related myelodysplastic syndrome; RT–PCR, reverse transcription–polymerase chain reaction.

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§The sequence reported in this paper has been deposited in the GenBank data base (accession no. L21756).

MATERIALS AND METHODS

Cells and Cultures. Peripheral blood cells from a patient with t-MDS and the t(3;21) were used for the construction of the cDNA library and for RT–PCR analysis. This patient has been described as patient 2 in ref. 7. Three Chinese hamster ovary (CHO)–human somatic cell hybrids were used in this study. The hybrid cell lines were 6918 (11), containing the short (p) arm of human chromosome 21 and that part of the long (q) arm of human chromosome 21 contained between the markers *D21S58* and *D21S17*, where the *AML1* locus has been located (12); UCH12 (13), containing human chromosome arm 3q; and UCTP2A3, containing the entire chromosome 3 as the only recognizable human material (H.A.D., unpublished data). In addition, the myeloid cell lines U937 and ML-1 and the CHO cell line UC2 were used. The CHO–human somatic cell hybrids were propagated in F12 medium supplemented with 5% (vol/vol) dialyzed fetal bovine serum. U937, ML-1, and UC2 cells were grown in RPMI 1640 medium supplemented with 5% fetal bovine serum. UC2 cells required added uridine for growth.

DNA and RNA Isolation and Southern and Northern Blot Analysis. DNA was extracted and manipulated as described (3). Polyadenylylated RNA was prepared from cell cultures or from the t(3;21) patient's frozen viable peripheral blood cell samples by use of the FastTrack mRNA preparation kit (Invitrogen) under conditions recommended by the manufacturer. Southern and Northern blot analyses were performed as described (14).

Construction and Screening of the cDNA Library. A cDNA library was constructed in λ Zap II (Stratagene) using the unidirectional cDNA cloning kit under conditions recommended by the supplier. The 0.6-kb probe used for screening of the library was AML1-0.6; it was PCR-amplified from plasmid pF4-9b (5) with primers I and II (Fig. 1*A*) and contained the promoter-proximal region of an *AML1* cDNA predicted to be maintained in a t(3;21) chimeric transcript (7). Conditions for PCR amplification have been described (3). The plasmids were rescued from the positive phage clones by using the *in vivo* excision protocol provided by the manufacturer.

cDNA Sequencing. Sequencing was performed with the Sequenase kit (United States Biochemical) according to Sanger's method with vector- and sequence-specific primers.

RESULTS

Identification and Characterization of Chimeric cDNA Clones. We screened 6.9×10^5 plaques of the t(3;21) cDNA library with the ^{32}P-labeled AML1-0.6 probe. Thirteen positive clones were identified and purified. Inserts were excised by restriction digestion and used as probes for Southern blot analysis of DNA from germ-line and somatic-cell hybrids. Three of the clones, pCRB1, pCRB5, and pCRB10B, hybridized to germ-line human DNA and to the DNA of the three CHO–human somatic cell hybrids UCH12, UCTP2A3, and 6918 (data not shown). The inserts were sequenced, and Fig. 1*A* shows the alignment of the nucleotide sequence of pCRB1 with the sequences of *AML1* and *EAP*, starting with the putative ATG codons. The diagrams of various normal and chimeric cDNAs are compared in Fig. 1*B*. pCRB1, pCRB5, and pCRB10B contained the same region of the published

A

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AML1   769          ATGCGTATCCCCGTA**********************************
pCRB1    1    ATGAATCCTTCTAGAGACGTCCACGATGCCAGCACGAGCCGCCGCTTCACGCCGCCTTCCA

AML1   821    ************************************************************
pCRB1   62    CCGCGCTGAGCCCAGGCAAGATGAGCGAGGCGTTGCCGCTGGGCGCCCCGGACGCCGGCG

AML1   881    ************************************************************
pCRB1  122    CTGCCCTGGCCGGCAAGCTGAGGAGCGGCGACCGCAGCATGGTGGAGGTGCTGGCCGACC
               ----------------> primer I

AML1   941    ************************************************************
pCRB1  182    ACCCGGGCGAGCTGGTGCGCACCGACAGCCCCAACTTCCTCTGCTCCGTGCTGCCTACGC

AML1  1001    ************************************************************
pCRB1  242    ACTGGCGCTGCAACAAGACCCTGCCCATCGCTTTCAAGGTGGTGGCCCTAGGGGATGTTC

AML1  1061    ************************************************************
pCRB1  302    CAGATGGCACTCTGGTCACTGTGATGGCTGGCAATGATGAAAACTACTCGGCTGAGCTGA

AML1  1121    ************************************************************
pCRB1  362    GAAATGCTACCGCAGCCATGAAGAACCAGGTTGCAAGATTTAATGACCTCAGGTTTGTCG
                                    primer V ----------------->

AML1  1181    ************************************************************
pCRB1  422    GTCGAAGTGGAAGAGGGAAAAGCTTCACTCTGACCATCACTGTCTTCACAAACCCACCGC

AML1  1241    ************************************************************
pCRB1  482    AAGTCGCCACCTACCACAGAGCCATCAAAATCACAGTGGATGGGCCCCGAGAACCTCGAA

AML1  1301    ************************************************************
pCRB1  542    GACATCGGCAGAAACTAGATGATCAGACCAAGCCCGGGAGCTTGTCCTTTTCCGAGCGGC

AML1  1361    ************************************************************
pCRB1  602    TCAGTGAACTGGAGCAGCTGCGGCGCACAGCCATGAGGGTCAGCCCACACCACCCAGCCC
EAP     52                                          ATGGCTCCTGTGAAAAAGCTTGT

AML1  1421    ************************************************************
pCRB1  662    CCACGCCCAACCCTCGTGCCTCCCTGAACCACTCCACTGCCTTTAACCCTCAGCCTCAGA
EAP     75    GGTGAAGGGGGGCAAAAAAAAGAAGCAAGTTCTGAAGTTCACTCTTGATTGCACCCACCC
                                                                      <---
                      ↓ ----------------> primer III
AML1  1481    ************GGAAGACACAGCACCCTGGAGATGTTAA
pCRB1  722    GTCAGATGCAGGAATCATGGATGCTGCCAATTTTGAGCAGTTTTTGCAAGAAAGGATCAA
EAP    135    TGTAGAAGATGG**********************************************
               ------------ primer II

pCRB1  782    AGTGAACGGAAAAGCTGGGAACCTTGGTGGAGGGGTGGTGACCATCGAAAGGAGCAAGAG
EAP    195    ************************************************************
                                        primer VI <----------------

pCRB1  842    CAAGATCACCGTGACATCCGAGGTGCCTTTCTCCAAAAGGTATTTGAAATATCTCACCAA
EAP    255    ************************************************************

pCRB1  902    AAAATATTTGAAGAAGAATAATCTACGTGACTGGTTGCGCGTAGTTGCTAACAGCAAAGA
EAP    315    ************************************************************

pCRB1  962    GAGTTACGAATTACGTTACTTCCAGATTAACCAGGACGAAGAAGAGGAGGAAGACGAGGA
EAP    375    ************************************************************
                                              primer IV <-----------

pCRB1 1022    TTAA
EAP    435    TTAA
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B

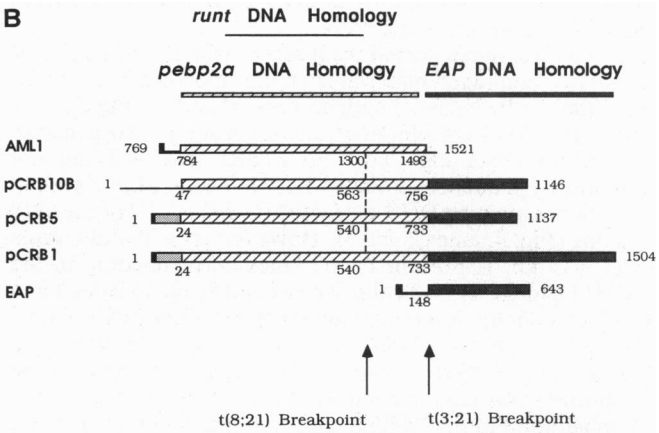

FIG. 1. (*A*) Alignment of pCRB1, *AML1*, and *EAP* nucleotide sequences. The sequences shown include the coding regions from the initiation to the termination codons (underlined). Only those nucleotides different from the pCRB1 sequence are indicated. Asterisks indicate identities. Numbering of the *AML1* and *EAP* sequences is as in refs. 4 and 9. The vertical arrow indicates the *AML1–EAP* cDNA junction after the t(3;21). The horizontal arrows indicate the position and the sequence (I, III, and V) or the complementary sequence (II, IV, and VI) of the primers used for PCR amplification. (*B*) Schematic sequence comparison of *AML1*, *EAP*, and the chimeric inserts of plasmids pCRB1, pCRB5, and pCRB10B. All of the sequences except that of pCRB10B begin with the starting ATG, indicated by a shaded box. Hatched boxes indicate homology with the gene for polyoma transcriptional factor *pebp2α*. Shaded segments indicate homology to *EAP*. Stippled bars in plasmids pCRB5 and pCRB1 are homologous but are not contained in *pebp2α* or pCRB10B. The thin line at the top of the diagram indicates the approximate region of homology with *runt*. Thick and thin lines indicate unique regions in the sequence. The two vertical arrows show the position of the breakpoint in the cDNA in the 8;21 and 3;21 translocations. Relevant nucleotide numbers of the published sequences of *AML1* and *EAP* are indicated (4, 9).

AML1 sequence from nt 784 to nt 1493 (Fig. 1*A*). The sequence upstream of position 784 was the same for pCRB1 and pCRB5. The sequence of pCRB10B was different, and the cloned fragment did not contain an ATG codon. Downstream of nt 1493 of *AML1*, the sequence of the three plasmids was identical but diverged from that of *AML1*. An NIH BLAST search of GenBank DNA sequences detected a 100% homology between these identical 300 bp distal to nt 1493 of *AML1* and a previously sequenced cDNA encoded by the *EAP* gene (9) (Fig. 1). All three plasmids contained the entire coding region of *EAP* as published (9). However, the 3′ ends of pCRB5 and pCRB10B were 14 nt apart and a few nucleotides past the stop codon, whereas pCRB1 continued for an additional 0.6 kb of the untranslated region (Fig. 1*B*; sequence not shown). This region is identical to the first 0.6 kb of the untranslated region of an *EAP* cDNA sequenced by David Toczyski and Joan Steitz (personal communication).

EAP codes for a small cellular protein associated with the Epstein–Barr virus small RNA EBER 1 (10) that was recently identified as the ribosomal protein L22 (David Toczyski, Greg Matera, David Ward, and Joan Steitz, personal communication). Analysis of the sequence junction indicated that the translocation had interrupted *EAP* at nt 148 and fused it to the 3′ end of *AML1* at nt 1494. The sequenced *AML1–EAP* junction, identical in all three chimeric plasmids, is shown in Fig. 2. Surprisingly, however, the fusion between the two genes was such that the exons of *EAP* were not in the same reading frame as *AML1*, and the fusion peptide contained 17 aa not related to the EAP. Fig. 3*A* shows the alignment of the putative amino acid sequences of AML1 and AML1–EAP, and Fig. 3*B* is a schematic diagram of the polypeptides derived from the various *AML1* cDNAs, from *EAP*, and from the *AML1–EAP* chimeric cDNA.

To confirm that a part of the insert of pCRB1, pCRB5, and pCRB10B contained material from chromosome 3, we PCR-amplified probe EAP-0.3 with primers III and IV (Fig. 1*A*) for use in the Southern blot analysis of germ-line and somatic-cell-hybrid DNA digested with *Eco*RI. EAP-0.3 did not contain any sequences from *AML1*. No bands were visible in the lanes containing DNA extracted from the UC2 or the 6918 cell line (Fig. 4, lanes 2 and 3). However, a band of the same size, ≈15 kb, is present in the lanes corresponding to the UCH12 and UCTP2A3 cells (lanes 4 and 5) and in lanes 1 and 6, which contain different amounts of germ-line DNA. Thus, this portion of the cDNA clone is, in fact, derived from chromosome 3. Hybridization of the EAP-0.3 probe to the germ-line DNA gave a complex pattern (Fig. 4, lanes 1 and 6); in addition to the 15-kb band present in lanes 4 and 5, we

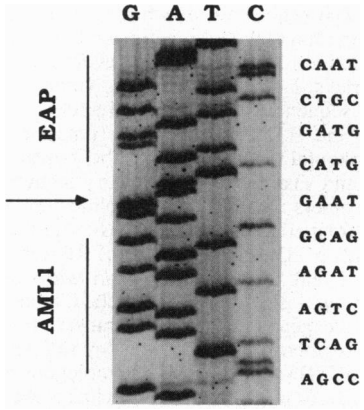

FIG. 2. Sequence analysis of the *AML1–EAP* junction in plasmid pCRB1. Lanes G, A, T, and C indicate the dideoxynucleotide sequencing track; the nucleotide sequence is read from bottom to top and from left to right; the breakpoint junction of the fusion transcript *AML1–EAP* is indicated by an arrow in the left margin.

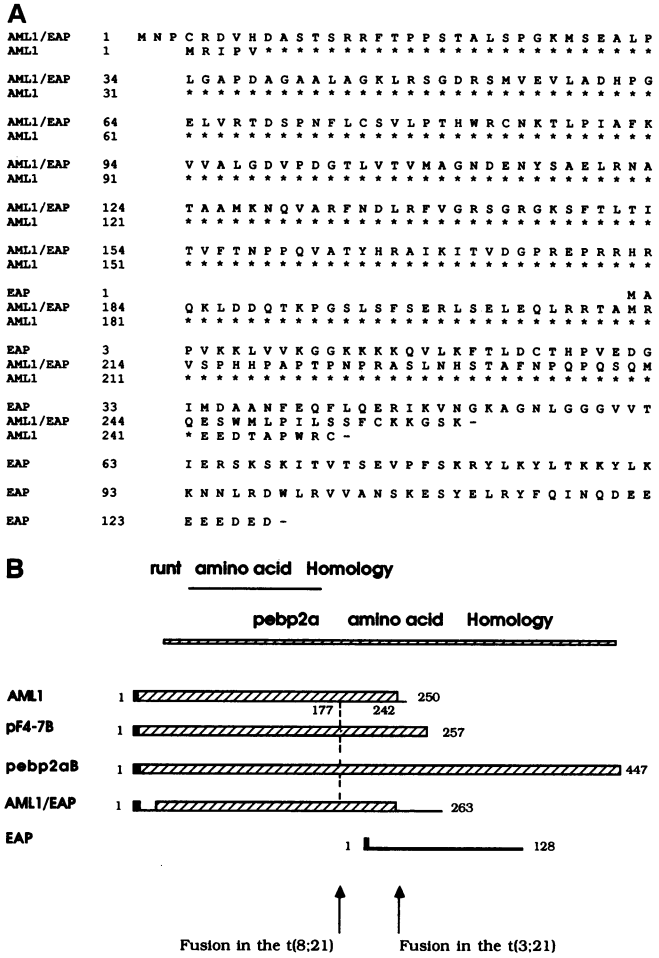

FIG. 3. (*A*) Alignment of the predicted amino acid sequences of EAP, AML1–EAP, and AML1. Only those residues that are not conserved between the three sequences are shown. Asterisks indicate identities. Numbering of EAP and AML1 is as in refs. 4 and 9. (*B*) Amino acid sequence comparison of AML1, pF4-7B, pebp2αB, EAP, and AML1–EAP. Common characteristics of the polypeptides are shown diagrammatically. Hatched boxes indicate homology to pebp2α. The thin line at the top of the diagram indicates the region of homology to runt. Thick and thin single lines indicate unique sequences. Solid boxes indicate the position of the starting methionine. The two vertical arrows indicate the position of the peptide fusion after the 8;21 or 3;21 translocation. Numbering of AML1, EAP, and pebp2αB is as in refs. 4, 8, and 9.

consistently identified at least 13 bands varying in size from 1.5 kb to >24 kb, suggesting that the probe hybridizes to a family of highly homologous genes. Results supporting the hypothesis that *EAP* belongs to a family of highly homologous genes were obtained by Kostyantin Soldatkin, David Toczyski, and Joan Steitz (personal communication) with the cloning of an *EAP* pseudogene.

RT–PCR Detection of a Chimeric Transcript from the t(3;21) t-MDS Patient's mRNA. One of the most convincing experiments proving that a chimeric gene is indeed transcribed in a cell with a specific chromosomal translocation is the Northern blot analysis of RNA extracted from normal cells and from cells harboring the translocation. We had only 1×10^7 peripheral blood cells available from the patient. We used most of this material and the cell lines U937 and ML-1 to prepare RNA for Northern blot analysis. The probe was EAP-0.3. Fig. 5 shows the results; not surprisingly, because of the very limited number of viable cells from the t(3;21) patient, no hybridization was evident in the patient's lane (Fig. 5, lane 3). Three bands of ≈0.6, 1.2, and 2.2 kb were

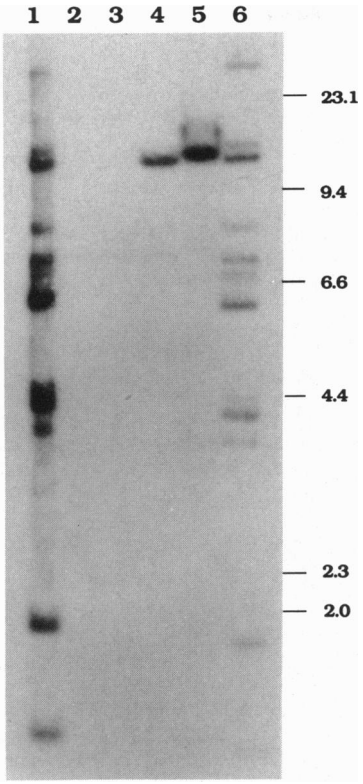

FIG. 4. Southern blot of *Eco*RI-digested DNA, demonstrating that the 0.3-kb fragment amplified from the cDNA of plasmid pCRB1 contains sequences from chromosome 3. Lanes: 1, germ-line DNA (13 μg); 2, UC2, CHO DNA only; 3, 6918, chromosome 21 DNA; 4 and 5, UCH12 and UCTP2A3, chromosome 3 DNA; 6, germ-line DNA (4 μg). The probe used was PCR-amplified from plasmid pCRB1 with primers III and IV (Fig. 1*A*). DNA size markers are indicated on the right side in kb.

detected for U937 (Fig. 5, lane 1) and ML-1 (Fig. 5, lane 2). These bands could represent either alternative EAP mRNAs or hybridization to the message of other genes of the family to which *EAP* belongs. To detect the chimeric transcript in the patient's cells, we used RT–PCR amplification of polyadenylylated RNA directly from a peripheral blood sample obtained from the patient at diagnosis. The location and sequence of the primers V and VI used for the amplification are indicated in Fig. 1*A*. We used ML-1 as a negative control and plasmid pCRB5 as a positive control in the RT–PCR. Fig. 6 shows the autoradiogram of the PCR-amplified cDNA from plasmid pCRB5 (lanes 1 and 2), from the patient (lanes 3 and 4), and for the cell line ML-1 (lane 5). The *AML1–EAP* probe was obtained by PCR amplification of pCRB1 with primers III and IV. The expected chimeric band of 0.46 kb was amplified correctly and hybridized to the *AML1–EAP* probe only for plasmid pCRB5 (Fig. 6, lanes 1 and 2) and for the patient's sample (Fig. 6, lanes 3 and 4) but not for the cell line ML-1 (Fig. 6, lane 5), confirming that the PCR amplification was specific for the chimeric message. Thus, the detection by RT–PCR of the chimeric transcript directly from the patient's polyadenylylated RNA confirmed that the *AML1–EAP* chimeric message was transcribed in the t(3;21) patient's cells. Southern blot analysis of the patient's PCR-amplified sample showed the presence of an additional band ≈0.15 kb larger than the major 0.46-kb band. This band was not visible in the ethidium bromide-stained gel (data not shown) but was weakly detected only by Southern blot analysis, suggesting a very low initial concentration of this cDNA. We have been unable to clone and to analyze this fragment.

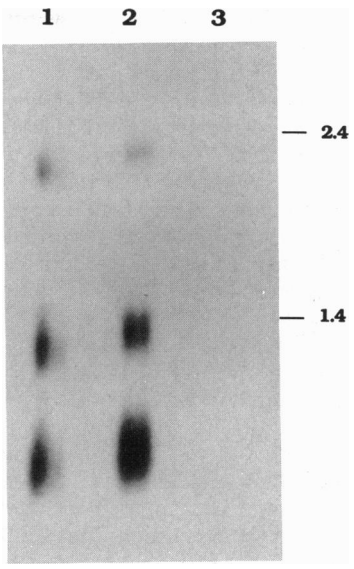

FIG. 5. Northern blot analysis of *EAP* expression. Polyadenylylated RNA isolated from U937 (lane 1), ML-1 (lane 2), or the t(3;21) patient's peripheral blood cells (lane 3) was hybridized to probe EAP-0.3. The position of RNA size markers is indicated on the right side in kb.

DISCUSSION

We have isolated a fusion cDNA from the abnormal cells of a t-MDS patient with the t(3;21). Our studies establish that the fusion occurs between two previously described genes, *AML1* on chromosome 21 and *EAP*. By Southern blot analysis, we have localized *EAP* to the long arm of chromosome 3. This is the second identified fusion gene involving *AML1* that is produced by a translocation, the first being the chimeric *AML1–ETO* cDNA isolated from a t(8;21) patient (5). Thus, *AML1* is a member of a small family of genes that produce different fusion transcripts as a consequence of translocations with more than one chromosome. For example, *E2A*, located at 19p13, can fuse to the *PBX1* gene in the t(1;19) (15) or to the *HLF* gene in the t(17;19) (16). The resulting E2A–PBX1 or E2A–HLF proteins are structurally similar in that the breakpoints occur in the same intron of *E2A* and maintain the transcription activation domain of E2A, whereas the DNA-binding domain of E2A is replaced by the homeobox region of PBX1 or by the basic zipper domain of HLF. Another example is the *MLL* gene, located at 11q23. *MLL* participates in a large number of common and rare chromosomal abnormalities, and one study has shown (17) that a 0.7-kb *MLL* cDNA probe could detect rearrangements in all of the leukemia patients or cell lines analyzed with >21

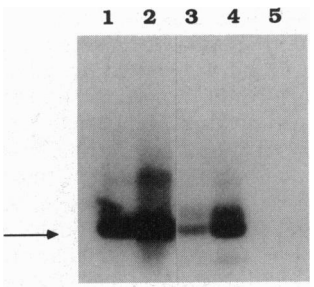

FIG. 6. Autoradiogram of the PCR-amplified *AML1–EAP* cDNA junction by use of primers V and VI from plasmid pCRB5 (lane 1, 2 μl; lane 2, 15 μl), from the patient's RNA (lane 3, 2 μl; lane 4, 15 μl), and from the cell line ML-1 (lane 5, 20 μl). The arrow indicates the expected size of the chimeric fragment. The probe used was PCR-amplified from plasmid pCRB1 with primers V and VI (Fig. 1*A*).

translocations involving 11q23 (17). This indicates that the breakpoints in *MLL* occur within an 8.3-kb *Bam*HI fragment of the gene (17). The breakpoint junctions that have been examined all lead to a fusion gene (18, 19).

AML1 appears to have more variable breakpoints. The fusion of *AML1* with *ETO* occurs just downstream of the *runt* homology, fusing a truncated *AML1* to *ETO* sequences. The *runt* homology segment coding for a motif with a DNA-binding property (8) is maintained, but the region homologous to the polyoma enhancer binding protein PEBP2 with transcription activation properties would be completely replaced by ETO sequences not yet characterized. The fusion of *AML1* to *EAP* in the t(3;21) occurs farther downstream in *AML1* but at a site of *AML1* that would still include the *runt* homology and exclude the *pebp2* homology. EAP is a small nuclear polypeptide of 128 aa that is abundant in all cells and was recently identified as the ribosomal protein L22 (David Toczyski, Greg Matera, David Ward, and Joan Steitz, personal communication). The polypeptide contains a nuclear localization signal at the N terminus that would be lost in the chimeric product with *AML1*. EAP is a highly hydrophilic molecule, with several charged residues, and ends with an unusual continuous stretch of nine aspartate and glutamate residues (9).

In the three chimeric cDNAs that we have sequenced, the fusion of *AML1* to *EAP* does not maintain the correct reading frame in the *EAP* exons; the putative chimeric polypeptide translated from our sequence would contain 17 out-of-frame residues with no homology to the EAP polypeptide fused to the truncated AML1.

There are three major hypotheses to explain how the *AML1–EAP* chimeric transcript can affect the cell. The fusion could result in a stable *AML1–EAP* transcript containing only the DNA-binding encoding region of *AML1*. This would result in the preferential accumulation in the cell of a truncated form of AML1 lacking the homology to PEBP2 that presumably contains the transcription-activating domain of the polypeptide. As a consequence, the truncated product could function as a dominant negative inhibitor of AML1. Alternatively, it is possible that the chimeric cDNA clone that we have isolated and sequenced represents the most abundant transcript but not the active one; the Southern blot analysis of the t(3;21) patient's polyadenylylated RNA after RT–PCR has shown the existence of a second larger band besides the expected junction fragment. It is possible that this fragment contains additional sequences, from either *AML1* or *EAP*, that would join the two genes in the correct reading frame. A last alternative to be considered is that ribosome frame shifting could occur during translation of the chimeric message. This nontriplet ribosome movement is a relatively frequent translation-reading anomaly that occurs in bacteria and in eukaryotic cells (20, 21) when stretches of identical ribonucleotides, such as those found in the *EAP* sequence, are read or certain aminoacyl-tRNAs are limiting (22). In either case, the binding of an incorrect aminoacyl-tRNA disturbs the translocation process in the ribosome, shifting the ribosome's reading frame either to the right or to the left. As a consequence, it could realign the *EAP* reading frame to that of *AML1*. At present, we have no clues as to the oncogenic potential of such a hypothetical chimeric molecule or where in the cell it would be active. However, fusion to a ribosomal protein can activate a cellular protooncogene, as shown by the activation of the human *TRK* protooncogene by recombination with the ribosomal large-subunit protein L7a (23).

We thank Dr. Stefan Bohlander for help with the computer search of the sequence and Dr. David Toczyski and Dr. Joan Steitz for helpful discussions. We thank Dr. Raju Chaganti and Dr. Eli Canaani for their critical review of this manuscript. This work was supported by The G. Harold and Leila Y. Mathers Charitable Foundation (J.D.R.), by Department of Energy Grant DE-FG02-86ER60408 (J.D.R.), and National Institutes of Health Grants CA 42557 (J.D.R.) and PO1-HD017449 (H.D.). G.N. was supported by National Institutes of Health Training Grant 5T32CA09273-14.

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