MOLECULAR AND CELLULAR BIOLOGY, Apr. 1996, p. 1349–1355 Vol. 16, No. 4
0270-7306/96/$04.00+0

The t(12;21) Translocation Converts AML-1B from an Activator to a Repressor of Transcription

SCOTT W. HIEBERT,[1]* WUHUA SUN,[1] J. NATHAN DAVIS,[1] TODD GOLUB,[2] SHEILA SHURTLEFF,[3] ARJAN BUIJS,[4] JAMES R. DOWNING,[1,3] GERARD GROSVELD,[4] MARTINE F. ROUSSEL,[1] D. GARY GILLILAND,[2] NOEL LENNY,[1] AND SHARI MEYERS[1]

Departments of Tumor Cell Biology,[1] Pathology,[3] and Genetics,[4] St. Jude Children's Research Hospital, Memphis, Tennessee 38105, and Division of Hematology-Oncology, Brigham and Women's Hospital, and Division of Pediatric Hematology/Oncology, Children's Hospital and Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts 02115[2]

Received 27 October 1995/Returned for modification 20 December 1995/Accepted 4 January 1996

The t(12;21) translocation is present in up to 30% of childhood B-cell acute lymphoblastic leukemias and fuses a potential dimerization motif from the *ets*-related factor *TEL* to the N terminus of *AML1*. The t(12;21) translocation encodes a 93-kDa fusion protein that localizes to a high-salt- and detergent-resistant nuclear compartment. This protein binds the enhancer core motif, TGTGGT, and interacts with the AML-1-binding protein, core-binding factor beta. Although TEL/AML-1B retains the C-terminal domain of AML-1B that is required for transactivation of the T-cell receptor beta enhancer, it fails to activate transcription but rather inhibits the basal activity of this enhancer. TEL/AML-1B efficiently interferes with AML-1B-dependent transactivation of the T-cell receptor beta enhancer, and coexpression of wild-type TEL does not reverse this inhibition. The N-terminal TEL helix-loop-helix domain is essential for TEL/AML-1B-mediated repression. Thus, the t(12;21) fusion protein dominantly interferes with AML-1B-dependent transcription, suggesting that the inhibition of expression of AML-1 target genes is critical for B-cell leukemogenesis.

AML1 is one of the most frequently mutated genes in human leukemia and is targeted either directly or indirectly in t(8;21), t(3;21), t(12;21), and inv(16). t(8;21) and inv(16) are present in up to 30% of de novo acute myeloid leukemias (AML) and target the two components of the AML-1 transcription factor complex, AML-1 and core-binding factor beta (CBFβ). In the t(8;21) translocation, AML-1 sequences including the DNA binding domain are fused to *ETO* (*MTG8*), a gene encoding the human homolog of the *Drosophila* gene *nervy* (7, 9, 33, 35). inv(16) fuses the non-DNA-binding factor CBFβ to a smooth muscle myosin heavy-chain gene, *MHY11* (25). t(3;21) is rare in de novo AML but is found in therapy-related AML and myelodysplasias and fuses AML-1 to three different genes, including the gene encoding the Evi-1 transcription regulator (32, 34, 36, 37, 44, 45). t(12;21) is present in up to 30% of childhood B-cell acute lymphoblastic leukemias and fuses a potential dimerization motif from the *ets*-related factor *TEL* to the N terminus of *AML1* (11, 12, 42, 43, 47). Thus, AML-1 is altered in both myeloid and B-cell acute leukemias.

Two independent lines of investigation identified *AML1* as an important regulator of transcription. The cloning of the t(8;21) breakpoint led to the isolation of the AML-1 cDNA clone and the realization that AML-1 could be the human homolog of a *Drosophila* pair-rule protein encoded by *runt* (5, 7). Recombinant AML-1 proteins were used to demonstrate that the *runt* homology domain of AML-1 is responsible for DNA binding of the protein and that AML-1 recognizes an important regulatory element, the enhancer core motif, TGTGGT (26, 27). Concurrently, the murine proteins that bind the enhancer core motif were purified, which led to the isolation of cDNA clones encoding the murine homolog of AML-1 (PEBP2αB or CBFα) as well as the highly related protein PEBP2αA1 (AML-3) (2, 23, 38, 53). Subsequently, low-stringency hybridization was used to identify a third family member, AML-2 (23). The cloning of the murine proteins indicated that the original AML-1 cDNA clone was a small, alternatively spliced mRNA; a larger, transcriptionally active form has been isolated and termed AML-1B (23, 30).

The enhancer core motif is required for the tissue-specific transcription of a number of genes, including those encoding the T-cell receptor (TCR) alpha, beta, gamma, and delta enhancers, myeloperoxidase, granulocyte-macrophage colony-stimulating factor, and interleukin-3 (reviewed in references 28 and 48). Although the enhancer core sequence is required for transcription of these genes, when attached to a heterologous promoter it cannot direct tissue-specific expression (28, 48). Likewise, AML-1B cannot activate an artificial promoter containing enhancer core binding sites (29, 30). Moreover, AML-1B is not expressed in a tissue-specific manner (31). Thus, it is possible that AML-1B and related proteins act as enhancer or promoter organizers to recruit tissue-specific factors leading to the activation of transcription. In each gene regulated by enhancer core motifs, flanking sites for ets or myb family transcription factors, which are also required for tissue-specific expression, are present. AML-1B may recruit these factors through physical interactions (10, 54).

Chromosomal translocations often target master regulatory genes (39, 40). The targeting of AML-1 by the t(12;21) translocation in pre-B-cell acute leukemia implies that AML-1 plays an important regulatory role in B cells (31). Although both *TEL/AML1B* and *AML1B/TEL* chimeric genes are formed as a result of this translocation, only the *TEL/AML1B* mRNA is consistently detected in these cases. This product results in the fusion of the first 333 amino acids of TEL, encompassing a putative helix-loop-helix domain but not the DNA binding domain, to residues 21 to 479 of AML-1B. We have demonstrated that the chimeric mRNA produces a 93-kDa fusion protein that fails to activate transcription from the TCRβ en-

* Corresponding author. Phone: (901) 495-2082. Fax: (901) 495-2381.

hancer but rather inhibits the basal activity of the enhancer. TEL/AML-1 efficiently interferes with AML-1B-dependent transactivation of the TCRβ enhancer, and the TEL helix-loop-helix domain is required for this function. Thus, TEL/AML-1B is a dominant interfering protein for AML-1-dependent gene regulation.

MATERIALS AND METHODS

Cells and construction of plasmids. C33A and Cos-7 cells were maintained in Dulbecco modified Eagle medium plus 10% fetal calf serum. The *TEL/AML1* fusion was reconstructed by using reverse transcription-PCR to isolate the breakpoint junction (12, 47), which was ligated to *TEL* and *AML1B* DNA fragments and cloned into the *Eco*RI site of pCMV5. The TEL/AML-1B deletion mutants were made by using PCR with 5′ oligonucleotides starting at amino acid residues 43, 155, and 274 and an AML-1 3′ oligonucleotide starting at amino acid 178 (of AML-1). Each of these mutants contains a methionine codon to initiate translation. The unique *Xba*I site in TEL/AML-1B was used to replace the wild-type 5′ end with the indicated mutant fragment.

Cellular localization of TEL/AML-1B. Cos-7 cells were transiently transfected with pCMV5 expressing AML-1, AML-1B, or TEL/AML-1B. After a 48-h incubation, cytoplasmic cell fractions were prepared by incubating the cells in ice-cold Iso-Hi buffer (140 mM NaCl, 25 mM Tris [pH 7.4], 1.5 mM $MgCl_2$) containing 0.5% Nonidet P-40 for 5 min and then subjecting them to low-speed centrifugation to collect the nuclei. Nuclei were incubated in high-salt (0.5 M NaCl) extraction buffer (6) for 1 h at 4°C. The DNA-particulate fraction was pelleted by microcentrifugation (15,000 rpm), washed once in high-salt buffer, solubilized in radioimmunoprecipitation assay buffer, and sonicated to sheer the DNA. Equal amounts of all fractions were precipitated with trichloroacetic acid, resuspended in protein sample buffer, and separated on a sodium dodecyl sulfate (SDS)–10% polyacrylamide gel. The proteins were detected by using affinity-purified AML-1 antibodies (27) and enhanced chemiluminescence (Amersham).

Synthesis of recombinant proteins and electrophoretic mobility shift assays. Plasmid DNA was transcribed in vitro by using 1 μg of supercoiled DNA and the Promega TNT coupled transcription-translation system. The glutathione *S*-transferase (GST)–CBFβ fusion protein was constructed and purified as described previously (22, 30). DNA binding reactions were performed at room temperature in a buffer containing 20 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES; pH 7.8), 1 mM $MgCl_2$, 0.1 mM ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), 0.4 mM dithiothreitol, 40 mM KCl, and salmon sperm DNA (60 μg/ml). The electrophoretic mobility shift assay was performed as previously described (27). The AML-1 binding site was created by annealing the complementary DNA oligomers 5′-AATTCGAGTATTGTGGT TAATACG-3′ and 5′-AATTCGTATTAACCACAATAAAACTCG-3′ (27). Annealed oligomers were labeled with [^{32}P]dATP, using the Klenow fragment of DNA polymerase I (20). Supershift experiments were performed with rabbit antisera directed against the N-terminal 17 amino acids of AML-1 in the presence or absence of the antigenic peptide (2 μg) (27, 30).

Transcriptional analysis. Construction of the TCRβ-chloramphenicol acetyltransferase (CAT) plasmid, kindly provided by Jeffrey Leiden, University of Chicago, has been previously described (15). Transfection by the calcium phosphate method was performed as described previously (14, 30). A plasmid expressing the gene for secreted alkaline phosphatase (SEAP) driven by the Rous sarcoma virus (RSV) long terminal repeat (LTR) was added (5 μg) as an internal control for transfection efficiency (3). CAT and SEAP activities were measured as described previously (20). CAT activity was quantified on a Molecular Dynamics PhosphorImager with Image-Quant software and was normalized with respect to the SEAP activity. If SEAP activities varied more than 50%, the sample was not used.

RESULTS

Cellular localization of the t(12;21) fusion protein. The t(12;21) chimeric mRNA encodes a predicted fusion protein of 791 amino acids. We reconstructed the full-length cDNA clone of TEL/AML-1B by using PCR fragments that span the t(12;21) breakpoint (12, 47). When expressed in Cos-7 cells, this cDNA produced a protein which migrated at approximately 93 kDa in an SDS-polyacrylamide gel and was detected by an anti-AML-1 serum (Fig. 1A). Because nearly all of AML-1B is retained in the fusion protein and AML-1B localizes to high-salt- and detergent-resistant nuclear fractions characteristic of the nuclear matrix, we determined the cellular localization of TEL/AML-1B. Cos-7 cells were transiently transfected with a plasmid expressing AML-1, AML-1B, or TEL/AML-1B, and the cells were fractionated into cytoplasmic, nuclear, and nuclear/high-salt-resistant fractions. Like AML-1B, TEL/AML-1B partitioned into fractions characteristic of the nuclear matrix (Fig. 1B).

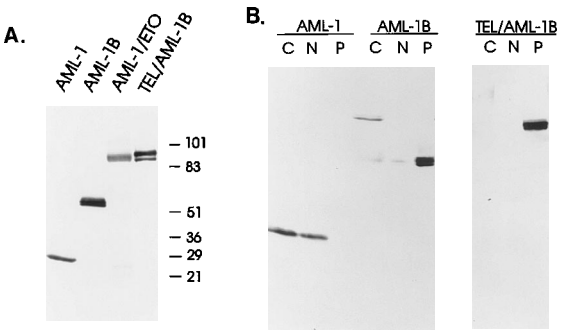

FIG. 1. Expression and cellular localization of the t(12;21) fusion protein. (A) t(12;21) encodes a 93-kDa fusion protein. Cos-7 cells were transiently transfected with the indicated plasmids expressed from the cytomegalovirus immediate-early promoter. Numbers to the right indicate the positions (in thousands) of Bio-Rad prestained molecular weight standards. (B) Cellular localization of TEL/AML-1B. Cos-7 cells were transfected as for panel A and separated into cytoplasmic (C), nuclear (N), and high-salt-resistant pellet (P) fractions. Proteins were detected by Western blot analysis using affinity-purified AML-1 antibodies.

TEL/AML-1B recognizes the enhancer core motif and binds CBFβ. The t(12;21) fusion protein lacks the ets-like DNA binding domain of TEL but retains the AML-1B *runt* homology domain, which mediates DNA binding and CBFβ interactions. Gel mobility shift analysis of TEL/AML-1B produced in vitro indicated that the fusion protein specifically binds the enhancer core motif, TGTGGT (Fig. 2A). The band identified as TEL/AML-1B was absent in unprogrammed reticulocyte lysates, was specifically eliminated with wild-type competitor DNA, and was ablated by the addition of the anti-AML-1 serum but not by the addition of antiserum in the presence of the antigenic peptide. Addition of GST-CBFβ fusion protein resulted in a shift in the migration of the TEL/AML-1B-DNA complex and an increase in DNA binding, indicating that the fusion protein can bind CBFβ and that this interaction enhances TEL/AML-1B DNA binding (Fig. 2B). The low level of TEL/AML-1B DNA binding in the absence of CBFβ may be due to incorrect folding in vitro, and CBFβ binding may help the protein assume the correct conformation.

t(12;21) encodes a dominant interfering protein. The enhancer core motif is necessary but not sufficient for the induction of genes containing this site (28, 48). In many situations, the AML-1 DNA binding site is flanked by sites for other DNA-binding factors, including ets-1, which can cooperate with core-binding factors to enhance DNA binding and transcriptional activity (10, 19, 54, 55). Because t(12;21) fuses the majority of an ets factor to AML-1B, we tested whether TEL/AML-1B could regulate transcription from a test enhancer that is normally controlled by both AML-1B and ets factors. The TCRβ enhancer contains three possible AML-1B binding sites and is activated by AML-1B in transient assays (15, 30, 38). C33A cells were used in these assays because of the low levels of enhancer core DNA binding activities in these cells (30). Comparison of the t(12;21) fusion protein with AML-1B showed that it failed to activate transcription from the TCRβ enhancer linked to the CAT gene (Fig. 3). In fact, in each experiment we observed a marked decrease in CAT activity upon increasing the concentration of TEL/AML-1B-expressing plasmid. At the highest levels of input DNA, transcription was reduced by 80%, suggesting that the fusion protein inhibited the basal activity of the enhancer. Importantly, TEL/AML-1B expression did not affect transcription of the RSV LTR used as an internal control for transfection efficiency.

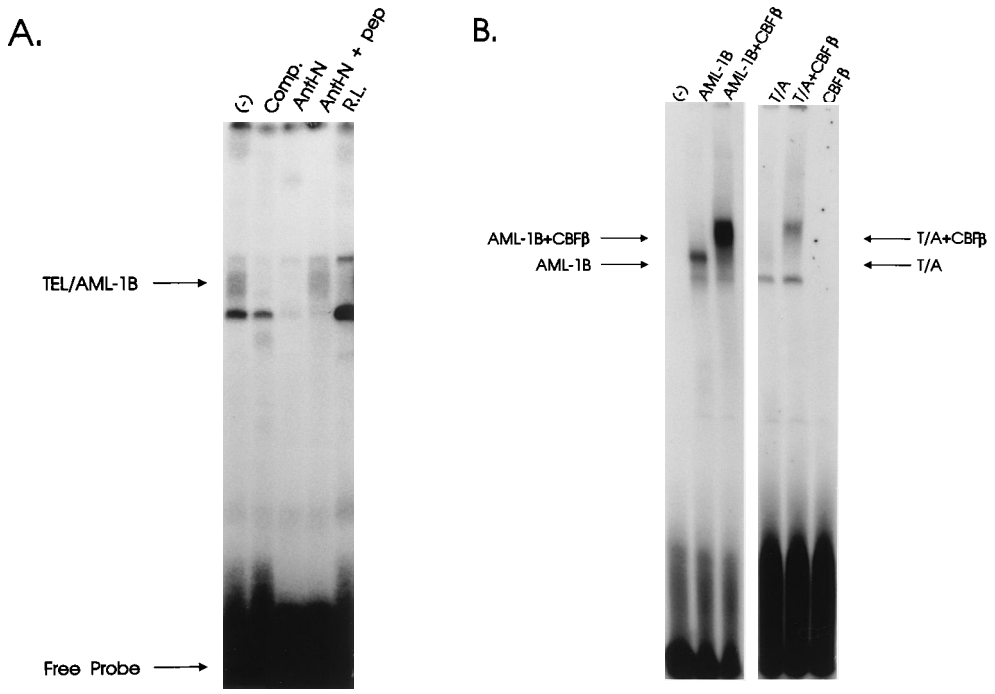

FIG. 2. The t(12;21) fusion protein binds the enhancer core motif and CBFβ. (A) TEL/AML-1B binds the consensus AML-1 binding site. TEL/AML-1B produced in vitro was used in gel mobility shift assays with a consensus AML-1 binding site, TGTGGT (27). TEL/AML-1B programmed lysates were used in the absence [(−)] or presence of specific competitor DNA (comp.), anti-N-terminal AML-1 serum, or antiserum plus antigenic peptide (Anti-N + pep). R.L., unprogrammed reticulocyte lysate. (B) TEL/AML-1B interacts with CBFβ. Reticulocyte lysates programmed with AML-1B or TEL/AML-1B mRNA were assayed by gel mobility shift in the absence or presence of GST-CBFβ. The sizes of the complexes are indicated with arrows. T/A, TEL/AML-1B; (−), no lysate.

The t(8;21) fusion protein, AML-1/ETO, dominantly interferes with AML-1B-dependent transactivation of the TCRβ enhancer (30). Therefore, we tested the ability of TEL/AML-1B to inhibit AML-1B activity. TCRβ expression was stimulated by the addition of wild-type AML-1B, and plasmids expressing TEL/AML-1B were added in increasing amounts (Fig. 4A). At 1 μg of input plasmid, TEL/AML-1B ablated transactivation by AML-1B; at higher levels, the fusion protein suppressed transcription to subbasal levels. Because the t(8;21) fusion protein also inhibits AML-1B function, we directly compared TEL/AML-1B and AML-1/ETO in this interference assay (Fig. 4B). At only 400 ng of input plasmid, both fusion proteins inhibited AML-1B-dependent transcription, indicating that both proteins may repress at substoichiometric levels of DNA. However, consistent with previous results (30), at higher levels AML-1/ETO failed to repress transcription below basal levels (data not shown). Importantly, expression from the internal control reporter plasmid (the RSV LTR) was not affected by either fusion protein.

Like many genes that are regulated by AML-1, the TCRβ enhancer contains ets factor binding sites. Therefore, we tested whether wild-type TEL could activate or repress basal transcription of this enhancer (Fig. 5A). Over a wide range of plasmid concentrations, overexpression of TEL had no effect on CAT activity. Therefore, TEL must be fused to the AML-1B DNA binding domain to inhibit transcription.

The domain of TEL that is fused to AML-1B contains a putative protein interaction motif. In t(5;12), this domain is fused to the platelet-derived growth factor beta receptor tyrosine kinase, and the helix-loop-helix domain of TEL is thought to mediate receptor dimerization and thereby activate protein tyrosine kinase activity (13, 18). This finding, coupled with the finding that the second allele of TEL is frequently

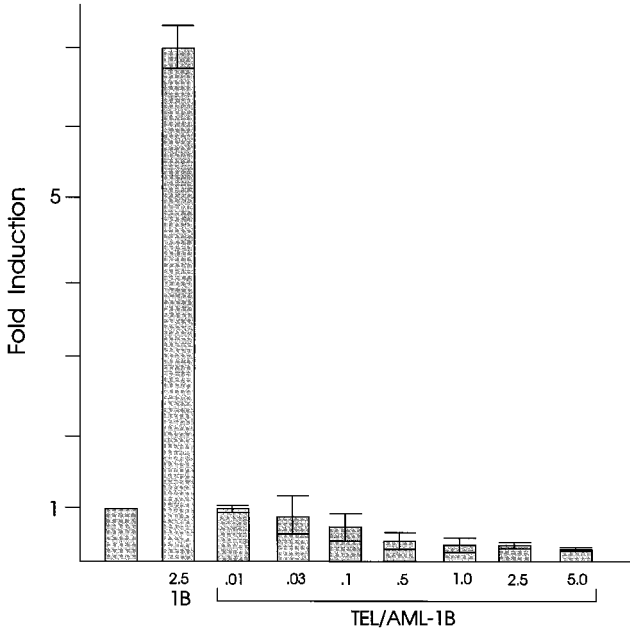

FIG. 3. Transcriptional activity of TEL/AML-1B. Increasing concentrations of pCMV5-TEL/AML-1B (indicated in micrograms) were cotransfected with 1.6 μg of the TCRβ-CAT reporter plasmid and compared with 2.5 μg of AML-1B-expressing plasmid (1B). Fold activation is expressed in relation to the basal activity of the TCRβ enhancer, which is set to 1. Values shown for 0.01, 0.03, 1.0, 2.5, and 5.0 μg are the averages of two experiments normalized by using SEAP expressed from the RSV LTR as an internal control for transfection efficiency. The error bars indicate the actual normalized levels for the two experiments. Values for AML-1B and 0.1 and 0.5 μg are the averages of at least three experiments, and the error bars indicate 1 standard deviation.

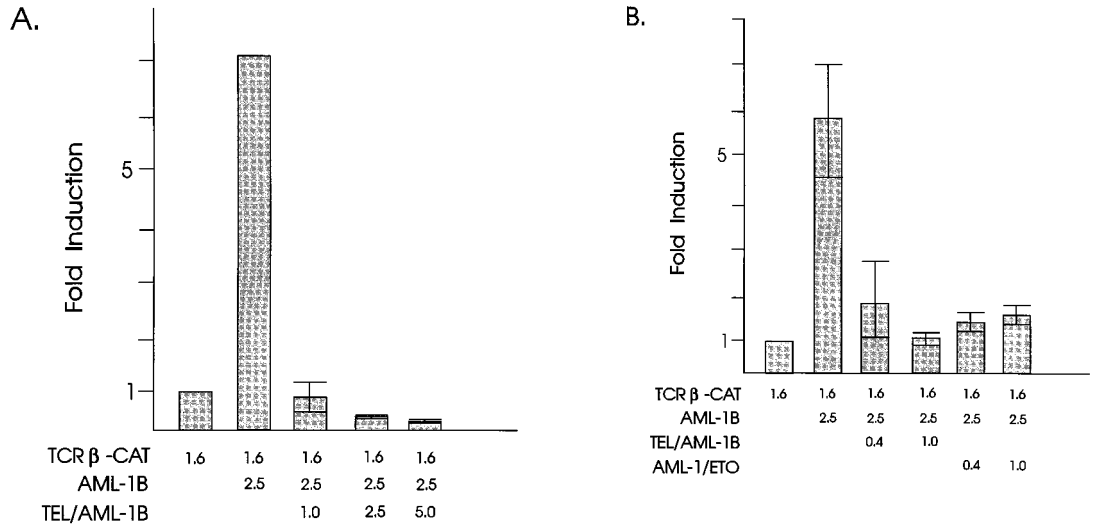

FIG. 4. TEL/AML-1B dominantly interferes with AML-1B-mediated transactivation. (A) Expression from the TCRβ enhancer was activated by using 2.5 μg of plasmid pCMV5-AML-1B in the absence or presence of 1.0, 2.5, and 5.0 μg of pCMV5-TEL/AML-1B. (B) Comparison of TEL/AML-1B and AML-1/ETO for the ability to interfere with AML-1B transactivation. Transfections were performed as for panel A with 400 ng or 1.0 μg of pCMV5 expressing TEL/AML-1B or AML-1/ETO. Values were corrected for SEAP activity and are the averages of at least three experiments; error bars indicate 1 standard deviation.

rearranged or deleted in cases containing t(12;21) (11, 12, 42, 43), prompted us to test whether coexpression of wild-type TEL could affect the function of TEL/AML-1B. Samples of transient transfections with plasmids expressing TCRβ, AML-1B, TEL/AML-1B, and increasing amounts of TEL were assayed for CAT activity. As a control, we used plasmids expressing TEL in the opposite orientation [TEL(−); Fig. 5B]. Although a wide range of concentrations of TEL plasmid was used, we observed no significant effect on TEL/AML-1B activity when TEL was expressed in the 5′-to-3′ rather than the 3′-to-5′ orientation (Fig. 5B). TEL expression from the sense but not the antisense orientation was confirmed by transfection into Cos-7 cells followed by Western blot (immunoblot) analysis (data not shown).

The helix-loop-helix domain of TEL is required for repression. The experiments described above indicate that the presence of TEL amino acids 1 to 333 converts AML-1B from a transcriptional activator to a dominant interfering protein. To directly test this possibility, we generated N-terminal deletion mutants of TEL/AML-1B (Fig. 6A). Plasmids expressing these proteins were transfected into Cos-7 cells, and their expression was measured by Western blot analysis using anti-AML-1 serum (Fig. 6B). Each plasmid expressed a protein of the expected size based on migration in an SDS-polyacrylamide gel.

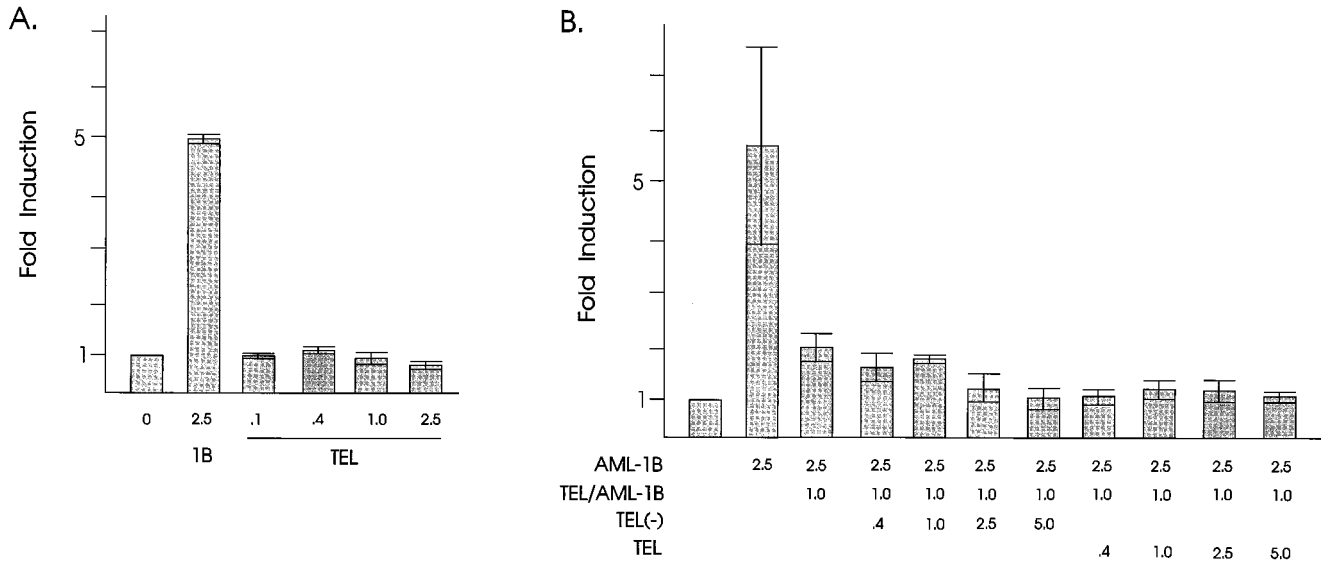

FIG. 5. Wild-type TEL does not affect TCRβ transcription. (A) Activity of TEL on the TCRβ enhancer. The ability of TEL to activate or repress transcription of the TCRβ enhancer was tested by cotransfecting 1.6 μg of TCRβ-CAT plasmid with increasing amounts of pCMV5-TEL. AML-1B (2.5 μg) was included for comparison (bar labeled 1B). (B) Wild-type TEL does not inactivate TEL/AML-1B. Transcriptional interference assays were set up as for Fig. 4, and increasing amounts of pCMV5 expressing TEL in the sense (TEL) or antisense [TEL(−)] orientation were added as indicated. Error bars indicate 1 standard deviation.

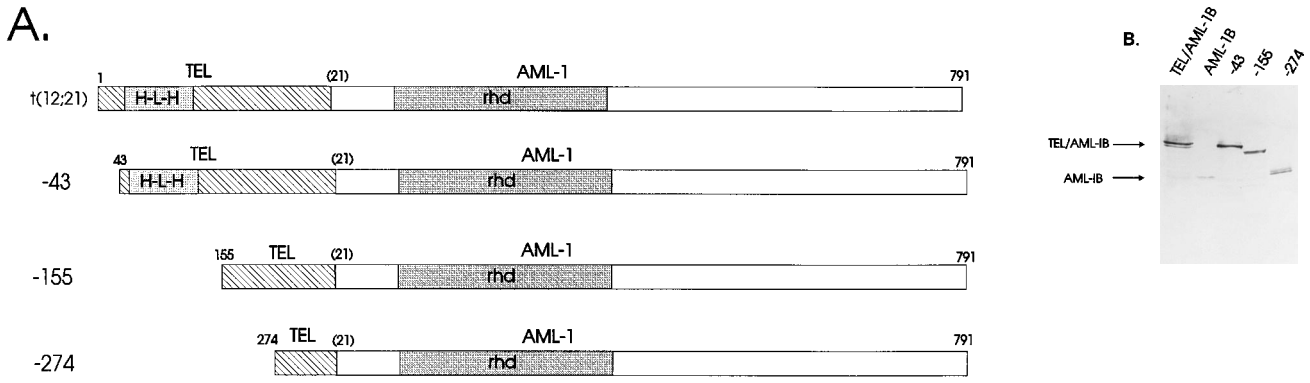

FIG. 6. Construction and expression of TEL/AML-1 deletion mutants. (A) Schematic representation of the t(12;21) fusion protein and the N-terminal deletion mutants. H-L-H, helix-loop-helix domain; (21), residue 21, the chromosomal breakpoint in AML-1B; rhd, *runt* homology domain. (B) Expression of TEL/AML-1B mutants. Cos-7 cells were transfected with the indicated pCMV5 plasmids, and cell extracts were analyzed by Western blotting with affinity-purified anti-AML-1 antibodies.

To determine if the deletion mutants activated, rather than inhibited, transcription, plasmids encoding each protein were cotransfected with the TCRβ-CAT reporter plasmid. Deletion of N-terminal sequences had little or no effect, but deletion of the putative helix-loop-helix domain (mutant -155) allowed activation of transcription (Fig. 7A). Further, deletion to residue 274 did not significantly enhance this activity. While the levels of activation achieved with mutants -155 and -274 did not reach that of AML-1B, these differences may be due to subtle differences in levels of expression. Importantly, the difference in activity between mutants -43 and -155 is nearly 10-fold.

Next, these mutants were tested for the ability to inhibit AML-1B-dependent transactivation. Again, deletion of the N-terminal 43 amino acids had little effect, but deletion of the helix-loop-helix domain abolished the ability of the t(12;21) fusion protein to act as a dominant interfering protein (Fig. 7B). Thus, TEL sequences are required for the ability of TEL/AML-1B to act as a dominant inhibitor.

DISCUSSION

The t(12;21) translocation is one of the most frequent events in childhood acute lymphoblastic leukemias (43, 47). This translocation fuses a putative protein interaction motif from TEL to the majority of AML-1B. The analogous domains in other ets factors (e.g., ets-1 and fli-1) are not transactivation domains but may modulate transcriptional activation (4, 41, 46, 50). In t(12;21), this domain from TEL converts AML-1B from an activator to a repressor of transcription. Thus, t(12;21), like t(8;21), creates a dominant negative protein that interferes with transcriptional activation by AML-1B. Recent observations indicate that AML-1B and AML-2 are expressed in both myeloid and B cells (31), that the consensus DNA binding site for AML-2 is nearly identical to that of AML-1, and that AML-2 can also transactivate the TCRβ enhancer (31). Therefore, a dominant inhibitory protein may be required to inactivate all of the AML-1 family members expressed in the target cell type, rather than inactivate both *AML1* alleles, for leukemogenesis.

Because t(8;21) and t(12;21) affect AML-1-dependent transactivation (30), we compared the sequences of ETO and TEL that are required for repression. Deletion mutagenesis localized the C-terminal boundary of ETO required for repression (22), and this region corresponds to a domain that is conserved in nervy, the *Drosophila* homolog of ETO (9, 28). This motif

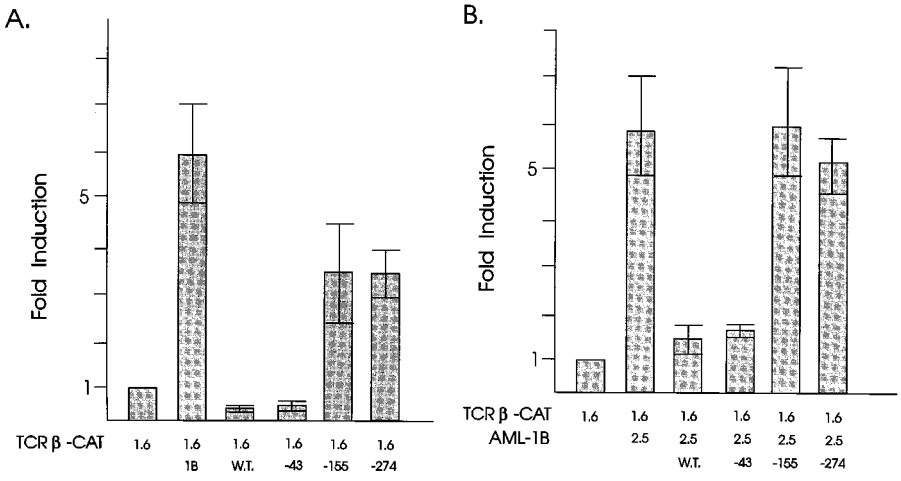

FIG. 7. The TEL helix-loop-helix domain is required for TEL/AML-1B-mediated repression. (A) Transactivation of the TCRβ enhancer by the TEL/AML-1B mutants. Each of the indicated pCMV5 plasmids (2.5 μg) was cotransfected with 1.6 μg of TCRβ-CAT plasmid. (B) AML-1B transcriptional interference by the TEL/AML-1B mutants. Transcription from the TCRβ enhancer was activated by cotransfection with 2.5 μg of plasmid pCMV5-AML-1B in the absence or presence of 1.0 μg of the indicated TEL/AML-1B plasmids. 1B, AML-1B; W.T., wild-type TEL/AML-1B. Error bars indicate 1 standard deviation.

can be modeled as an amphipathic helix, and in other proteins this type of structure mediates protein-protein interactions (8, 28). The work presented here demonstrates that the helix-loop-helix domain of TEL, a putative protein interaction motif, is required for transcriptional repression by the t(12;21) fusion protein. Interestingly, the first helix of this domain can also be modeled as an amphipathic helix, implying that t(12;21) and t(8;21) may fuse similar functional domains to AML-1 to induce leukemogenesis.

Although these studies have not been aimed at identifying the precise mechanism for repression, some conclusions can be inferred. Enforced expression of full-length ETO (31) or TEL has no effect on transcriptional regulation of AML-1 target genes, indicating that ETO and TEL sequences must be tethered to AML-1 for transcriptional repression. Moreover, the fusion proteins did not affect transcription from the RSV LTR (our internal control plasmid). Therefore, it is unlikely that repression is due to the titration of a required transcriptional coactivator (i.e., squelching). Because both fusion proteins may act at substoichiometric levels of input DNA and are much more effective repressors than AML-1, which can bind DNA but lacks the transcription activation domain (30, 38), simple competition for DNA binding sites is also unlikely. Additionally, the observed repression requires putative protein interaction motifs in both fusion proteins, suggesting that interactions with either surrounding activating factors (i.e., quenching) or the basal transcription machinery (the direct mechanism) block transcription. Alternatively, the fusion protein may recruit a general repressing factor (i.e., a corepressor). Given that TEL/AML-1 expression affects both activated and basal activities of the TCRβ enhancer, either the direct or corepressor model may apply for t(12;21) (24). Interestingly, several regulatory transcription factors interact with corepressors through amphipathic helices. For example, the Mad/Max heterodimer represses transcription by recruiting the mammalian Sin3a (mSin3a) or mSin3b corepressor to target genes (1), and the interaction between Mad and mSin3 is mediated by amphipathic helices. In yeast cells, sin3 does not bind DNA but mediates transcriptional repression of a wide range of genes and can repress when tethered to DNA through interactions with site-specific DNA-binding proteins (51, 52). Likewise, the yeast Ssn6 protein is recruited to promoters by Matα2/MCM-1 heterodimers to repress transcription (21). Thus, it is possible that TEL/AML-1B and AML-1/ETO recruit members of this new class of transcriptional regulators to inhibit AML-1-dependent transcription.

We have used the TCRβ enhancer in this study because it contains multiple AML-1B binding sites and is activated by AML-1B. Thus, we cannot rule out the possibility that a myeloid cell- or B-cell-specific promoter would be regulated differently by AML-1/ETO or TEL/AML-1B fusion proteins. However, the ability of these fusion proteins to repress transcription, given their strikingly different architectures, argues that part of their ability to induce leukemogenesis is due to dominant interference with the transactivation functions of the AML-1 transcription factor family. Moreover, the t(3;21) fusion protein, AML-1/Evi-I (49), and the inv(16) fusion protein, CBFβ-MYH11, also inhibit AML-1-dependent transcription (16). inv(16) also transforms NIH 3T3 cells, and coexpression of CBFα (murine AML-1B) inhibits CBFβ-MYH11-mediated transformation (17). Thus, translocations that target AML-1 directly and indirectly may induce leukemogenesis by the same mechanism.

While both alleles of *AML1* are rarely affected in either myeloid or lymphoid leukemias, the second allele of *TEL* is rearranged in approximately 30% of the cases containing t(12;21). Thus, wild-type TEL function may inhibit the action of the fusion protein, or TEL may function as a tumor suppressor whose inactivation allows the fusion protein to transform (12, 42, 43). Our data indicate that TEL alone does not regulate the expression of an AML-1 target gene containing ets factor binding sites, nor does coexpression of TEL and the t(12;21) fusion protein affect the repression mediated by TEL/AML-1B. Thus, loss of function of the *TEL* gene may be more relevant to other aspects of pathogenesis than simply the ability of TEL to interfere with TEL/AML-1B function. Given that the N-terminal domain of TEL acts as a repressor domain when fused to a heterologous transcriptional activator (AML-1B), we speculate that TEL may represent a natural inhibitor for other ets factors, many of which stimulate cellular proliferation. Notably, in vitro, TEL can homodimerize (data not shown), suggesting that TEL could inhibit transcription either by binding ets sites within target genes or by heterodimerizing with positive-acting ets factors that contain the conserved helix-loop-helix motif. Further characterization of the normal role of TEL will be required to answer these important questions.

ACKNOWLEDGMENTS

We thank David Strom for many helpful discussions and Zhongling Cai for technical assistance.

This work was supported by grants CB-39 and JFRA-591 from the American Cancer Society and RO1 CA-64140 from the National Institutes of Health to S.W.H., by National Institutes of Health grant CA 57261 and a Stephen Birnbaum Leukemia Society of America Scholar award to D.G.G., by a Burroughs-Wellcome Career Award in Biomedical Sciences to T.G., by NIH grant RO1 CA-56819 to M.F.R., by Cancer Center Support CORE grant 5 P30 CA 21765, and by the American Lebanese Syrian Associated Charities of St. Jude Children's Research Hospital.

REFERENCES

1. **Ayer, D. E., Q. A. Lawrence, and R. N. Eisenman.** 1995. Mad-Max transcriptional repression is mediated by ternary complex formation with mammalian homologs of yeast repressor Sin3. Cell **80:**767–776.
2. **Bae, S. C., Y. Yamaguchi-Iwai, E. Ogawa, M. Maruyama, M. Inuzuka, H. Kagoshima, K. Shigesada, M. Satake, and Y. Ito.** 1993. Isolation of PEBP2αB cDNA representing the mouse homolog of human acute myeloid leukemia gene, AML1. Oncogene **8:**809–814.
3. **Berger, J., J. Hauber, R. Hauber, R. Geiger, and B. R. Cullen.** 1988. Secreted placental alkaline phosphatase: a powerful new quantitative indicator of gene expression in eukaryotic cells. Gene **66:**1–10.
4. **Chumakov, A. M., D. L. Chen, E. A. Chumankova, and H. P. Koeffler.** 1993. Localization of the c-ets-2 transactivation domain. J. Virol. **67:**2421–2435.
5. **Daga, A., J. E. Tighe, and F. Calabi.** 1992. Leukaemia/Drosophila homology. Nature (London) **356:**484. (Letter.)
6. **Dignam, J. D., R. M. Lebovitz, and R. G. Roeder.** 1983. Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei. Nucleic Acids Res. **11:**1475–1489.
7. **Erickson, P., J. Gao, K. S. Chang, T. Look, E. Whisenant, S. Raimondi, R. Lasher, J. Trujillo, J. Rowley, and H. Drabkin.** 1992. Identification of breakpoints in t(8;21) acute myelogenous leukemia and isolation of a fusion transcript, AML1/ETO, with similarity to Drosophila segmentation gene, *runt*. Blood **80:**1825–1831.
8. **Fairman, R., R. K. Beran-Steed, S. J. Anthony-Cahill, J. D. Lear, W. F. Stafford, III, W. F. DeGrado, P. A. Benfield, and S. L. Brenner.** 1993. Multiple oligomeric states regulate the DNA binding of helix-loop-helix peptides. Proc. Natl. Acad. Sci. USA **90:**10429–10433.
9. **Feinstein, P. G., K. Kornfeld, D. S. Hogness, and R. S. Mann.** 1995. Identification of homeotic target genes in Drosophila melanogaster including nervy, a proto-oncogene homologue. Genetics **140:**573–586.
10. **Giese, K., C. Kingsley, J. R. Kirshner, and R. Grosschedl.** 1995. Assembly and function of a TCR alpha enhancer complex is dependent on LEF-1-induced DNA bending and multiple protein-protein interactions. Genes Dev. **9:**995–1008.
11. **Golub, T., T. McLean, K. Stegmaier, J. Ritz, S. Sallen, D. Neuberg, and D. G. Gilliland.** 1995. TEL-AML-1: the most common gene rearrangement in childhood ALL. Blood **86**(Suppl.)**:**597a.
12. **Golub, T. R., G. F. Barker, S. K. Bohlander, S. W. Hiebert, D. C. Ward, P. Bray-Ward, S. C. Raimondi, J. D. Rowley, and D. G. Gilliland.** 1995. Fusion

of the Tel gene on 12p13 to the AML1 gene on 21q22 in acute lymphoblastic leukemia. Proc. Natl. Acad. Sci. USA **92:**4917–4921.
13. **Golub, T. R., G. F. Barker, M. Lovett, and D. G. Gilliland.** 1994. Fusion of PDGF receptor beta to a novel ets-like gene, tel, in chronic myelomonocytic leukemia with t(5;12) chromosomal translocation. Cell **77:**307–316.
14. **Gorman, C. M., L. F. Moffat, and B. H. Howard.** 1982. Recombinant genomes which express chloramphenicol acetyltransferase in mammalian cells. Mol. Cell. Biol. **2:**1044–1051.
15. **Gottschalk, L. R., and J. M. Leiden.** 1990. Identification and functional characterization of the human T-cell receptor β gene transcriptional enhancer: common nuclear proteins interact with the transcriptional regulatory elements of the T-cell receptor α and β genes. Mol. Cell. Biol. **10:**5486–5495.
16. **Hajra, A., P. P. Liu, Q. Wang, C. A. Kelley, T. Stacy, R. S. Adelstein, N. A. Speck, and F. S. Collins.** 1995. The leukemic core binding factor beta-smooth muscle myosin heavy chain (CBFbeta-SMMHC) chimeric protein requires both CBFbeta and myosin heavy chain domains for transformation of NIH 3T3 cells. Proc. Natl. Acad. Sci. USA **92:**1926–1930.
17. **Hajra, A., P. P. Lui, N. A. Speck, and F. S. Collins.** 1995. Overexpression of core-binding factor α (CBFα) reverses cellular transformation by the CBFβ-smooth muscle myosin heavy chain chimeric oncoprotein. Mol. Cell. Biol. **15:**4980–4989.
18. **Heldin, C.-H., A. Ernlund, C. Rorsman, and L. Ronnstrand.** 1989. Dimerization of B-type platelet-derived growth factor receptors occurs after ligand binding and is closely associated with receptor kinase activation. J. Biol. Chem. **264:**8905–8912.
19. **Hernandez-Munain, C., and M. S. Krangel.** 1995. c-Myb and core-binding factor/PEBP2 display functional synergy but bind independently to adjacent sites in the T-cell receptor delta enhancer. Mol. Cell. Biol. **15:**3090–3099.
20. **Hiebert, S. W.** 1993. Regions of the retinoblastoma gene product required for its interaction with the E2F transcription factor are necessary for E2 promoter repression and pRb-mediated growth suppression. Mol. Cell. Biol. **13:**3384–3391.
21. **Keleher, C. A., M. J. Redd, J. Schultz, M. Carlson, and A. D. Johnson.** 1992. Ssn6-Tup1 is a general repressor of transcription in yeast. Cell **68:**709–719.
22. **Lenny, N., S. Meyers, and S. W. Hiebert.** 1995. Functional domains of the t(8;21) fusion protein, AML-1/ETO. Oncogene **11:**1761–1769.
23. **Levanon, D., V. Negreanu, Y. Bernstein, I. Bar-Am, L. Avivi, and Y. Groner.** 1994. AML1, AML2, and AML3, the human members of the runt domain gene-family: cDNA structure, expression, and chromosomal localization. Genomics **23:**425–432.
24. **Levine, M., and J. L. Manley.** 1989. Transcriptional repression of eukaryotic promoters. Cell **59:**405–408.
25. **Liu, P., S. A. Tarle, A. Hajra, D. F. Claxton, P. Marlton, M. Freedman, M. J. Siciliano, and F. S. Collins.** 1993. Fusion between transcription factor CBFβ/PEBP2β and a myosin heavy chain in acute myeloid leukemia. Science **261:**1041–1044.
26. **Melnikova, I. N., B. E. Crute, S. Wang, and N. A. Speck.** 1993. Sequence specificity of the core-binding factor. J. Virol. **67:**2408–2411.
27. **Meyers, S., J. R. Downing, and S. W. Hiebert.** 1993. Identification of AML-1 and the t(8;21) translocation protein (AML-1/ETO) as sequence-specific DNA-binding proteins: the runt homology domain is required for DNA binding and protein-protein interactions. Mol. Cell. Biol. **13:**6336–6345.
28. **Meyers, S., and S. W. Hiebert.** Direct and indirect alteration of transcriptional regulation in cancer. Crit. Rev. Eukaryotic Gene Expression, in press.
29. **Meyers, S., and S. W. Hiebert.** 1995. Unpublished data.
30. **Meyers, S., N. Lenny, and S. W. Hiebert.** 1995. The t(8;21) fusion protein interferes with AML-1B-dependent transcriptional activation. Mol. Cell. Biol. **15:**1974–1982.
31. **Meyers, S., N. Lenny, W.-H. Sun, and S. W. Hiebert.** Unpublished data.
32. **Mitani, K., S. Ogawa, T. Tanaka, H. Miyoshi, M. Kurokawa, H. Mano, Y. Yazaki, M. Ohki, and H. Hirai.** 1994. Generation of the AML1-EVI-1 fusion gene in the t(3;21)(q26;q22) causes blastic crisis in chronic myelocytic leukemia. EMBO J. **13:**504–510.
33. **Miyoshi, H., K. Shimizu, T. Kozu, N. Maseki, Y. Kaneko, and M. Ohki.** 1991. t(8;21) breakpoints on chromosome 21 in acute myeloid leukemia are clustered within a limited region of a single gene, *AML1*. Proc. Natl. Acad. Sci. USA **88:**10431–10434.
34. **Morishita, K., D. S. Parker, M. L. Mucenski, N. A. Jenkins, N. G. Copeland, and J. N. Ihle.** 1988. Retroviral activation of a novel gene encoding a zinc finger protein in IL-3-dependent myeloid leukemia cell lines. Cell **54:**831–840.
35. **Nisson, P. E., P. C. Watkins, and N. Sacchi.** 1992. Transcriptionally active chimeric gene derived from the fusion of the AML1 gene and a novel gene on chromosome 8 in t(8;21) leukemic cells. Cancer Genet. Cytogenet. **63:** 81–88.
36. **Nucifora, G., C. R. Begy, H. Kobayashi, D. Roulston, D. Claxton, J. Pedersen-Bjergaard, E. Parganas, J. N. Ihle, and J. D. Rowley.** 1994. Consistent intergenic splicing and production of multiple transcripts between AML1 at 21q22 and unrelated genes at 3q26 in (3;21)(q26;q22) translocations. Proc. Natl. Acad. Sci. USA **91:**4004–4008.
37. **Nucifora, G., D. J. Birn, R. I. Espinosa, P. Erickson, M. M. LeBeau, D. Roulston, T. W. McKeithan, H. Drabkin, and J. D. Rowley.** 1993. Involvement of the AML1 gene in the t(3;21) in therapy-related leukemia and in chronic myeloid leukemia in blast crisis. Blood **81:**2728–2734.
38. **Ogawa, E., M. Maruyama, H. Kagoshima, M. Inuzuka, J. Lu, M. Satake, K. Shigesada, and Y. Ito.** 1993. PEPB2/PEA2 represents a family of transcription factors homologous to the products of the Drosophila runt gene and the human AML1 gene. Proc. Natl. Acad. Sci. USA **90:**6859–6863.
39. **Rabbits, T. H.** 1991. Translocations, master genes, and differences between the origins of acute and chronic leukemias. Cell **67:**641–644.
40. **Rabbits, T. H.** 1994. Chromosomal translocations in human cancer. Nature (London) **372:**143–149.
41. **Rao, V. N., T. Ohno, D. D. K. Prasad, G. Bhattacharya, and E. S. P. Reddy.** 1993. Analysis of the DNA-binding and transcriptional activation functions of human Fli-1 protein. Oncogene **8:**2167–2173.
42. **Romana, S. P., M. Mauchauffe, M. Le Coniat, I. Chumakov, D. Le Paslier, R. Berger, and O. A. Bernard.** 1995. The t(12;21) of acute lymphoblastic leukemia results in a tel-AML1 gene fusion. Blood **85:**3662–3670.
43. **Romana, S. P., H. Poirel, M. Leconiat, M.-A. Flexor, M. Mauchauffe, P. Jonveaux, E. A. Macintyre, R. Berger, and O. A. Bernard.** 1995. High frequency of t(12;21) in childhood B lineage acute lymphoblastic leukemia. Blood **86:**4263–4269.
44. **Rubin, C. M., R. A. Larson, J. Anastasi, J. N. Winter, M. Thangavelu, J. W. Vardiman, J. D. Rowley, and M. M. LeBeau.** 1990. t(3;21)(q26;q22) translocation: a recurring chromosomal abnormality in therapy-related myelodysplastic syndrome and acute myeloid leukemia. Blood **76:**2594–2598.
45. **Sacchi, N., P. E. Nisson, P. C. Watkins, F. Faustinella, J. Wijsman, and A. Hagemeijer.** 1994. AML1 fusion transcripts in t(3;21) positive leukemia: evidence of molecular heterogeneity and usage of splicing sites frequently involved in the generation of normal AML1 transcripts. Genes Chromosomes Cancer **11:**226–236.
46. **Schneikert, J., Y. Lutz, and B. Wasylyk.** 1992. Two independent activation domains in c-Ets-1 and c-Ets-2 located in non-conserved sequences of the ets gene family. Oncogene **7:**249–256.
47. **Shurtleff, S. A., A. Buijs, F. G. Behm, J. E. Rubnitz, S. C. Raimondi, M. L. Hancock, G. C.-F. Chan, C.-H. Pui, G. Grosveld, and J. R. Downing.** 1995. Tel/AML1 fusion resulting from a cryptic t(12;21) is the most common genetic lesion in pediatric ALL and defines a subgroup of patients with an excellent prognosis. Leukemia **9:**1985–1989.
48. **Speck, N. A.** PEBP2/CBF, a new transcription factor family associated with human acute myeloid leukemias. Crit. Rev. Eukaryotic Gene Expression, in press.
49. **Tanaka, T., K. Mitani, M. Kurokawa, S. Ogawa, K. Tanaka, J. Nishida, Y. Yazaki, Y. Shibata, and H. Hirai.** 1995. Dual functions of the AML1/Evi-1 chimeric protein in the mechanism of leukemogenesis in t(3;21) leukemias. Mol. Cell. Biol. **15:**2383–2392.
50. **The, S. M., X. Xie, F. Smyth, T. S. Papas, D. K. Watson, and R. A. Schulz.** 1992. Molecular characterization and structural organization of D-elg, an ets proto-oncogene-related gene of Drosophila. Oncogene **7:**2471–2478.
51. **Vidal, M., R. Strich, R. E. Exposito, and R. F. Gaber.** 1991. RPD1 (SIN3/UME4) is required for maximal activation and repression of diverse yeast genes. Mol. Cell. Biol. **11:**6306–6316.
52. **Wang, H., and D. J. Stillman.** 1993. Transcriptional repression in *Saccharomyces cerevisiae* by a Sin3-LexA fusion protein. Mol. Cell. Biol. **13:**1805–1814.
53. **Wang, S., Q. Wang, B. E. Crute, I. N. Melnikova, S. R. Keller, and N. A. Speck.** 1993. Cloning and characterization of subunits of the T-cell receptor and murine leukemia virus enhancer core-binding factor. EMBO J. **13:**3324–3339.
54. **Wotton, D., J. Ghysdael, S. Wang, N. A. Speck, and M. J. Owen.** 1994. Cooperative binding of ets-1 and core binding factor to DNA. Mol. Cell. Biol. **14:**840–850.
55. **Zhang, D.-E., C. J. Hetherington, S. Meyers, K. L. Rhoades, C. J. Larson, H.-M. Chen, S. W. Hiebert, and D. G. Tenen.** 1996. CCAAT enhancer-binding protein (C/EBP) and AML1 (CBFα2) synergistically activate the macrophage colony-stimulating factor receptor promoter. Mol. Cell. Biol. **16:**1231–1240.