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Expression of the human acute myeloid leukemia gene *AML1* is regulated by two promoter regions

M. C. GHOZI, Y. BERNSTEIN, V. NEGREANU, D. LEVANON, AND Y. GRONER*

Department of Molecular Genetics, The Weizmann Institute of Science, Rehovot 76100, Israel

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ABSTRACT **The human chromosome 21 AML1 gene is expressed predominantly in the hematopoietic system. In several leukemia-associated translocations AML1 is fused to other genes and transcription of the fused regions is mediated by upstream sequences that normally regulate the expression of AML1. The 5′ genomic region of AML1 was cloned and sequenced. The two 5′ untranslated regions (UTRs) previously identified in AML1 cDNAs were located in this region and the distance between them was established. The distal 5′ UTR maps over 7 kb upstream of the proximal one. Using primer extension with mRNA, transcription start sites were identified at two distinct sites above these 5′ UTRs. Sequence analysis revealed the absence of a TATA motif and the presence of Sp1, PU.1, Oct, CRE, Myb, Ets, and Ets-like binding sites in both upstream regions. Several initiator elements (Inr) that overlap the transcription start sites were also identified. These proximal and distal upstream regions and their deletion mutants were cloned in front of a luciferase reporter gene and used in transfection assays. We demonstrate that both upstream regions function as promoters in hematopoietic (Jurkat) and nonhematopoietic (HEK) cell lines. The activity of both promoters was orientation dependent and was enhanced, in a cell-type specific manner, by a heterologous enhancer sequence. These results indicate that additional control elements, either negative or positive, regulate the tissue-specific expression of AML1.**

The human *AML1* (also designated core-binding factor α subunit 2, *CBFA2*) gene belongs to a recently identified gene family of transcription factors sharing a highly conserved region of 128 aa termed the runt domain (reviewed in refs. 1 and 2). The family includes the *Drosophila* segmentation gene runt, the human genes *AML1*, *AML2*, and *AML3* and their respective mouse homologues *PEBP2αB*, *CBFA3*, and *PEBP2αA* (3–9). The runt domain embodies regions that are involved in binding to DNA and in protein–protein interaction (1). The putative transcriptional activation domain of the AML1 proteins lies 3′ to the runt region (ref. 2 and references therein). The *AML1* gene resides on chromosome 21 and is involved in several leukemia-associated translocations (reviewed in refs. 2 and 10). In the t(8;21) translocation, which is most common among patients with acute myeloid leukemia (AML)-M2, and in the t(3;21) translocation, found in therapy-related AML and myelodysplastic syndrome, the 5′ part of the *AML1* gene, which contains the promoter region, is fused to other genes not normally expressed in myeloid cells (ref. 2 and references therein). Fused RNA transcripts spanning *AML1* and a partner gene, *ETO*, *EAP*, *MDS1*, or *EVI1* were identified in AML patients (2), highlighting the potential importance of the transcriptional control elements of the *AML1* gene in leukemogenesis. The molecular mechanisms underlying leukemogenesis in t(8;21) and t(3;21) translocations are not fully known but thought to involve inhibition of the normal functions of AML1 by the chimeric proteins (2). Interestingly, we and others identified in normal blood a number of *AML1* mRNA species that encode shortened proteins similar in size to the *AML1* segment in the fused t(8;21) and t(3;21) products (3, 11–13). When transfected into cells, they dominantly suppress activity of the full-length AML1 protein (2, 14, 15). When overexpressed due to gene dosage, these mRNAs may have a significant role in the Down syndrome-associated AML, in which the chromosomal defect is trisomy 21 (16, 17).

AML1 is expressed mainly in the hematopoietic system as a complex pattern of mRNAs ranging in size between 2 and 8 kb (2, 3, 6). Several of these transcripts differ in their 5′ and 3′ untranslated regions (UTRs) (13, 18). There are two distinct 5′ UTRs that exhibit different expression patterns in hematopoietic tissues and in response to mitogenic stimulation (13). These data raise the possibility that *AML1* expression is transcriptionally regulated by two discrete promoter regions and prompted us to investigate the genomic regions upstream of these 5′ UTRs. Here we describe the structural/functional analysis of these two regions and demonstrate that they harbor the *AML1* mRNA start sites and serve as transcriptional promoters that regulate expression of the *AML1* gene.†

MATERIALS AND METHODS

Library Screening, Phage DNA Mapping, and Sequencing. A genomic library enriched for human chromosome 21 was screened as described (6). Phages containing the 5′ region of *AML1* were isolated, fragments of their inserts were subcloned in pBluescript (Stratagene), and both strands were sequenced by the automated *Taq* dideoxynucleotide chain-termination method on a model 373A Applied Biosystem apparatus.

Primer-Extension Analysis. Two primers were used for each 5′ UTR. The reverse primers for the proximal region (Fig. 1*BI*) span nucleotides 74–104 and 121–150 relative to +1 of the *AML1-a* cDNA (6), while those used for the distal region (Fig. 1*BII*) correspond to nucleotides 85–110 and 198–223 of *AML1-g* (13). Primers were labeled at the 5′ end by [γ^{32}P]ATP; 40 fmol of each primer was mixed with 1 μg of Jurkat cell poly(A)$^+$ RNA in TE buffer/125 mM KCl, and annealed (70°C for 20 min). To extend the cDNA, reverse transcriptase buffer and 30 units of enzyme (Promega) were added, followed by a 1-h incubation at 42°C. Extended products were analyzed by sequencing PAGE (6%) with a parallel sequencing ladder obtained using the same primers annealed to the relevant genomic fragments.

Construction of AML1 Luciferase Plasmids and Site-Directed Mutagenesis. Relevant genomic fragments were cloned in the corresponding polylinker sites of the promoterless luciferase vector pGL2-Basic (Promega). The proximal promoter constructs shown in Fig. 2 were derived by using convenient restriction sites, from the *Xho* I/*Avr* II fragment

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Abbreviations: TCRβ, T-cell receptor β; UTR, untranslated region; Inr, initiator element; RA fragment, *Rsa* I/*Avr* II fragment.

*To whom reprint requests should be addressed.

†The sequences reported in this paper have been deposited in the GenBank data base (accession nos. U39636 and U39637).

that spans most of the region between exons 6 and 7 (see Fig. 1*A*) including 111 bp of 5′ UTR. The initiator elements were mutated using PCR and two synthetic oligonucleotides with the sequence: GCACGTTTGTTTCCAGGGGCTCTTCTTCATAG in forward and reverse orientations. These primers introduced mutations, marked by boldface letters, at positions −1 + 1 of the initiator elements. Similarly the PU.1/Ets-like sites and Oct motifs were modified using the corresponding primers GAATTAATCGATCCGGCCGGG and GCCCCTAGGCAAACGAGC in both orientations. Deletion constructs of the distal promoter used in Fig. 3 were generated by subcloning PCR fragments derived from the distal upstream region shown in Fig. 1*A*. Primers used for PCR were modified to include a 5′ *Kpn* I site and a 3′ *Bgl* II site. The following oligonucleotides were used for site-directed mutagenesis of the PU.1 and CRE sites and the oligoA spacer: CAGCACACAAA, AATCGGCAGCGCTT, and AAAGGAAGCAAT, respectively. Constructs containing the T-cell receptor β (TCRβ) enhancer were generated by introducing a 572-bp *Bam*HI fragment of the mouse TCRβ enhancer (positions 39–610 in ref. 19) into the pGL2 downstream of the luciferase gene. All constructs were analyzed by mapping and/or sequencing.

Cell Culture and DNA Transfection Assays. Cells were grown as described (13); 5×10^6 cells were transfected by DEAE-dextran with 5 μg of Luc plasmid DNA in the presence of 0.5 μg of pSV-βgal as an internal control for transfection efficiency. Forty-eight hours after transfection, cells were collected and assayed for luciferase activity using a Turner (Palo Alto, CA) model TD-20e luminometer. β-Galactosidase was determined as described (20) and the results of the luciferase activity assay were normalized for transfection efficiency.

RESULTS

Transcription of *AML1* Starts at Two Distinct 5′ Regions. Two different types of 5′ UTRs were found by sequence analysis of human *AML1* cDNAs: one exceedingly long (1.58 kb) containing 15 upstream AUGs, and a shorter one of 0.257 kb with only a single upstream AUG (13). The genomic organization of the 5′ upstream region of *AML1* is shown in Fig. 1*A*, which depicts the genomic regions that were sequenced, the structures of the two 5′ UTRs, and the locations of primers I and II used for extension analyses of mRNAs. Analysis revealed that the proximal 1.58-kb 5′ UTR (no. 7 in Fig. 1*A*) and the region encoding the first 90 amino acids (no. 8) are included in a single exon that undergoes in-exon splicing. Specifically, we and others isolated cDNAs in which most of the coding region of this exon is spliced to other 5′ genomic sequences (13, 15, 18, 21) as shown schematically in Fig. 1*A* (exons 4–6). Consistent with this, the sequence around the junction point at nucleotide 1596 corresponds to a consensus 3′ acceptor splice site, and 32 nucleotides upstream the sequence TCCTAACT appears, which matches the consensus for a lariat branch point (22). The distal 5′ UTR, while much shorter than the proximal, consists of several exons that are alternatively spliced to generate a variety of *AML1* mRNAs (Fig. 1*A* exons 1–4). Several *AML1* cDNAs were isolated in which various combinations of 5′ UTR exons and coding sequences are joined (13, 18), thereby further increasing the repertoire of AML1 proteins with distinct N termini. Based on restriction enzyme analyses of λ genomic phages, the distance between the two different 5′ UTRs exceeds 7 kb (Fig. 1*A*).

To identify and map the transcription start sites of the two 5′ UTRs, primer-extension analysis was performed. For the proximal 5′ UTR, poly(A)$^+$ RNAs from Daudi and Jurkat cells were annealed to two different primers from the 5′ end of the UTR (Fig. 1*A*) and the reverse transcriptase-elongated DNAs were analyzed as described. From Jurkat RNA, the primed cDNAs gave, with both primers, one strong band designated +1 and a fainter band at position −6 (Fig. 1*BI*). A few additional extended products at −16, −20, and −28 were seen upon longer exposure of the film, indicating transcription initiation at more upstream sequences (Fig. 1*CI*). As presented below, the +1, −6, and −16 start sites closely resemble the initiator consensus sequence (reviewed in refs. 23 and 24) and are vital for the promoter function of this region. In parallel primer-extension experiments with RNA from Daudi cells, no signal was visible. In these cells, the level of mRNA species with the proximal 5′ UTR is much lower than in Jurkat cells (6). Two primers complementary to the 5′ end of the distal UTR were utilized, with Jurkat RNA, for detecting the 5′ termini of these mRNAs (Fig. 1 *A* and *BII*). Four products were obtained, indicating transcription initiations at sites +1, −10, −15, and −24 nucleotides upstream of the distal cDNA sequence (Fig. 1*CII*). A doublet of fainter bands corresponding to transcripts starting at −40 was also detected (data not shown).

The Two 5′ Upstream Sequences of *AML1* Contain Hallmarks of Promoter Regions. Based on the mapping of mRNA 5′ ends, the region between the transcription start sites and the 3′ end of exon 6 (Fig. 1*A*) has been tentatively defined as the proximal promoter. Sequence analysis and a computer search (SIGNAL SCAN; ref. 25) have identified several features of this region that are characteristic of promoters. It is particularly rich in G+C (>70%) and contains several Sp1 binding sites (Fig. 1*CI*). As in numerous other G+C-rich promoters, this region lacks a TATA box but contains three initiator (Inr) elements that overlap the transcription start sites (Fig. 1*CI*). These three elements fit the loose sequence requirements for a functional Inr as described by Javahery *et al.* (24). Significantly, they share sequence similarities with the consensus sequence (T/G)C**A**(T/C)T(C/T)N(C/T)**A**, in which the underlined A corresponds to +1 of the primed DNA start sites (Fig. 1*BI*) and the boldface nucleotides are fully conserved. Two CCAAT boxes, at −95 (inverted) and at −154, and several potential binding sites of hematopoietic transcription factors were also identified including Oct proteins (26), Ets family proteins (27), and a PU.1 subtype (28) (Fig. 2*CI*).

The distal promoter was defined in a region spanning ≈1 kb upstream of the distal 5′ UTR (Fig. 1*A*) and encompassing the transcription start sites. It has a significantly lower G+C content (40%) than the proximal promoter but likewise lacks a correctly spaced TATA box and contains a CCAAT box (−84; Fig. 1*CII*). Two of the four start sites, +1 and −10, are within the above-noted loose consensus sequence of Inr elements (23, 24). Putative binding sites of transcription factors found in this region include GATA (29), PU.1 (30), CRE (29), and Myb (29). Interestingly, several elements of the PEBP2/CBF binding site (29) recognized by AML1 are present in both promoter regions, at positions −294 and −311 of the proximal promoter and at positions −69 and −305 of the distal promoter.

The 5′ Regions of *AML1* Function as Transcriptional Promoters. To evaluate promoter functions of the two 5′ regions, reporter plasmids were constructed with various DNA segments cloned into the promoterless luciferase vector pGL2-Basic. Plasmids were transiently transfected into two human cell lines: the T-cell line Jurkat, which expresses high levels of *AML1*, and the embryonic kidney cell line HEK, in which *AML1* expression is barely detectable. Transfection of the XHA-Luc construct that contains the proximal 5′ 1.072-kb *Xho* I/*Avr* II fragment (Fig. 2) into Jurkat cells yielded levels of luciferase activity >150-fold above the pGL2-Basic control, confirming that this segment contains promoter activity. Consistent with this, inversion of XHA orientation markedly reduced luciferase activity of the construct (data not shown). To locate more precisely the region required for activity of the proximal promoter, seven consecutive deletions were produced in the XHA-Luc construct (Fig. 2). When the *Xho* I/*Not*

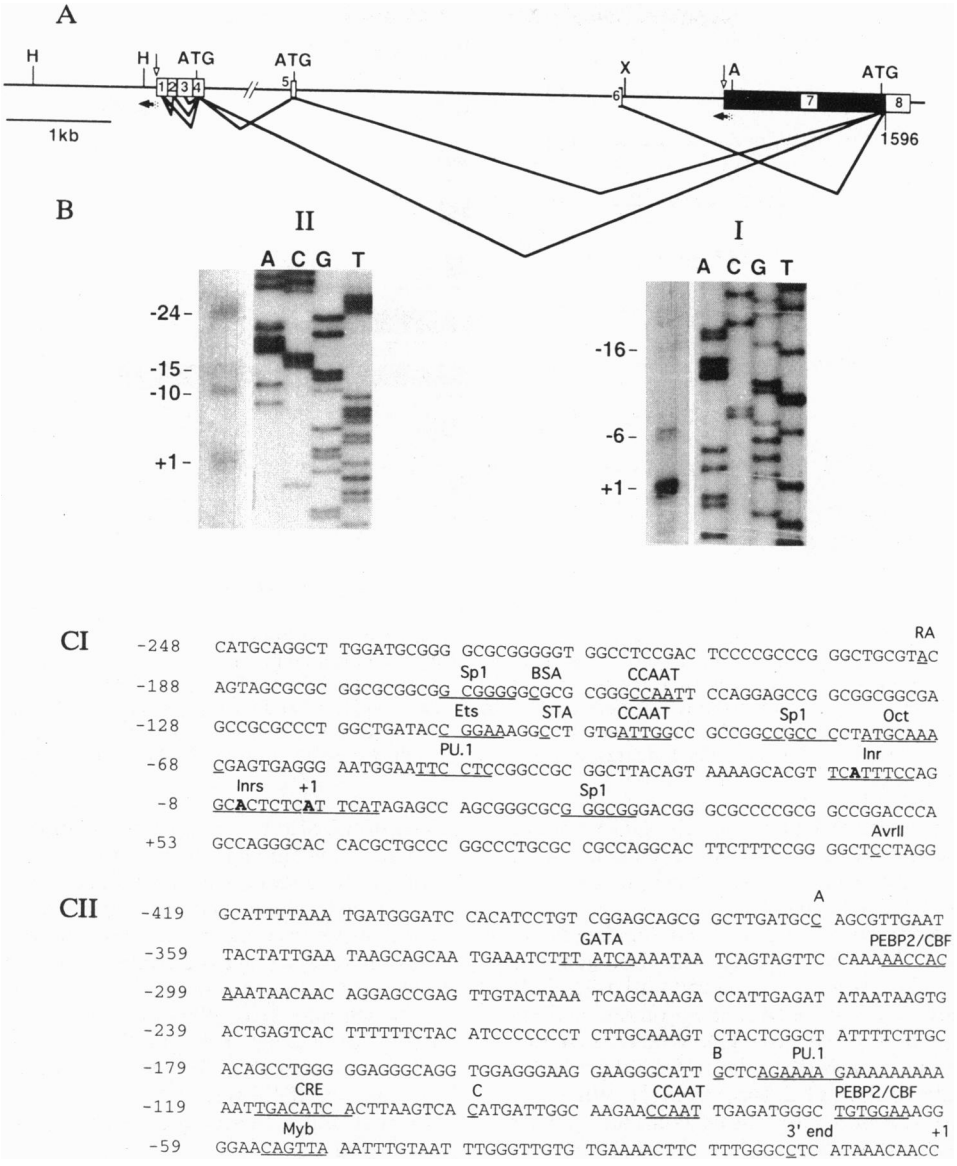

FIG. 1. Mapping of *AML1* transcription start sites and sequence analysis of promoter regions. (*A*) Schematic map of the 5′ region of the *AML1* gene showing the genomic organization, the exons that comprise the two 5′ UTRs, and the various combinations of alternative splicing. Vertical arrows indicate 5′ ends of the UTRs. The *Avr* II and *Xho* I sites (-A and -X, respectively) delineate the proximal promoter region. *Hae* III sites (-H) roughly define the distal promoter region. Location of primers used for the extension experiment in *B* are denoted by small arrows with asterisks. (*B*) Poly(A)$^+$ RNA from Jurkat cells was annealed to labeled primers corresponding to the (*I*) proximal or (*II*) distal 5′ UTR. Two distinct primers were utilized for each region and the extended products were analyzed on a 6% polyacrylamide gel along with a sequencing ladder of the same primers annealed to the relevant genomic fragments. +1, Position of the most 3′ start site. (*C*) Sequence of the immediate 5′ regions encompassing the transcription start sites. (*I*) Proximal region spanning *Rsa* I/*Avr* II (the RA fragment). (*II*) Fragment A of the distal region. The +1 start site is indicated. Inr elements and potential binding sites are underlined.

I fragment (−961 to −499) was removed, the activity was reduced 3-fold, suggesting that this region contains positive regulatory elements. Subsequent deletions of sequences from the 610-bp *Not* I/*Avr* II (NA) fragment down to 368 bp of *Apa* I/*Avr* II (APA) produced a further decrease in promoter activity compared to XHA. Interestingly, the activity of the *Rsa* I/*Avr* II (RA) fragment was 26% greater than that of XHA, raising the possibility that the region spanning APA–RA (−257 to −190) contains binding sites for inhibitory factors (Fig. 2). The two final deletions down to *Stu* I/*Avr* II (STA) reduced the activity to 15%, suggesting a positive role for sequences located between RA and STA (−190 to −101). Of note, the three Inr motifs of the proximal transcriptional start sites are retained in the 212-bp STA (Fig. 1*CI*). XHA-Luc and the seven deletion constructs were also transfected into HEK cells. Overall, the response pattern was similar to that seen for Jurkat cells (Fig. 2). A notable exception was the RA-Luc construct, the activity of which was 2-fold greater in Jurkat cells than in HEK cells. Furthermore, insertion of a TCRβ enhancer element (19) into the RA-Luc, 3′ to the luciferase gene, produced in Jurkat cells a >2-fold increase of enzyme activity but had no effect on the activity in HEK cells (Fig. 2). The results indicate that RA is capable of responding to a cell-specific enhancer element.

To further assess the importance of the RA fragment for promoter activity, several consensus binding sites within this region (see Fig. 1*CI*) were modified by site-directed mutagenesis. Mutations in the three Inr sequences in their most critical site, the −1/+1 position (24), led to marked reduction of promoter activity in both Jurkat cells (to 20%) and HEK cells (to 26%) (Table 1). Similarly, a modified nucleotide in the second position of the putative Oct element reduced activity

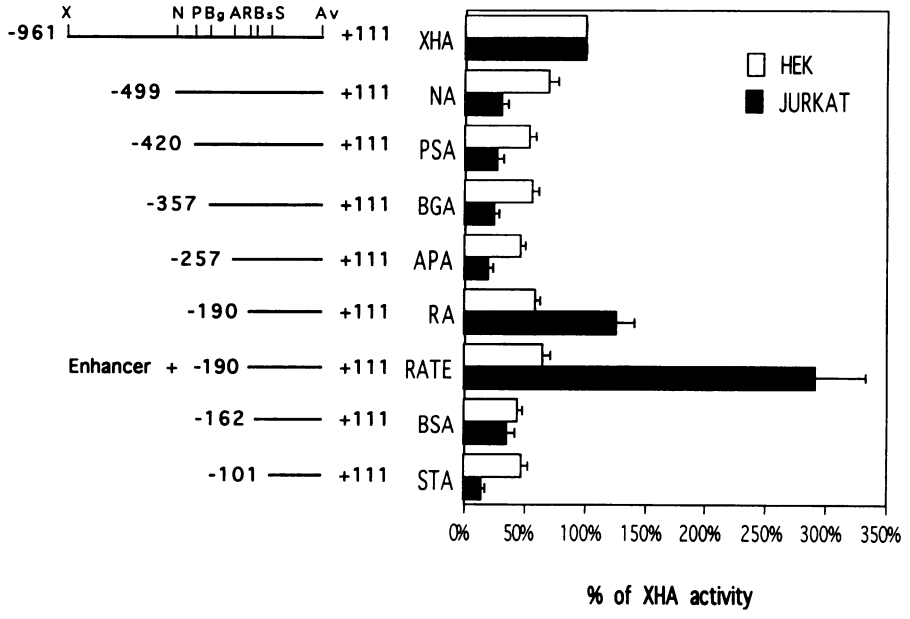

FIG. 2. Functional analysis of proximal *AML1* promoter region in Jurkat and HEK cell lines. The 1072-bp XHA (*Xho* I −961/*Avr* II +111) fragment was sequentially deleted from its 5′ end, as shown schematically, using the indicated restriction sites: N, *Not* I; P, *Pst* I; Bg, *Bgl* II; A, *Apa* I; R, *Rsa* I; Bs, *Bss*HII; S, *Stu* I. The fragments and constructs so produced were, respectively, NA, PSA, BGA, APA, RA, BSA, and STA. Luciferase reporter constructs containing these fragments were transfected into cells, Jurkat (solid bars) and HEK (open bars), and resultant luciferase activity was normalized for transfection efficiency against pSV-βgal. Histograms depict activities of plasmids relative to the longest promoter construct XHA. Results are given as means ± SE of six independent transfection experiments for each construct. RATE, RA construct with TCRβ enhancer inserted 3′ to the luciferase gene.

in both cell lines. On the other hand, when the GGA core of the PU.1 binding site and the adjacent Ets-like sequence were altered, promoter activity in Jurkat was reduced to 22% and almost not affected in HEK (Table 1).

Four luciferase constructs were generated for functional analysis of the distal 5′ region (Fig. 3). The four genomic fragments designated Z, A, B, and C were produced using PCR and relevant oligomers. We first analyzed the promoter activity of the 359-bp fragment A (−370 to −12). When the A-Luc construct was transfected into Jurkat cells, it yielded luciferase activity >180-fold above the pGL2-Basic control. When the orientation of A was inverted within A-Luc, construct activity was abolished (data not shown). When fragment A was replaced with the 1.126-kb long fragment Z (−1137 to −12), promoter activity was not affected; when fragment A was replaced by the shorter fragment B (−139 to −12), the activity was reduced by merely 25% (Fig. 3). These results suggested that sequences within the 1-kb region spanning fragments Z–B play only a modest role in modulating the activity of the distal promoter. More revealing was the pronounced decrease (to 5%) in luciferase activity of the C-Luc construct (Fig. 3). Since fragment C (−99 to −12) was obtained by deleting 40 bp from the 5′ terminus of fragment B, we gathered that this short segment encompasses sequences important for promoter activity of the distal region. As noted above, these 40 bp contain two adjacent binding sites, for the transcription factors PU.1 and CRE, separated by 11 residues of adenine (Fig. 1*CII*). Interestingly, when each of the two sites as well as the oligoA spacer were modified by site-directed mutagenesis, the result was only small changes of promoter activity in both Jurkat and HEK cells (data not shown). Transfection of the four Z–C constructs into HEK cells gave an expression pattern similar to that in Jurkat cells, including the sharp drop in activity of the C-Luc construct (Fig. 3). The only difference was the lack of response, in HEK cells, to the TCRβ enhancer element, which in Jurkat cells increased the activity of the A-Luc construct by 3-fold (Fig. 3).

DISCUSSION

Several observations strongly suggested that transcription of *AML1* mRNAs is initiated at two distinct genomic sites, raising the possibility that expression of the gene is transcriptionally regulated by sequences within these regions (see Fig. 1*A*). We isolated and analyzed genomic sequences spanning the 5′ upstream regions of the two *AML1* 5′ UTRs. RNA-extension experiments, employing two different primers for each UTR, revealed several transcription start sites (Fig. 1*B*). In the

Table 1. Site-directed mutagenesis of binding sites in the proximal promoter

Mutated element	Position in Fig. 1*CI*	Sequence	Effect of mutation as % wt	
			Jurkat	HEK
Initiators	−20	wt GTT**CA**TTTCCAGG**CA**CTCT**CA**TTC mut TG GG TG	19.5 ± 3.5	26.6 ± 5.7
PU.1 and Ets-like	−60	wt GG**A**AT**GG**AAT**TCC**TC mut T ΔT CGA	22.6 ± 2.4	93.0 ± 15.4
Octamer	−75	wt A**T**GCAAAC mut G	21.7 ± 2.2	38.2 ± 4.6

Mutations (mut) were introduced as described. Positions in the wild-type (wt) sequence are denoted by underlined boldface letters with altered nucleotides indicated below. Luciferase activity was determined as in Fig. 2. Results of six experiments are expressed as means ± SE relative to activity of the parental plasmid.

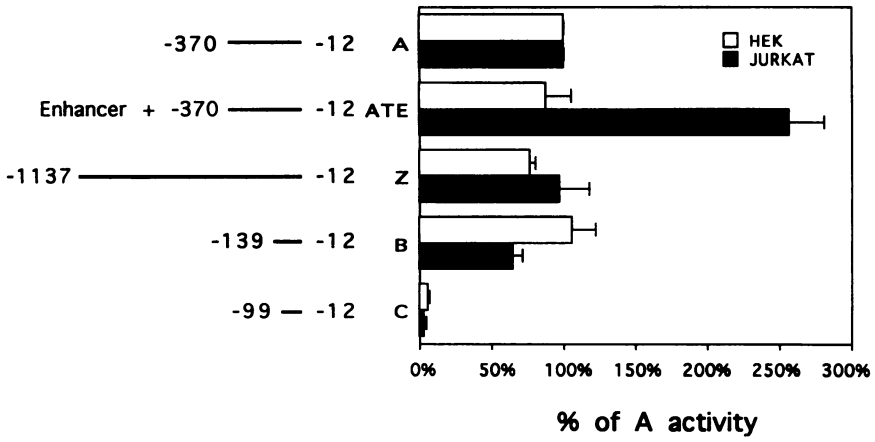

FIG. 3. Functional analysis of distal *AML1* promoter region in Jurkat and HEK cells. Reporter plasmids include the 1126-bp fragment Z (−1137 to −12) and three deletion products (A, B, and C). Mean luciferase activity of six transfections was determined as in Fig. 2 and related to the activity of the A-Luc construct. ATE, A construct with TCR β enhancer.

proximal 5′ region, three of the start sites are embodied within an Inr consensus sequence known to participate as a core promoter element in the assembly of transcription complexes (reviewed in ref. 31). Site-directed mutagenesis and transfection assays demonstrated the functional importance of these elements for transcription of *AML1* (Table 1). Transcription complex formation requires Inr sequences to a greater degree in TATA-less promoters on which Inr mediates localization of TFIID through interaction with Inr-binding proteins (32). Significantly, it was recently shown (33) that the activator Sp1, for which several binding sites are present in the proximal region (Fig. 1*CI*), functions most effectively with Inr, especially in the absence of a TATA box. No Sp1 binding sites were identified in the distal region, but since it is relatively A+T-rich it is possible that the Inr motifs, in conjunction with an A+T-rich sequence, facilitate assembly of the preinitiation complex in this region (32).

The function of the 5′ regions in transcription was analyzed using luciferase reporter plasmids, and the locations of promoters in the regions upstream of the transcription start sites were confirmed. Deletion analysis of the two AML1 promoters identified DNA regions that potentially contain positive and negative regulatory elements. In the proximal promoter, activity was greatly reduced when sequences between *Xho* I (−961) and *Not* I (−499) were deleted, while activity was restored and enhanced when sequences spanning −257 to −190 were removed (Fig. 2). The latter deletion is of particular interest because increased activity occurred only in Jurkat cells and not in HEK cells. Further analyses are needed to identify the negative elements within this region. Deletions of an Sp1 binding site and a CCAAT box may be responsible for the decrease in promoter activity observed when the upstream fragment was further shortened to the 212-bp STA (Fig. 1*CI* and 2). Site-directed mutagenesis revealed that while the two binding sites of Oct and PU.1/Ets-like were essential for promoter activity in Jurkat cells, only Oct was required in HEK cells (Table 1). Negative cell-type specificity could be imposed on the proximal promoter by mutating the PU.1/Ets-like sites. In the distal *AML1* promoter, a vast reduction in activity was created by shortening of fragment B (Fig. 3). Since the deleted sequences contain binding sites for PU.1 and CRE (Fig. 1*CII*), we assumed that these sites are important for activity of the distal promoter. When these two sites and the oligoA spacer between were mutated, no significant difference in activity was observed comparable to the marked reduction seen with fragment C (Fig. 3). A potentially interesting finding is that both promoter regions contain binding sites for *AML1* (PEBP2/CBF); hence, *AML1* expression may be autoregulated by either AML1 proteins or other members of the gene family—e.g., *AML2* and *AML3*.

The transfection assays presented in Figs. 2 and 3 show that each of the two promoter regions functions at approximately the same level in Jurkat cells and in HEK cells. Hence, although these regions are involved in *AML1* transcription, they are not sufficient to mediate cell-type specificity. The only indications of cell-type specificity were obtained with the RA fragment and the mutated PU.1/Ets-like binding sites in the proximal promoter. These data suggest that sequences of cell-type-specific functions are yet to be identified in the *AML1* promoters. These may include cell-specific enhancer/silencer elements, DNA methylation sites, or chromatin structure.

A lack of cell-type specificity was previously observed for the CD34 hematopoietic stem cell antigen promoter (34, 35). This promoter has many features in common with the proximal promoter of *AML1*. Promoters of this type were originally classified as housekeeping, G+C-rich, TATA-less promoters (reviewed in ref. 36). Recently, it became clear that G+C-rich promoters are also found upstream of certain tissue-specific genes; in this case, there is usually a housekeeping core promoter, and the specificity of expression is under the negative control of repressors in nonexpressing cells. The pattern of AML1 expression in various cells and hematopoietic tissues agrees with this premise; the distal promoter region upstream of the short 5′ UTR is active in cells (Daudi) and tissues (thymus) where the proximal G+C-rich promoter is repressed (6, 13). More experiments are needed in order to clarify the functional significance of the two *AML1* promoter regions and their role in leukemogenesis. As has been noted above, the fusion genes generated by the *AML1*-associated translocation are not normally expressed in myeloid cells and their transcription as chimeric RNAs is under control of the *AML1* promoters (2). It will therefore be interesting to determine whether sequences within the fusion genes cause alteration in the native pattern of tissue-specific expression of *AML1*.

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