The granulocyte-macrophage colony-stimulating factor (GM-CSF) gene promoter contains a consensus sequence for the polyomavirus enhancer binding-protein 2 (PEBP2) transcription factor, which consists of α and β subunits. There are at least two genes, αA and αB, encoding the α subunit. αB is the mouse homologue of human AML1 gene detected at the breakpoints of t(8;21) and t(3;21) myeloid leukemias. We examined αA1 (an αA-gene product) and αB1 and αB2 (two αB-encoded isomers) for their effects on the GM-CSF promoter. PEBP2αA1, αB1, and αB2 proteins bound the PEBP2 site within the mouse GM-CSF promoter. PEBP2αA1 and αB1 enhanced the expression of the GM-CSF promoter-driven reporter plasmid in unstimulated and 12-O-tetradecanoylphorbol 13-acetate/phytohemagglutinin-stimulated human Jurkat T cells. In contrast, the promoter activity was suppressed by αB2. Coexpression of αB1 and αB2 showed that the promoter activity could be determined by the αB1/αB2 ratio. Jurkat cell extract contained PEBP2 site-binding protein(s) that cross-reacted with anti-mouse αA1 antibodies. Northern blot analysis indicated the expression of human PEBP2αA, αB (AML1), and β genes in Jurkat cells. Although further studies are required to determine the precise role of PEBP2 in the GM-CSF promoter activity, the present findings suggested the importance of the relative ratio of different PEBP2 isoforms in regulating the levels of the promoter activity.

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Submitted May 13, 1994; accepted March 5, 1995.


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been identified at the breakpoint of inversion 16 translocation leukemias. Also, the human homologue of 21 of 8;21 and 3;21 translocations observed in myeloid leukemias. Also, the human homologue of PEBP2β has been identified at the breakpoint of inversion 16 translocation associated with a subset of AML.

PEBP2 has been implicated in the T-cell–specific gene regulation. Northern blot analysis of the RNAs obtained from mouse tissues and in situ hybridization analysis of the mouse tissue sections showed that PEBP2αA and αB mRNAs are expressed in immature and mature thymocytes, suggesting the involvement of PEBP2 in transcriptional regulation throughout the T-cell development. The potential role of PEBP2 in T-cell ontogeny has been suggested by the multiple PEBP2 isoforms generated by alternative splicing from mouse tissues and in situ hybridization analysis of the mouse embryonal carcinoma cells were maintained and transfected with pEFBos-αA1, -αB1, and -αB2 plasmids as described. In brief, P19 cells, plated at 1 × 10⁵ cells per 6-cm dish 24 hours earlier, were transfected with 10 µg of plasmid DNAs using a modified calcium phosphate precipitation procedure and were harvested 48 hours posttransfection.

In the present study, we examined the effects of exogenously expressed mouse AML1/PEBP2α cDNAs on the GM-CSF promoter activity. We found that PEBP2αA proteins bind to the GM-CSF promoter region and that some PEBP2 consensus sequences are represented by the GM-CSF promoter.

Fig 1. (A) A comparison of human and mouse GM-CSF promoter regions and Py enhancer A element is shown. PEBP2 consensus sequences are boxed. CLE0 element within GM-CSF promoter, AP-1, and ets sites within Py enhancer are also indicated. (B) Nucleotide sequences of wt and mutant forms (61.62, 59.60, and 57.58) of synthetic oligonucleotides that span the PEBP2 consensus within the mouse GM-CSF promoter are shown. Only one strand is represented for each DNA. PEBP2 consensus sequence is underlined.

MATERIALS AND METHODS

Plasmids. Wild-type (wt; pSVGM-95CAT) and mutant (pSVGM-61.62CAT) reporter plasmids had been constructed by inserting the -95 to +24 segment of mouse GM-CSF promoter into the pSV0CAT plasmid. pEFBos-αA1, -αB1, and -αB2 are expression plasmids for PEBP2αA1, αB1, and αB2 cDNAs driven by the human elongation factor 1α promoter.

DNA-binding assay. In vitro transcription and translation of PEBP2αA1 and αB2 were performed as described previously. P19 mouse embryonal carcinoma cells were maintained and transfected with pEFBos-αA1, -αB1, and -αB2 plasmids as described. In brief, P19 cells, plated at 1 × 10⁵ cells per 6-cm dish 24 hours earlier, were transfected with 10 µg of plasmid DNAs using a modified calcium phosphate precipitation procedure and were harvested 48 hours posttransfection. Jurkat human T cells (clone JP112; 1 × 10⁵) were incubated with or without TPA (10 ng/mL; Sigma, St Louis, MO) and PHA-M-form (PHA-M; 0.5%; GIBCO, Grand Island, NY) for 12 to 48 hours in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum (10%FCS-RPMI). Whole cell extracts (WCEs) of Jurkat and P19 cells were prepared as described.

The synthetic oligonucleotide containing a wt PEBP2 consensus sequence was either radiolabeled with [α-³²P]deoxyctydine triphosphate using Klenow DNA polymerase (Toyobo, Osaka, Japan) or end-labeled with [γ-³²P]adenosine triphosphate using T4 polynucleotide kinase (Toyobo) and was used as a probe. DNA-binding reactions and electrophoretic mobility shift assay (EMSA) were performed as described with some modifications. WCEs (10 to 20 µg) or in vitro translated αB1 or αB2 proteins (approximately 1 fmol) mixed with Escherichia coli-produced β2 protein (approximately 10 fmol) were incubated with a radiolabeled DNA probe (10⁶ cpm) in a 10-µL reaction mix containing 20 mmol/L HEPES (pH 7.9), 20% glycerol, 0.1 mmol/L KCl, 0.2 mmol/L EDTA, 0.5 mmol/L phenylmethylsulfonyl fluoride, 0.5 mmol/L dithiothreitol, 100 µg/mL bovine serum albumin, 0.01% Nonidet P40 (Boehringer Mannheim, Germany), and 1 µg poly(dI-dC) (Boehringer Mannheim). After 30 minutes at room temperature, the reaction mixture was analyzed on a 5% or 6% polyacrylamide gel in 0.25× TBE (22 mmol/L Tris, 22 mmol/L boric acid, and 0.6 mmol/L EDTA). After electrophoresis at 350 V (10 to 30 mA) for 1 hour at 4°C, the gel was dried and analyzed by image analyzer (Fuji Photo Film Co, Ltd, Tokyo, Japan). Preparation and detailed properties of the anti-PEBP2αA1 rabbit serum used in antibody supershift experiments are described elsewhere.
Transfection and chloramphenicol acetyltransferase (CAT) assay. A CAT reporter plasmid (4 μg) and an effector plasmid (0 to 5 μg) were cotransfected into Jurkat cells\(^{47,51}\) (2 to 5 × 10\(^6\) cells) using a modified diethyl aminoethyl-dextran method.\(^{52}\) After 24 hours, cells were incubated in the absence or presence of TPA (10 ng/mL) and PHA-M (0.5%)\(^{18}\) in 10% FCS-RPMI for an additional 24 hours. The CAT activity in the cell extract was examined at 48 hours post-transfection as described\(^{30}\) and was corrected for the protein concentration of the extract as determined by the Bradford method (Bio-Rad protein assay kit; Bio-Rad, Hercules, CA). Total amount of plasmid DNAs transfected was adjusted to 10 μg by addition of the pEF-Bos backbone vector\(^{46}\) for expression plasmids. The pRSV-βGal plasmid\(^{41}\) (1 μg) was also included in all the samples to monitor the efficiency of transfection.

Northern blotting. Total cytoplasmic RNA was prepared from unstimulated and TPA/PHA-stimulated Jurkat cells by acid guanidinium thiocyanate/phenol-chloroform extraction procedure.\(^{35}\) Poly(A)\(^+\) RNA selected by an oligo(dT) cellulose column (Collaborative Research, Bedford, MA) was electrophoresed in a formaldehyde/1% agarose gel, transferred to a nylon membrane (Hybond N; Amersham, Buckinghamshire, UK), and fixed by UV-cross-linking. The human homologues of PEBP2αA (Zhang et al, unpublished observations) and PEBP2β (Oka and Ito, unpublished observations) cDNAs have been cloned recently from a human T-cell cDNA library. C6E6H2 probe from the human AML1 gene (kindly provided by Dr M. Ohki, National Cancer Center Research Institute, Tokyo, Japan), a 0.82-kb HindIII-EcoRI fragment of human αA1 cDNA, and a Pvu II-Pvu II fragment of human PEBP2β cDNA (25 ng each) were \(^{32}\)P-labeled by a random priming method (Multiprime labeling kit; Amersham) and used as probes. Hybridization and washing of the blots were performed according to a phosphate buffer containing 0.5 mol/L Na,HPO\(_4\) (pH 7.2), 7% sodium dodecyl sulfate, and 1 mmol/L EDTA and transferred to a sealable bag containing the hybridization buffer and a radiolabeled probe. After overnight hybridization at 65°C, the membrane was washed for 5 minutes 3 times at room temperature and for 30 minutes once at 65°C in a washing buffer containing 40 mmol/L Na,HPO\(_4\) (pH 7.2) and 1% sodium dodecyl sulfate. After exposure to x-ray films, all the membranes were washed to strip off radioactivities and were reprobed with human β-actin cDNA to assess the integrity and quantity of the loaded RNA.

RESULTS

Binding of PEBP2 to the GM-CSF promoter. We first examined by EMSA whether the mouse PEBP2 proteins bind the PEBP2 consensus sequence within the mouse GM-CSF promoter. Synthetic oligonucleotides encompassing nucleotides -71 to -51 of the mouse GM-CSF promoter and their mutants (shown in Fig 1B) were used as labeled probes or competitors. PEBP2αB1 and αB2 (Fig 2) were translated in vitro and mixed with β2 protein, the most abundant isoform encoded by the β gene, which had been expressed in E coli and purified.\(^{30}\) Figure 3 shows an EMSA pattern obtained using radio-labelled wt oligonucleotides as a probe. A single major protein-DNA complex was observed when the αB1 and β2 proteins were mixed (Fig 3, lane 3). WCEs of αB1-transfected P19 cells created a complex that comigrated with it (data not shown). aB1 alone in the absence of β2 created a faster-migrating and less dense band as observed before (data not shown).\(^{35}\) Sequence specificity of the binding was assessed by adding 66-fold molar excess of unlabelled wt or mutant oligonucleotide competitors. The com-

![Fig 3. Binding of mouse PEBP2α proteins to PEBP2 site within mouse GM-CSF promoter. Wt oligonucleotide from the GM-CSF promoter (Fig 1B, wt), \(^{32}\)P-labeled by Klenow enzyme, was used as a probe. PEBP2αB1 (lanes 3 to 6) and αB2 proteins (lanes 7 to 10) were translated in vitro and mixed with PEBP2β protein expressed in E.coli. PEBP2αA1 protein was overexpressed in P19 cells, and their WCE (20 μg) was used (lanes 11 to 14). WCE was also obtained from P19 cells transfected with backbone vector for expression plasmids (PEF-Bos; lane 15). EMSA was performed using 6% non-denaturing polyacrylamide gels. (-), no competitor added; wt, 61.62, 59.60, a 66-fold molar excess of unlabelled oligonucleotides added as competitors (lanes 4 to 6, 8 to 10, and 12 to 14). Reaction mixture using WCE (10 μg) of Jurkat cells that were unstimulated (lane 1) or TPA/PHA-stimulated for 12 hours (lane 2) was electrophoresed on the same gel for comparison. The arrows on the left denote the positions of the specific complexes corresponding to PEBP2 binding.](http://www.bloodjournal.org/content/609/11/10840/F2.large.jpg)
plex formation was abolished by the wt competitor (Fig 3, lane 4), whereas it was not affected by the 61.62 or 59.60 mutants (Fig 3, lanes 5 and 6). The αB2 protein formed a faster-migrating complex (Fig 3, lane 7). In this case, the mutant competitors, 61.62 and 59.60, weakly competed with the band formation (see Fig 3 and compare lanes 9 and 10 with lane 7). This is most likely because of the fact that αB2 binds to DNA 2 to 3 times more strongly than αB1 and that each of these two mutations did not completely abolish the binding site. PEBP2αA1 (Fig 2) cDNA was transfected into P19 cells that express neither αA nor αB, and their WCE was prepared. αA1 protein also generated a complex having similar PEBP2 site-binding property (Fig 3, lanes 11 to 14). It migrated more slowly than the complex formed by the αB1 and β2 proteins. A faster-migrating doublet (Fig 3, lane 11), which migrated at the same position as αB2 and was also observed with WCE from P19 cells not transfected with PEBP2α (Fig 3, lane 15), did not show PEBP2-site specificity (see Fig 3 and compare lanes 12, 13, and 14 with lane 11). Because the doublet was also detected in Jurkat cells (Fig 3, lane 1) and the intensity of the doublet was reduced after the TPA/PHA stimulation (Fig 3, lane 2), we rigorously examined whether they are related to PEBP2. By performing EMSA using the oligonucleotides as probes or competitors containing the consensus PEBP2 site as well as mutated versions, including the one harboring the completely mutated PEBP2 site (GTGCGGAG compared with the original TGTGGTGC), and by testing the reactivity of the anti-αA1 antiserum, we came to the conclusion that they are unrelated to PEBP2 and, thus, represent nonspecific DNA-binding proteins (data not shown). These results indicated that the PEBP2αB1, αB2, and αA1 proteins bind the PEBP2 site within the mouse GM-CSF promoter at least in vitro, most likely as a heterodimer with the β protein.

Effect of PEBP2 on the GM-CSF promoter activity. pSVGM-95CAT reporter plasmid encodes the CAT gene driven by nucleotides −95 to +24 of the mouse GM-CSF promoter region containing CLE2/CK-2,11,12,20 GM-αB,15,21,22 GC box,11,12 PEBP2 consensus, CATT(A/T) repeat,13 and CLE04,1,12 elements. The pSVGM-95CAT was cotransfected into Jurkat T cells with expression plasmids for PEBP2αA1, αB1, or αB2. The transfected cells were stimulated with TPA/PHA or left unstimulated. As shown in Fig 4A, expression of αA1 or αB1 stimulated the promoter activity of the reporter plasmid in a dose-dependent manner. In contrast, αB2 inhibited the promoter activity by about 50% in both unstimulated and stimulated cells. The promoter activity of the effector plasmids was not affected by TPA/PHA treatment as assessed by the expression of pEFBos-CAT in unstimulated and stimulated cells (data not shown). To confirm that the effects of PEBP2 on the promoter activity were mediated by its binding to the PEBP2 site, the 61.62 mutation in Fig 1B was introduced into the CAT plasmid. The αA1 and αB1 failed to activate the promoter activity of the mutant pSVGM61.62CAT plasmid (Fig 4B). The transcriptional repression by the αB2 was also abolished by the mutation (Fig 4B). These results suggested that PEBP2 regulates the GM-CSF promoter activity, positively or negatively depending on its isoforms, by binding to its cognate binding site in vivo.

The opposite effects of αB1 and αB2 prompted us to examine the possibility that they can antagonize with each other in the regulation of the promoter activity. αB1 and αB2 were cotransfected at various ratios into Jurkat cells. As the αB1/αB2 ratio increased, there was a gradual increase in the CAT activity in both TPA/PHA-stimulated (Fig 5) and unstimulated Jurkat cells (data not shown). This result suggested that there is a regulatory mechanism that involves the competition among PEBP2α proteins with distinct transactivation or repression potentials on the promoter activity.

Expression of PEBP2 site-binding proteins in Jurkat T cells. When WCE of unstimulated Jurkat cells was incubated with the labeled GM-CSF promoter oligonucleotides (Fig 1B, wt), two major protein-DNA complexes were observed, the faster-migrating one being a doublet as described...
Fig 5. Effect of cotransfection of PEBP2αB1 and αB2 at various ratios on the GM-CSF promoter activity. Jurkat cells (4.5 × 10⁶) were cotransfected with indicated amounts of pEFBos-αB1 and -αB2 expression plasmids together with 4 μg of pSVGM-95CAT and were TPA/PHA-stimulated for 24 hours. CAT activities were assayed using one sixth of the cell extracts. The value for percentage of chloramphenicol acetylation is shown above each lane.

above (Fig 6, lane 1). The formation of the slower-migrating complex indicated by an arrow was abolished by the addition of unlabeled wt oligonucleotides in the reaction (Fig 6, lane 2). In contrast, competitors containing mutated PEBP2 sites, 61.62 and 59.60, did not affect the complex formation (Fig 6, lanes 3 and 4). The mutation 57.58, located at the margin of the PEBP2 site, partially inhibited the complex formation (Fig 6, lane 5). Thus, the complex indicated by the arrow showed the property of a PEBP2 site-specific protein-DNA complex. The faster-migrating doublet was considered nonspecific (see above). When the Jurkat cells were stimulated with TPA/PHA for 24 or 48 hours, the specific band was also detected, but its intensity was slightly diminished (Fig 6, lanes 6 and 7). Comparison of the gel-shift mobility showed that the Jurkat complex comigrated with the protein-DNA complex formed by the mixture of the mouse αB1 and β2 proteins (Fig 3, lanes 1 to 3). The results indicated the presence of endogenous protein(s) that bind to the PEBP2 site within the GM-CSF promoter in unstimulated and TPA/PHA-stimulated Jurkat cells.

To assess whether PEBP2α proteins, when exogenously expressed in Jurkat cells, could compete with the endogenous PEBP2 site-binding protein(s) for DNA binding, WCE of PEBP2αA1-overexpressed P19 cells was mixed with Jurkat WCE in EMSA. As the amount of the αA1-containing extract was increased, the Jurkat complex gradually decreased in amount, and the αA1 band increased in intensity (Fig 7A, lanes 2 to 5). Addition of the αB2-containing extract also gave a similar result (Fig 7B, lanes 2 to 5). The Jurkat complex was not diminished by addition of WCE from P19 cells transfected with pEF-Bos backbone vector (data not shown). These results established that there are endogenous PEBP2 site-binding protein(s) in Jurkat cells that have the DNA-binding properties similar to αA1 and αB2. These results also suggested that αA1, αB1, and αB2 transfected into Jurkat cells exerted their transcriptional regulatory activity by competing with endogenous Jurkat PEBP2 site-binding protein(s) for DNA binding.

Finally, we tested the reactivity of the Jurkat complex to rabbit polyclonal antiserum against mouse αA1 protein, which also cross-reacts with mouse αB1.⑨ As expected, the
antiserum induced the supershift of the authentic mouse αA1- or αB1-containing protein-DNA complex (Fig 8, lanes 5 to 8). Addition of the antiserum, but not the preimmune serum, caused the supershift of the Jurkat complex formed by WCE of unstimulated or TPA/PHA-stimulated Jurkat cells (Fig 8 lanes 1 to 4). This indicated that the PEBP2 site-binding protein(s) present in Jurkat cells are antigenically related to the mouse PEBP2αA1 and αB1 proteins. The faster-migrating bands in Fig 8, lanes 5 to 8, did not react with the antiserum. Therefore, they were considered nonspecific.

**PEBP2αA, αB, and β mRNA expression in Jurkat T cells.** EMSA showed that PEBP2 site-binding protein(s) structurally related to mouse PEBP2α proteins were present in Jurkat cells. Therefore, we performed Northern blot analysis to examine the expression of human PEBP2 genes. The human αB/AML1 probe showed at least four major species of mRNA (Fig 9, lane 1) similar to those described for human peripheral blood cells.36 After Jurkat cells were stimulated with TPA/PHA for 24 hours, the expression of human αB/AML1 was markedly downregulated. In the case of αA mRNA, increased amounts of poly(A)+ RNA had to be blotted to detect the signals, suggesting that its expression in Jurkat cells was much less than that of human αB1/AML1.

The human αA probe showed a cluster of three major mRNA species of 7.1, 6.7, and 6.1 kb (Fig 9, lane 3). The human αA mRNA was also markedly decreased in TPA/PHA-stimulated Jurkat cells. A single major species of the human PEBP2β mRNA was detected in unstimulated Jurkat cells (Fig 9, lane 5). Again, TPA/PHA stimulation significantly reduced the level of the human PEBP2β expression. These results suggested that the human homologue(s) of the αB and possibly αA proteins constitutes at least a part of the Jurkat complex shown by EMSA. The biologic significance of the TPA/PHA-induced downregulation of αA, αB/AML1, and β expression remains to be clarified.

**DISCUSSION**

Sequence analysis of the GM-CSF promoter had shown a putative binding site for PEBP2 that is totally conserved between the mouse and the human genes. We showed that PEBP2 indeed binds the GM-CSF promoter and that the exogenously expressed αA1 and αB1 activate and αB2 inhibits, through the PEBP2 site, the transcription from the transfected GM-CSF promoter. Human T-cell line, Jurkat, expresses low levels of αA and much higher levels of αB mRNAs. By EMSA, PEBP2 site-binding protein(s) was de-
REGULATION OF GM-CSF PROMOTER BY PEBP2

Fig 8. Reactivity of Jurkat PEBP2 site-specific protein-DNA complex to anti-PEBP2αA1 serum. WCE (10 μg) from Jurkat cells, unstimulated (lanes 1 and 2) or TPA/PHA-stimulated for 24 hours (lanes 3 and 4), or WCE (20 μg) from P19 cells transfected with PEBP2αA1 (lanes 5 and 6) or αB1 (lanes 7 and 8), was preincubated with preimmune (lanes 1, 3, 5, and 7) or anti-PEBP2αA1 (lanes 2, 4, 6, and 8) rabbit serum for 15 minutes on ice. EMSA was performed using end-labeled wt probe and a 5% polyacrylamide gel. The positions of PEBP2 proteins to DNA (E. Ogawa and Y. Ito, unpublished observation). Figure 4 shows that the basal level of GM-CSF promoter activity before the TPA/PHA stimulation is reduced by 50% by exogenous expression of αB2. This effect is not observed when the reporter plasmid containing the 61.62 mutation is used. Therefore, αB2 must have interfered with the GM-CSF promoter activity in a transdominant negative fashion, strongly suggesting that the endogenous PEBP2 is contributing to the GM-CSF promoter activity significantly. Although the magnitude of the reduction was only 50%, it was not surprising because αB2 is 25% to 45% as active as the full-size protein, αB1.35 When the cells were stimulated by TPA/PHA and the overall GM-CSF promoter activity increased by about fivefold, exogenously expressed αB2 also showed the PEBP2 site-dependent reduction of the activity. Once again, the result shows the involvement of the endogenous PEBP2 in the promoter activity. In both states of the cells (ie, before and after the TPA/PHA stimulation), the 61.62 mutation did not affect the basal level of transcription. We interpreted the result to mean that particular ratios of the αA1, αB1, and αB2 proteins, as well as perhaps as yet unknown PEBP2 α isoforms. There are other mRNA species of PEBP2α isoforms. It remains to be determined that is antigenically related to mouse αA1 and αB1 proteins.

The present study indicated that PEBP2αB2 had a repressor-like activity when transfected into Jurkat cells. This seems to be contradictory to our previous report that αB2 is a transactivator when assayed in P19 cells.35 αB1 is the full-size αB isoform with a maximum transactivation potential. αB2, on the other hand, is only 25% to 45% as active as αB1 as a transcriptional activator. Although αB2 functions as a transactivator when tested in the cells, there is no evidence that this endogenous PEBP2 activity is stronger than that of αB2. Several examples of negative regulation by a weaker transactivator have been reported.37 Alternatively, the repressor-like function of αB2 may be specific for the GM-CSF promoter. In the context of the TCRβ enhancer linked to herpesvirus thymidine kinase gene promoter,35 the potential negative regulatory function would be alleviated by interaction of αB2 with other factors bound to adjacent sites.

The overall promoter activity of the reporter plasmid was increased by fivefold by the TPA/PHA stimulation. Transcription factors interacting with the elements located between −91 to −73 and −54 to −40 have been shown to be responsible for this process.12,14,15,22,23 What could be the role of PEBP2? It has recently been shown that PEBP2/CBF and another transcription factor, Ets-1, cooperatively bind to DNA.36 We have evidence that PEBP2 hardly stimulates transcription by itself but it functionally cooperates with Ets-1 in vivo. Therefore, PEBP2 appears to stimulate transcription by stabilizing the binding of Ets-1 or other activator proteins to DNA (E. Ogawa and Y. Ito, unpublished observation).

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clarified whether other genes exist that encode PEBP2 site-binding proteins. Cloning of all cDNAs of the PEBP2 family, characterization of proteins encoded by them, assessment of their effects on the activities of the promoter or enhancer of target genes, and analysis of their expression in various cells and tissues are clearly necessary.

Our present findings have suggested that the GM-CSF promoter is one of the targets of the PEBP2. A single binding-site within the upstream element seems to be sufficient for its transcriptional regulatory activity. By computer-aided sequence search, we further noted that PEBP2 consensus sequences also occur within the promoters of various genes, such as interleukin-3, MIP1α/LD78, myeloperoxidase, and the human eosinophil Charcot-Leyden crystal protein. In fact, it has recently been reported that PEBP2/CFB sites in interleukin-3 and myeloperoxidase promoters are functionally important. The promoter activity could be regulated by positive or negative regulators generated from several distinct PEBP2α genes through differential posttranscriptional and, possibly, posttranslational modifications. Accumulating evidence suggests that proliferation and differentiation of hematopoietic cells are controlled by ordered regulation of gene expression by transcription factors and involve a controlled balance between positively and negatively acting factors. A subversion of this controlled equilibrium may underlie the leukemogenesis by chromosomal translocations creating chimeric proteins, which may have unusual transcriptional regulatory properties. Further study on the function of each PEBP2α protein in the transcriptional control of each target gene would show the roles of the PEBP2 transcription factors in physiology and pathophysiology, such as embryonic development, T-cell differentiation, and leukemogenesis.

ACKNOWLEDGMENT

We thank Dr M. Ohki (National Cancer Center Institute, Tokyo, Japan) for AML1 probe. We also thank Y. Murakami, E. Ogawa, H. Ishikawa, C. Sakakura, and T. Kanda (from the Institute for Virus Research, Kyoto University, Kyoto, Japan) for helpful discussions and suggestions; M. Kumamoto for technical assistance; and Y. Fujita for secretarial assistance.

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Positive and negative regulation of granulocyte-macrophage colony-stimulating factor promoter activity by AML1-related transcription factor, PEBP2

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