AML1 (RUNX1) is one of the most frequently disrupted genes in human leukemias. AML1 encodes transcription factors, which play a pivotal role in hematopoietic differentiation, and their inappropriate expression is associated with leukemic transformation of hematopoietic cells. Previous studies demonstrated that the transcription cofactor p300 binds to the C-terminal region of AML1 and stimulates AML1-dependent transcription during myeloid cell differentiation. Here, we report that AML1 is specifically acetylated by p300 in vitro. Mutagenesis analyses reveal that p300 acetylates AML1 at the two conserved lysine residues (Lys-24 and Lys-43). AML1 is subject to acetylation at the same sites in vivo, and p300-mediated acetylation significantly augments the DNA binding activity of AML1. Disruption of these two lysines severely impairs DNA binding of AML1 and reduced the transcriptional activity and the transforming potential of AML1. Taken together, these data indicate that acetylation of AML1 through p300 is a critical manner of posttranslational modification and identify a novel mechanism for regulating the function of AML1.

AML1 (PEBP2αB, core binding factor α2, or RUNX1) and its cofactor PEBP2β/core binding factor β are the most frequent targets of chromosomal translocations in human leukemias (1). The AML1 gene was identified through its involvement in the (8;21) translocation, which rearranges the AML1 gene on chromosome 21q22 and the ETO (MTG8) gene on chromosome 8q22, resulting in the generation of the AML1-ETO fusion protein (2–4). AML1 is also involved in human leukemias carrying t(3;21) or t(12;21) translocation, suggesting that it plays an important role in leukemogenesis (5–8).

The AML1 gene encodes a transcription factor containing an N-terminal DNA-binding domain that is highly homologous to the Drosophila pair-rule protein Runt, which is called the Runt domain (9). AML1 binds to the core enhancer DNA sequence, TG/Tc/GGT, called the PEBP2 site, through the Runt domain. Its affinity for DNA is markedly increased by heterodimerization with PEBP2β (10–13). This heterodimeric complex regulates transcription of a large number of hematopoietic lineage-specific genes (14, 15). Targeted disruption of either AML1 or PEBP2β has demonstrated that both AML1 and PEBP2β are essential for all lineages of definitive hematopoiesis in the murine fetal liver (16–18). In addition, AML1 exhibits the transforming activity when expressed in fibroblasts, and this activity requires both the Runt domain and the C-terminal transcriptional regulatory domain called the PST region (19). At least four forms of the AML1 proteins are produced by alternative splicing, termed AML1a, AML1b, AML1c, and AML1N (20, 21). Among them, AML1b is one of the transcriptionally active forms, which contain both the Runt domain and the PST region (22). We simply refer to this alternative form as AML1 hereafter.

Previously, Kitabayashi et al. (23) demonstrated that AML1 associates with a transcription cofactor p300 in vivo and that p300 potentiates AML1-dependent transcriptional activation. On the other hand, AML1 synergizes with a variety of transcription factors, including CCAAT/enhancer binding protein-α, AP-1, Ets-1, PU.1, and c-Myc, which regulate cellular proliferation and differentiation (24–30). Conversely, AML1 can repress transcription by associating with corepressors such as Groucho/transducin-like Enhancer of split and mSin3A (31–33). Thus, AML1 appears to act as an “organizing” factor of transcription by interacting with a wide variety of transcription regulators. In contrast, regulatory mechanisms for AML1 function remain elusive thus far. Previously, we reported that AML1 is phosphorylated through the extracellular signal-regulated kinase (ERK) (34). ERK-dependent phosphorylation potentiates the transcriptional activity and the transforming capacity of AML1 through regulating interaction between AML1 and mSin3A (35). Thus, the function of AML1 is also regulated through the signal transduction pathways.

Acetylation has recently emerged as the central mode of regulation for a significant number of transcription factors (36, 37). p300 and the related protein CBP are highly conserved proteins that have a pivotal role in transcriptional regulation, bridging a wide variety of DNA-binding proteins to components of the general transcriptional machinery (38). In addition, p300
AML1 Acetylation by p300

...and CBP possess histone acetyltransferase (HAT) activity, which is able to acetylate histone and non-histone proteins. Histone acetylation is linked to transcriptional activation and participates in the nucleosomal remodeling that accompanies gene activity (39). Recently, HATs have been shown to acetylate a significant number of non-histone proteins, which include transcription factors such as p53, Drosophila T-cell receptor, erythroid kruppel-like factor, GATA-1, GATA-3, and the high mobility group protein I/Y (40, 41). Acetylation of these factors leads to changes in protein-protein and protein-DNA of AML1, K24R/K43R and K24A/K43A into the pGEX2T vector, respectively. For construction of FLAG-tagged P/CAF-HAT/Br and K182R/K188R were obtained by replacing the lysine residues with arginines and alanines, respectively, by the site-directed mutagenesis method (46). For construction of the retroviral vector that harbors AML1 or K24A/K43A, the 1.8-kb EcoRI fragment encoding AML1 or K24A/K43A was deprived of the polyadenylation signal by digestion with BamH I and cloned into the pSRaMSVtkgeo vector (19). pM-CSF-R-luc containing 416 to +71 of the human M-CSF receptor promoter was described previously (47). peDEF3-p300 was kindly provided by Dr. Miyazono and Dr. Kawabata. The glutathione S-transferase (GST) fusion constructs of AML1, FLAG-tagged p300-HAT, P/CAF-HAT/Br, and GCN5 were purified as described previously (44, 45).

In Vivo Acetylation Assays—GST fusion proteins or histones (Roche Applied Science) were collected on glutathione-Sepharose beads (Amersham Biosciences) incubated at 30 °C for 1 h in the buffer containing 50 mM Tris, pH 8.0, 10% glycerol, 1 mM dithiotreitol, 10 mM sodium butyrate, 1 μg of protein/ml, 1 μg of pepstatin/ml, 1 μg of leupeptin/ml, 0.2 mM phenylmethylsulfonyl fluoride followed by incubation for 30 min on ice. Whole cell lysates containing 100 μg of proteins were subjected to SDS-PAGE and transferred to polyvinylidene difluoride membranes (Immobilon, Millipore). The membranes were blocked with 10% skim milk, treated with anti-p300 (RW128, Upstate Biotechnology), anti-AML1, anti-FLAG (M2, Sigma), or anti-PEBP2β, washed, and reacted with the mouse or rabbit anti-IgG antibody coupled to horseradish peroxidase. The blots were visualized using the enhanced chemiluminescence (ECL) system (Amersham Biosciences). For immunoprecipitation, cells were lysed in the above buffer and subjected to immunoprecipitation with anti-PST or anti-AML1 (PC284L, Oncogene) followed by incubation with protein A-Sepharose (Sigma). Immunoprecipitates were washed, separated by SDS-PAGE, and analyzed with anti-p300 as described above.

In Vivo Sodium [3H]Acetate Labeling—MOLT-4 cells were grown to 2 × 10⁶ cells, washed twice with cold phosphate-buffered saline, resuspended in RPMI labeling medium (1 mL of [3H]sodium acetate (Amer sham Biosciences) per ml and 50 μM trichostatin A (Wako)) and then incubated at 37 °C for 90 min. pME18S-AML1 and mutants either with or without FLAG tag were transfected with peDEF3-p300 as described above into COS7 cells. After 30 h, cells were exposed to 1 μCi of sodium [3H]acetate/ml in the presence of 50 μm trichostatin A for 90 min. Lysates were prepared and processed using either anti-PST or anti-FLAG as described above. SDS-PAGE and blotting was performed, followed by electrotransferred onto polyvinilidene difluoride membrane (Immobilon, Millipore) and analyzed using BAS2000 Image Analyzer (Fuji Film).

Electrophoretic Mobility Shift Assays (EMSA)—The M4 probe contains a partial A core of the polyomavirus enhancer was produced as described elsewhere (50). Five micrograms of GST fusion proteins were collected on glutathione-Sepharose beads, incubated with purified FLAG-p300-HAT in the presence or absence of 10 μM acetyl-CoA (Amersham Biosciences). Then, reaction mixtures were eluted from the beads in glutathione elution buffer (10 mM reduced glutathione in 50 mM Tris-HCl (pH 8.0)). GST-PEBP2β collected on glutathione-Sepharose beads was incubated in the thrombin (Amersham Biosciences) reaction mixture at room temperature for 16 h, and then centrifuged, and supernatant containing PEBP2β cleaved off from GST was collected. The recovered proteins were quantified by Coomassie staining, and 100 ng of these proteins were incubated with 1 ng of M4 probe in the buffer containing 20 μM Heps (pH 7.6), 4% Ficoll (W/V), 10 mM EDTA, 0.5 mM dithiothreitol, 300 ng of poly(dI-dC) for 30 min at room temperature in the presence or absence of PEBP2β. Seventy ng of unlabeled M4 probe were added as a cold competitor. Reaction mixtures were subjected to EMSA as described previously (45). Nuclear extracts were obtained from COS7 cells transfected with full-length AML1 or K24R/K43R in pME188 either alone or together with PEBP2β by the DEAE-dextran method, as described previously (19). The procedures for EMSA were presented previously (45). For radiosotope labeling, [α-32P]dCTP was incorporated into the M4 probe by incubating with the Klonev fragment.

Luciferase Assays—HeLa cells were transfected by using SuperFect (Qiagen) with pM-CSF-R-luc and plasmids expressing wild type AML1, K24R/K43R, or K24A/K43A in the presence or absence of p300 expression plasmids. Fifty ng/ml trichostatin A was added 8 h prior to harvest. Luciferase activity was determined 48 h later, as described previously (51). A plasmid expressing β-galactosidase was co-transfected as an internal control of transfection efficiency, and the data were normalized to the β-galactosidase activity, as described previously (51). Soft Agar Assays—Soft agar assays were performed according to procedures described elsewhere (19, 52). Colonies were counted after 14 days of culture in soft agar if they were larger than 0.25 mm in diameter.

RESULTS

AML1 Interacts with p300 in Vivo—p300 and CBP are known to interact with a variety of transcriptional factors as coactivators. Recently, a physical interaction between AML1 and p300 was demonstrated (23). To confirm the interaction of endogenous AML1 with p300 in hematopoietic cells, we performed immunoprecipitation experiments using M1 cells, a murine leukemic cell line. Whole cell lysates were prepared from M1 cells and subjected to immunoprecipitation with the anti-AML1 antibody or control preimmune serum. Immunoblot...
AML1 Acetylation by p300

Fig. 1. AML1 interacts with p300 in vivo. Co-immunoprecipitation (IP) of endogenous AML1 with p300 in lysates from M1 cells is shown. Whole cell lysates were precipitated with preimmune serum ([K]) or anti-PST serum (lane 3), and the precipitate was subjected to immunoblot (IB) analysis with anti-p300.

analysis with the p300-specific antibody showed that the precipitate with anti-AML1 contained p300, whereas p300 protein was never detected in the precipitate obtained with control preimmune serum (Fig. 1). These results indicate that AML1 forms complexes with p300 in vivo in agreement with previous findings (23).

p300 Specifically Acetylates AML1 in Vitro—Association of p300 with AML1, together with the recent demonstration of its acetyltransferase activity on a variety of transcription factors, prompted us to determine whether AML1 is a substrate for acetylation by p300. AML1 possesses nine lysine residues, which become potential targets for acetylation. To examine whether AML1 could be acetylated by p300 directly, the in vitro acetylation assay using purified forms of p300-HAT was performed. GST-AML1-[1–189], which contains all nine lysine residues, and the GST fusion protein was expressed in the BL21 bacterial host and purified (Fig. 2A). GST-AML1-[1–189] was incubated with p300-HAT in the presence of [3H]acetate-CoA. Use of these highly purified recombinant proteins eliminates possible contamination by other HATs. As shown in Fig. 2B, GST-AML1-[1–189] was specifically labeled with [3H]acetate in the presence of p300-HAT, whereas no signal was detected for GST alone. The faster migrating bands were considered to represent degraded products of the acetylated AML1 protein because the purified GST-AML1-[1–189] that served as a substrate contains identical migrating bands. In contrast, GST-AML1-[1–189] was never labeled without p300-HAT. We further investigated whether other HATs can acetylate AML1 using P/CAF and its close homologue GCN5. The HAT/bromodomain of P/CAF (P/CAF-HAT/Br) and full-length GCN5 were tagged with FLAG, expressed bacterially, and subsequently used in the in vitro acetylation assays. The results in Fig. 2B show that neither P/CAF-HAT/Br nor GCN5 acetylated GST-AML1-[1–189] at all. As shown in Fig. 2C, the HAT activities of p300-HAT, P/CAF-HAT/Br, and GCN5 were confirmed by using histones as substrates. p300-HAT could acetylate histones H2A, H2B, H3, and H4, whereas only histones H3 and H4 were acetylated by P/CAF-HAT/Br and GCN5, which is in agreement with the previous reports (42). Taken together, these results unequivocally indicate that AML1 can be a specific substrate for p300-mediated acetylation in vitro.

In Vivo Acetylation of AML1—Next, we went on further to examine whether acetylation of AML1 occurs in vivo to manifest a physiological relevance of in vitro acetylation of AML1. First, we wished to determine whether endogenous AML1 in hematopoietic cells was also acetylated. For these experiments, we pulse-labeled MOLT-4 cells, a human acute lymphoblastic leukemia cell line, with [3H]acetate in the presence of a histone deacetylase inhibitor (trichostatin A) and then subjected them to cell lysis and immunoprecipitation with anti-AML1. As shown in Fig. 3A, anti-AML1, but not control preimmune serum, precipitated [3H]acetate-labeled AML1 (lanes 2 and 3). Immunoblot analysis with the same antibody revealed migration of endogenous AML1 (lane 1). These results directly provide evidence that AML1 is endogenously acetylated in hematopoietic cells.

To define the positions within AML1 to be acetylated by p300, we then employed transient transfection into COS7 cells. First, cells were transfected with FLAG-tagged full-length AML1 and p300 and labeled with sodium [3H]acetate in the same manner performed above. Lysates were immunoprecipitated with the anti-FLAG antibody and subjected to immunoblot analysis. As shown in Fig. 3B, AML1 was efficiently recovered from AML1-transfected cells with anti-FLAG. The immunoprecipitated AML1 protein was specifically labeled with [3H]acetate as in MOLT-4 cells (Fig. 3B, right). Next, we employed three types of serial deletion mutants to cover all nine lysines (Fig. 2A). These FLAG-tagged mutants were expressed in COS7 cells together with p300 and subjected to the in vivo acetylation assays. Fig. 3B shows that these mutants are expressed in COS7 cells in the anticipated sizes. Acetylation of AML1 was retained when amino acids 47–172 in the Runt domain or amino acids 173–188 in C-terminal region adjacent to the Runt domain are deleted (lanes 9 and 10). In contrast, deletion of N-terminal region (amino acids 23–64) flanked by the Runt domain completely abolished AML1 acetylation by p300 (lane 8). This region contains two lysine residues, Lys-24 and Lys-43. These two lysines are highly conserved among the Runt-containing protein family, with Lys-24 being completely conserved from the Zebrafish Runx1 to the human AML1 family members. These results suggest that either or both of these two N-terminal lysines are potentially acetylated by p300.

AML1 Is Acetylated by p300 on N-terminal Two Lysine Residues—To precisely determine the target residues of AML1 for p300-mediated acetylation, Lys-24 and Lys-43 were substituted with arginines or alanines either individually or in combination. Substitution of either Lys-24 or Lys-43 with arginine (K24R or K43R) significantly reduced the level of acetylation. The level of acetylation in K24R was significantly lower than that in K43R. Furthermore, substitution of both Lys-24 and Lys-43 by arginines or alanines almost completely abolished acetylation of AML1 (Fig. 4B). In contrast, substitution of C-terminal lysines (Lys-182 and Lys-188) did not alter in vivo acetylation of AML1, indicating that these residues are not involved in acetylation. These findings suggest that Lys-24 and Lys-43 are preferentially acetylated in vivo in agreement with the results obtained from the deletion mutants. We also performed an in vitro acetylation assay using purified forms of GST-K24R/K43R-(1–189) and GST-K24A/K43A-(1–189) in which Lys-24 and Lys-43 of AML1-(1–189) were substituted by arginines and alanines, respectively. Although GST-AML1-(1–189) was efficiently acetylated by p300, no acetylation was detected for GST-K24R/K43R-(1–189) and GST-K24A/K43A-(1–189) (Fig. 4C), which is consistent with the results of the in vivo acetylation assays. Autoacetylated p300-HAT was observed in the very top in all lanes except the one without p300-HAT. To preclude the possibility that substitution of Lys-24 and Lys-43 can disrupt the interaction between AML1 and p300, we performed immunoprecipitation experiments by transiently expressing p300 with AML1 and K24A/K43A in 293T cells. The mutant formed a complex with p300 as effi-
ciently as wild type AML1 in 293T cells (data not shown). These results imply that loss of acetylation in the Lys-24/Lys-43 mutant does not result from their inability to associate with p300 but suggest that these two lysines are authentic targets for acetylation by p300.

Acetylation Augments Site-specific DNA Binding of AML1—Having identified an AML1 mutant that cannot be acetylated by p300, we set out to use this mutant to dissect the functional consequence of AML1 acetylation. For a growing number of transcription factors, it has been suggested that acetylation...
plays a key role in the regulation of sequence-specific DNA binding (40, 42). Since acetylation can lead to a change in the charge and the size of the lysine residues (53), it is likely that acetylation impinges on the affinity of AML1 for DNA. To test this possibility, electrophoretic mobility shift assays were performed with purified proteins for GST-AML1-(1–189) or its lysine mutant, both of which possess the Runt domain that is responsible for binding to the PEBP2 site (11, 12). Each protein was incubated with bacterially produced p300-HAT and radiolabeled M4 probe, double-stranded oligonucleotide bearing the PEBP2 site, and DNA binding abilities were evaluated. When M4 probe was incubated with GST-AML1-(1–189) and p300-HAT in the absence of acetyl-CoA, we observed a shifted band that was not seen for GST (Fig. 5A, left). The shifted band was significantly reduced when an excess of the cold probe was added, indicating that the AML1-DNA complex was formed through specific binding of AML1 to the PEBP2 sequence. We then determined the effect of p300-mediated acetylation on DNA binding of AML1. The addition of acetyl-CoA greatly augmented the shifted band (Fig. 5A, left, lane 4), indicating DNA binding of AML1 specifically enhanced by acetylation. In contrast, K24R/K43R substitution in GST-AML1-(1–189) significantly abolished the DNA binding ability of AML1, and no increase in DNA binding was found even upon the treatment of acetyl-CoA (Fig. 5A, left, lanes 5 and 6). Coomassie staining of each recombinant protein indicated the presence of equal amounts of the GST-AML1 proteins (Fig. 5A, right).

Because PEBP2β is a key regulator for DNA binding of AML1, we next tested the effect of AML1 acetylation on DNA binding in the presence of PEBP2β. As shown in Fig. 5B (left), GST-AML1-(1–189) in the presence of bacterially produced PEBP2β showed a sequence-specific DNA-binding complex that was markedly diminished by the addition of the cold probe. K24A/K43A mutation abolished the DNA binding ability of AML1 even in the presence of PEBP2β (Fig. 5B, left). The K24R/K43R mutant, which maintains the positive charge also exhibited severely impaired DNA binding, indicating that al-
AML Acetylation by p300

Acetylation by p300 Stimulates Transcriptional Activation of AML1—Because DNA binding of AML1 is stimulated in an acetylation-dependent manner, it is tempting to speculate that the transcriptional activity of AML1 can potentially be regulated by acetylation. To determine this, we examined whether substitution of Lys-24 and Lys-43 could modify transcriptional responses induced by AML1. For these experiments, we employed a reporter plasmid pM-CSF-R-luc in which the M-CSF receptor promoter is linked to the luciferase gene because it is efficiently activated by exogenous expression of AML1 (29). Wild type AML1, K24R/K43R, or K24A/K43A was introduced into HeLa cells, which lack endogenous AML1 activity, together with the reporter plasmid, and then luciferase activities were evaluated. As shown in Fig. 6B, we observed a 7-fold activation of pM-CSF-R-luc when wild type AML1 was expressed. In contrast, both K24R/K43R and K24A/K43A showed a significantly reduced transcriptional activation. Although p300 expression further enhanced the transcriptional activity of wild type AML1, impaired transcription by K24R/K43R and K24A/K43A was not restored even in the presence of p300. We confirmed that both K24R/K43R and K24A/K43A were ex-

cantly enhanced DNA binding of the AML1-PEBP2β complex. Coomassie staining of each recombinant protein indicated the presence of equal amounts of the GST-AML1 proteins (Fig. 5B, right). These results indicate that PEBP2β cannot fully overcome a decrease in DNA binding of the AML1 mutants defective for acetylation.

To obtain further evidence that acetylation of target lysines alters DNA binding of AML1, we examined the effect of acetylation on sequence-specific DNA binding using full-length AML1 and its arginine mutant (K24R/K43R) in the presence of PEBP2β. We performed EMSA with nuclear extracts of COS7 cells transfected with mock, full-length AML1, and K24R/K43R together with PEBP2β. When the M4 probe was incubated and electrophoresed with nuclear extracts containing wild type AML1 in the presence of PEBP2β, a distinct band was observed that was not recognized in the lane loaded with the mock transfectant (Fig. 5C, left, lanes 1 and 2). This band became undetectable when a cold probe was co-incubated with a labeled probe (lane 3), indicating that it represents a specific AML1-DNA complex. We again found that DNA binding of K24R/K43R was significantly reduced, although it still remained at a detectable level. Immunoblot analysis of nuclear extracts confirmed the presence of equal amounts of the AML1 proteins in all samples (Fig. 5C, right). Taken as a whole, these results demonstrated that DNA binding of AML1 could be regulated by acetylation of Lys-24 and Lys-43.

Heterodimerization with PEBP2β Is Not Modulated by Acetylation of AML1—The affinity of AML1 for DNA is markedly increased by heterodimerization through the Runt domain with PEBP2β, which could not interact with DNA by itself (10–13). It is possible that the altered interaction with PEBP2β could determine the DNA binding property of acetylated AML1. We asked, therefore, whether mutation of Lys-24 and Lys-43 in AML1 affects the interaction with PEBP2β. COS7 cells were transfected with wild type AML1, K24R/K43R, or K24A/K43A together with PEBP2β. The cells were lysed and immunoprecipitated with anti-PST and then subjected to immunoblot analysis using anti-PEBP2β. As shown in Fig. 6A, K24R/K43R and K24A/K43A associated with PEBP2β as effectively as wild type AML1. The expression level of each construct was confirmed by immunoblotting of whole cell lysates. These data indicate that Lys-24 and Lys-43, the target residues for acetylation, are dispensable for heterodimerization between AML1 and PEBP2β, suggesting that acetylation of AML1 does not affect the affinity for PEBP2β.

Acetylation by p300 alters DNA binding of AML1 rather than a fundamental change in the conformation of the mutants. Impaired DNA binding in these mutants was not restored by the addition of acetyl-CoA, which signifi-
pressed as efficiently as wild type AML1 in HeLa cells (data not shown). These data strongly suggest that p300-mediated acetylation of AML1 on Lys-24 and Lys-43 is required for the optimal transcriptional activation of AML1.

Disruption of the Target Lysines Impairs Fibroblast-transforming Activity of AML1—Previously, we reported that overexpression of AML1 induces neoplastic transformation of NIH3T3 cells depending on the DNA binding ability and the

Fig. 6. A, interactions of wild type and the lysine mutant of AML1 with PEBP2β. COS7 cells were transfected with pME18S, wild type AML1, K24R/K43R, or K24A/K43A, together with PEBP2β as indicated. Cells were lysed, immunoprecipitated (IP) with anti-PST, and subjected to immunoblotting (IB) with anti-PEBP2β (top). Expression of each protein is monitored by immunoblotting of whole cell lysates with either anti-PEBP2β or anti-AML1 (middle and bottom). B, acetylation of AML1 stimulates its transcriptional activity. pM-CSF-R-luc was co-transfected into HeLa cells with each set of expression plasmids with or without co-expression of p300, and the cells were analyzed for luciferase activity. Trichostatin A was added where indicated to a final concentration of 50 ng/ml at 8 h prior to harvest. Values of the relative luciferase activity and error bars represent the means and the standard deviations, respectively, for three independent experiments. C, soft agar assays with NIH3T3 cells expressing AML1 and the K24A/K43A mutant. NIH3T3 cells infected with retroviruses for AML1 or K24A/K43A of a comparable titer were seeded in soft agar after G418 selection and cultured for 14 days. Cells infected with AML1 gave larger average colony sizes and increased colony numbers, whereas cells infected with K24A/K43A created barely macroscopic colonies in agar as well as mock-infected (mock) cells. D, comparison of transforming activities of AML1 and K24A/K43A. Colonies greater than 0.25 mm in diameter were counted as positive. Numbers and error bars show the means and standard deviations of colony counts, respectively, for three independent experiments.
transcriptional activity (19). Furthermore, functional modulation of AML1 such as ERK-dependent phosphorylation significantly alters the transforming activity of AML1 (34). To study a role of acetylation in the in vivo function of AML1, we compared transforming activities of the wild type and the lysine mutant of AML1. Replication-deficient retroviruses for AML1 and K24A/K43A were generated by hyperexpression of the corresponding plasmid in COS7 cells. NIH3T3 cells were infected with these retroviruses, and soft agar assays were performed on G418-resistant populations. As shown in Fig. 6, C and D, wild type AML1 rapidly produced a number of macroscopic colonies in soft agar. In contrast, replacement of Lys-24 and Lys-43 to alanines remarkably impaired the transforming activity of AML1, presumably because of the inability to bind to DNA efficiently. AML1 and K24A/K43A showed equivalent expression levels in NIH3T3 cells (data not shown). These results suggest that p300-mediated acetylation on Lys-24 and Lys-43 is also important for the biological activity of AML1 in vivo.

DISCUSSION

In this study, we showed that AML1 interacts with p300 and is acetylated on the two conserved lysines in the N terminus adjacent to the Runt domain. Acetylation increases sequence-specific DNA binding of AML1 and is needed for efficient transcriptional activation by AML1. Furthermore, acetylation plays a key role for the transforming activity of AML1 in fibroblasts.

Acetylation of proteins is shown to have both stimulatory and inhibitory effects on transcription (54, 55). As for histones, acetylation is reversible and affects the strength of protein-DNA or protein-protein interactions. In addition, acetylation of several transcription factors, such as p53 and MyoD, enhances transcription of their target genes (56, 57). In contrast, acetylation of Drosophila T-cell receptor, high mobility group protein I/Y, and activator of thyroid and retinoic acid receptor results in decreased transcription (58–60). Thus, acetylation plays bipartite roles in the regulation of gene expression. Recently, it was reported that acetylation of E2F enhances its function via multiple mechanisms including protein half-life other than the increased DNA binding activity and transcriptional activation. These findings suggest that acetylation may affect transcription factors at multiple steps (61). In the present case, one can envision several models for the regulatory mechanisms of AML1 by acetylation. First, p300-mediated acetylation may stabilize AML1 through a prolonged protein half-life. However, we could not observe that mutation of Lys-24 and Lys-43 causes a significant difference in protein stabilization when compared with wild type (Fig. 4). Secondly, acetylation may directly increase the affinity of AML1 for DNA. Significant in this regard is our demonstration that the residues of AML1 acetylated by p300 are located in the negative regulatory region for DNA binding N-terminal to the Runt domain (NRDBn) (25, 62). It might be expected that acetylation could induce a conformational change in NRDBn that unMASKs the DNA-binding interface of the Runt domain, resulting in potentiation of sequence-specific DNA binding. Schematic model for this hypothesis is shown in Fig. 7. However, Gu et al. (63) reported the controversial results that sequences N-terminal to the Runt domain do not affect DNA binding, which does not support this hypothesis. Another possibility is that acetylation may cause an increase in heterodimerization of AML1 with PEBP2β (25, 62). However, substitution of Lys-24 and Lys-43 does not affect the affinity of AML1 to PEBP2β, indicating that acetylation of AML1 does not contribute to heterodimerization with PEBP2β (Fig. 6A). Therefore, the increase in DNA binding by p300-mediated acetylation would reflect the altered interaction of AML1 itself with DNA rather than the lack of enhancing effect of the β protein. Finally, acetylation could affect protein-protein interactions, as described for binding of histone tails to the yeast transcriptional repressor Tup1 (64). Along these lines, further studies are in progress to elucidate the effect of AML1 acetylation on binding to other transcription factors such as Ets-1, CCAAT/enhancer binding protein-α, and PU.1.

In contrast to the remarkable effect of p300 acetylation on DNA binding of AML1, an impact on the transcriptional activation is relatively small. This discrepancy could be explained in several ways. First, AML1 manifests its transcriptional activation by participating in the assembly of a high order enhancer complex including other transcription factors as described above. These proteins in the complex may partially compensate for the decrease in DNA binding of AML1, which prevents a total loss of transcriptional activation. In this regard, it should be noticed that DNA binding of AML1 K24R/K43R can be detected to some extent in EMSA using nuclear extracts of COS7 cells, whereas it is much less detectable in EMSA using recombinant proteins (Fig. 5). Since many cooperating factors that associate with AML1 are supposed to exist in the nuclear extracts in contrast to highly purified recombinant proteins, it is reasonable to speculate that the formation of such a complex can partially compensate for decrease in DNA binding of AML1, which may blunt the effect of AML1 acetylation in transcriptional responses. Further investigation is needed to determine the existence of other possible intermolecular interactions. Another possibility is that other functional modifications of AML1, such as phosphorylation and methylilation, may dampen the consequence of acetylation in transcriptional responses. In particular, phosphorylation is a critical modification that regulates the DNA binding activity, nuclear localization, protein interaction, and transactivation of various transcription factors. For example, p53 is phosphorylated in response to DNA damage, leading to stabilization and stimulated DNA binding in vitro (65, 66). Acetylation of C terminus of p53 is also observed in response to DNA damage. Furthermore, C-terminal acetylation of p53 has been shown to be regulated through its N-terminal phosphorylation induced by DNA damage, indicating an intimate cascade between phosphorylation and acetylation (56, 67, 68). Previously, we demonstrated that transcriptional activation of AML1 is regulated through phosphorylation by ERK at the specific serine residues (Ser-246 and Ser-266). Phosphorylation of AML1 is induced by cytokine stimulation in hematopoietic cells. Taken together with our present studies, it is now clear that AML1 undergoes
two types of posttranslational modifications (Fig. 7). A potential association between phosphorylation and acetylation of AML1 remains to be further investigated.

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AML1 Is Functionally Regulated through p300-mediated Acetylation on Specific Lysine Residues
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