HoxA10 Represses Gene Transcription in Undifferentiated Myeloid Cells by Interaction with Histone Deacetylase 2*

YuFeng Lu‡§¶, Inna Goldenberg‡¶, Ling Bei‡§¶, Jelena Andrejic, and Elizabeth A. Eklund**

From the ‡Fineberg School of Medicine and the ¶Robert H. Lurie Comprehensive Cancer Center, Northwestern University, Chicago, Illinois 60611, and the ¶Robert R. M. F. Veterans Affairs Hospital, Chicago, Illinois 60611, and the ¶Birmingham Veterans Affairs Hospital, Birmingham, Alabama 35294

The homeodomain proteins, HoxA10 and Phx1a, interact with negative cis elements to repress gene transcription in undifferentiated myeloid cells. The CYBB and NCF2 genes, which encode the gp91PHOX and p67PHOX proteins, are two such HoxA10-Phx1a target genes. In previous studies, we found that HoxA10-Phx1a represses transcription of these genes by two mechanisms: competition for DNA binding with transcriptional activators and endogenous repression activity. In these studies, we identify a novel molecular mechanism of endogenous transcriptional repression by HoxA10-Phx1a. Endogenous repression activity of other Hox-Phx1a complexes requires recruitment of transcriptional co-repressor proteins by Phx1a. In contrast, our investigations have determined that HoxA10 has Phx1a-independent endogenous repression activity. We find that this transcriptional repression activity is abrogated by histone deacetylase inhibitors, suggesting involvement of co-repressor proteins. Consistent with this, we identify HoxA10 amino acids 224–249 as a Phx1a-independent repression domain, which interacts with histone deacetylase 2. We have determined that this HoxA10 domain is not conserved with other Abd Hox proteins, although homology exists with other transcription factors and co-repressors. Understanding the roles different Hox proteins play in myeloid differentiation is a challenging problem. Our results suggest that insight into this problem can be obtained from biochemical characterization of the various molecular mechanisms of Hox protein function.

Hox proteins are homeodomain transcription factors that play a crucial role in hematopoiesis. Genes encoding Hox proteins are organized in four paralogous groups (A–D) on four chromosomes (1). Hox expression is tightly regulated during embryonic and definitive hematopoiesis (1). Specifically, Hox 1–4 are expressed in hematopoietic stem cells, whereas committed myeloid progenitors express the Abd HoxA proteins 9–13 (2). A number of investigations suggest that tight regulation of Hox expression is necessary for normal hematopoiesis. For example, overexpression of HoxB4 in murine bone marrow cells results in CD34+ cell expansion ex vivo and induces myeloid leukemia in mice in the presence of overexpressed Phx1a (3, 4). In contrast, HoxA9 overexpression immortalizes committed murine myeloid progenitor cells, ex vivo (5). In the presence of overexpression of either Phx1a or Meis1 proteins, HoxA9 overexpression leads to murine myeloid leukemia (6, 7). Similarly, HoxA10 overexpression immortalizes bone marrow myeloid cells, ex vivo. However, HoxA10 overexpression in murine bone marrow induces myeloid leukemia, independent of Phx1a (8). Phx and Meis proteins increase the DNA binding affinity of various Hox proteins (9). However, Phx1a also interacts directly with transcriptional co-repressor proteins (10), indicating a number of potential functional activities in Hox-containing protein complexes.

Functional disparities suggest that Hox proteins are not redundant during myelopoiesis. Such differences could be due to variable target gene affinities. Consistent with this, binding site selection assays document different DNA-binding affinities among Hox proteins, despite highly conserved homeodomains (9). Further studies indicate that, similar to homologous Drosophila Hox proteins (11), Hox proteins function as transcriptional activators or repressors, depending on context. For example, a HoxB1-Phx1a complex either activates or represses transcription of artificial promoter constructs (12). Activation requires HoxB1 recruitment of CBP (12). In contrast, repression of the HOXB7 promoter by HoxB1 requires recruitment of histone deacetylase 1 (HDAC1), Sin3a, NCoR-1, and SMRT by the Hox-Phx complex (12).

We previously found that HoxA10-Phx1a represses transcription of the CYBB and NCF2 genes, via cis elements homologous to derived Hox-Pbx consensus sequences (13). The CYBB and NCF2 genes, which encode the respiratory burst oxidase proteins gp91PHOX and p67PHOX, are expressed in differentiating myeloid cells after the promyelocyte stage (14, 15). We found HoxA10-Phx1a binds the CYBB and NCF2 cis elements, in undifferentiated myeloid cells and prevents DNA binding of transcriptional activators to positive cis elements, immediately adjacent in the promoters (27). However, we also found that HoxA10, expressed as a fusion protein with the GAL4 DNA-binding domain, represses an artificial promoter containing GAL4 DNA-binding sites (13). Therefore, we identified two mechanisms for HoxA10-Phx1a repression of these genes: competition with transcriptional activators and endogenous repression activity (13). We found that HoxA10-binding affinity to the CYBB and NCF2 genes is decreased by HoxA10 tyrosine phosphorylation (13). Since HoxA10 is tyrosine-phos-
phorylated during differentiation, this provides a mechanism regulating repression activity during myelopoiesis (16).

Functional differences between Hox proteins could reflect variable mechanisms of transcriptional repression or activation. In the current studies, we find that, in contrast to previously described Hox proteins, HoxA10 transcriptional repression is Pbx1a-independent. We identify a novel transcriptional repression region in HoxA10 that interacts with HDAC2, which has homology to domains in other transcriptional repressor and co-repressor proteins. These results suggest that various Hox proteins participate in different protein-protein interactions, which may confer some of the dissimilarity of function between these proteins.

**MATERIALS AND METHODS**

**Plasmids and PCR Mutagenesis—** Artificial promoter/reporter constructs were generated as previously described (13) in the minimal promoter/reporter vector, p-TATACAT (17) (obtained from Dr. A. Kraft, University of Colorado, Denver). Constructs were generated with four copies (in the forward direction) of the −94 to −134 bp sequence from the CYBB promoter (p-cybbTATACAT). This CYBB promoter sequence has previously been demonstrated to function as a repressor element in mammalian cells per the manufacturer's instructions (Stratagene). Wild type HoxA10 cDNA sequence was subcloned into the pDNAamp vector for in vitro translation, and the pSR and pDNA5.1his vectors for expression in mammalian cells. Wild type HoxA10 was also subcloned into the pM2 vector for expression in mammalian cells as a fusion protein with the DNA-binding domain of the yeast transcription factor GAL4, as previously described (13).

HoxA10 5′ truncation mutant cDNAs were generated by PCR, using primers that incorporate the genuine HoxA10 Kozak consensus sequence and an ATG. Truncation mutants were generated that included HoxA10 amino acids (aa) 1–207, 207–393, 269–393, 293–393, 314–393, 269–297, 207–275, 207–275, 207–297, 207–297, 269–314, 207–297, 207–233, 224–249, 250–275, and 297–314. These mutant cDNA sequences were subcloned into pDNAamp vector for in vitro translation, into pSRs for overexpression in mammalian cells, and into the pM2 vector for expression in mammalian cells as a fusion protein with the Gal4 DNA-binding domain, and into pcDNA3.1his vectors for expression in yeast. In these oligonucleotides, the HoxA10 core is in gray. In these oligonucleotides, the HoxA10 core is in gray. 

Immunoprecipitation and Western Blotting—

In vitro translated proteins and nuclear proteins were tyrosine-phosphorylated with Yop protein-tyrosine phosphatase (New England Biolabs, Beverly, MA). Proteins (either 10 µl of in vitro translated proteins or 2 µg of nuclear proteins) were incubated for 30 min at 30 °C in a 20-µl reaction volume with 50 units of Yop and 1× reaction buffer, according to the manufacturer's instructions. Control proteins were incubated similarly in 1× reaction buffer without Yop.

EMSAs with the in vitro translated proteins was performed as described (13). The amount of mutant and wild type HoxA10 proteins in DNA-binding reactions was quantified by SDS-PAGE of [35S]methionine-labeled proteins.

**Transfection and Reporter Gene Assays—** Cells were transfected by electroporation as described (13). U937 cells (32 × 10⁶ cells/sample) were transfected with 70 µg of p-TATACAT or p-cybbTATACAT; 30 µg of pSRs, HoxA10/pSRSa, N312A/W313T HoxA10/pSRSb, 207–393 aa HoxA10/pSRSb, 269–393 aa HoxA10/pSRSb, 314–393 aa HoxA10/pSRSa; and 15 µg of p-CMV-β-gal (to normalize for transfection efficiency). Transfectants were incubated for 24 h at 37 °C, 5% CO₂, followed by 24 h with or without trichostatin A (TSA) (500 or 1000 ng/ml). Prepa-ration of cell extracts, β-galactosidase, and chloramphenicol acetyl transferase (CAT) assays were performed as described (25, 26).

In other experiments, cells were transfected with 30 µg of p-GAL4/TKCAT and 60 µg of a vector to express HoxA10 or various mutants as fusion proteins with the DNA-binding domain of the yeast Gal4 transcription factor. The amount of Gal4 fusion protein expression vector used in these experiments was determined in preliminary studies in which the various HoxA10 expression plasmids were transfected into U937 cells. The amount of GAL4 expression vector that resulted in a maximal repression activity (10, 30, 60, and 120 µg). For all constructs, maximal repression activity was obtained with 60 µg of expression vector, consistent with our previous investigations with this assay (13).

**Immunoprecipitation and Western Blotting—** Immunoprecipitation was performed with 300 µg of nuclear proteins, extracted from U937 cells. Proteins were immunoprecipitated with HoxA10 antisera (Covance Research Products) or control rabbit preimmune serum (11). Proteins were immunoprecipitated for 4 h at 4 °C, with 2 µl of HoxA10 antiserum or 2 µl of control rabbit preimmune serum, followed with a 1-h incubation with 30 µl of 50% staphylococcus protein A-Sepharose bead slurry, as described (13). Immunoprecipitated proteins were washed three times with lysis buffer, eluted in SDS sample buffer, and separated on 12% SDS-PAGE. Immunoprecipitated proteins were transferred to nitrocellulose, and the blots were sequentially probed with anti-HoxA10 (Santa Cruz Biotechnology), anti-Pbx1 (Santa Cruz Biotechnology), or anti-HDAC2 (Santa Cruz Biotechnology).

Immunoreactive proteins were detected by chemiluminescence, according to the manufacturer's instructions (Amersham Biosciences).

In other experiments, lysate proteins (1.5 mg) from U937 transfections with HoxA10/GAL4 or various truncation mutants of HoxA10/GAL4, vector control, or sham-transfected cells were immunoprecipi-tated with anti-GAL4 DNA-binding domain antibody (rabbit polyclonal antibody from Santa Cruz Biotechnology) or irrelevant control antibody (mouse monoclonal IgG from Santa Cruz Biotechnology), as described above. Immunoprecipitated proteins were analyzed by Western blots, sequentially probed with antibodies to GAL4 DNA-binding domain (mouse monoclonal antibody from Santa Cruz Biotechnology) or HDAC2, as described above.

**Northern Blots—** Total cellular RNA was extracted from U937 cells, treated under various conditions, by the “single step” method, as previously described (27). RNA was separated on denaturing formaldehyde gels and transferred to nylon membranes, according to standard tech-niques. Probes were generated by the random primer method, using cDNA sequences from genes of interest as a template. Membrane blots from three independent experiments were quantitated using the UN-SCAN-IT software.

**Electrophoretic Mobility Shift Assays (EMSA)—** Nuclear extract proteins were prepared by the method of Dignam (23) with protease inhibitors (as described) (24). Oligonucleotide probes were prepared, and EMSA and antibody supershift assays were performed, as described (13). Antiserum to HoxA10 (not cross-reactive with other Hox proteins) was obtained from Covance Research Products (Richmond, CA) and from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Antibodies to HDAC1, HDAC2, and the GALA DNA-binding domain were obtained from Santa Cruz Biotechnology. An antibody to Pbx1 that does not react with other Pbx proteins was also obtained from Santa Cruz Biotechnology.
**Histone Deacetylase Assays**—Histone deacetylase assays were performed using the HDAC kit from Upstate Biotechnology, Inc. (Lake Placid, NY) according to the manufacturer’s instructions. Substrates for the assays were U937 nuclear proteins (50 μg), isolated after TSA treatment (as described in the experimental design). In other assays, HDAC2 or HDAC1 was immunoprecipitated from U937 nuclear proteins (300 μg) under various TSA treatment conditions and assayed as above.

**GST Fusion Protein “Pull-down” Assays**—JM109 E. coli transformed with HoxA10 and various truncation mutants in the pGEX vector were grown to log phase, supplemented to 0.1 mM isopropyl-β-D-thiogalactopyranoside, and incubated for 3 h at 37 °C with shaking. The cells were harvested and resuspended in HN buffer (20 mM HEPES (pH 7.4), 0.1 mM NaCl, 2 mM MgCl₂, 0.1 mM EDTA, 0.5% Nonidet P-40, 0.1% Triton X-100, 2 mM phenylmethylsulfonyl fluoride, 5 mM NaN₃) and sonicated on ice (13). Debris was removed by centrifugation, and the lysate was incubated for 30 min at 4 °C with glutathione-agarose beads (Sigma) and washed extensively with HN buffer. The beads were preincubated for 30 min at 4 °C with 5 μl of control rabbit reticulocyte lysate and then for 1 h with 20 μl of [³⁵S]methionine-labeled in vitro translated protein and washed extensively in HN buffer. Proteins were eluted with SDS-PAGE sample buffer and separated on 15% SDS-PAGE, and an autoradiograph was performed.

**RESULTS**

**HoxA10 and Pbx1a Recruit HDAC2-negative Cis Elements in Genuine Target Genes**—In these studies, we hypothesize that the DNA-bound HoxA10-Pbx1a complex recruits transcriptional co-repressors to target genes in undifferentiated myeloid cells. Since Pbx1a can interact directly with transcriptional co-repressors (10), we also hypothesized that endogenous repression activity would be Pbx1a-dependent. To pursue this hypothesis, we further investigated the protein complex interacting with the negative CYBB cis elements. We performed EMSA, using the proximal CYBB repressor element as a labeled DNA probe and nuclear proteins from undifferentiated U937 cells (Fig. LA). Previously, we demonstrated that antibodies to either HoxA10 or Pbx1a disrupt the low mobility complex that interacts with this probe (13). Therefore, we investigated the ability of antibodies to transcriptional co-repressors to disrupt or supershift this DNA-protein complex. We found that an antibody to HDAC2 specifically disrupts this complex, although antibodies to histone deacetylase 1 and pre-immune serum have no effect.

To reconfirm this interaction, we performed immunoprecipitation of HoxA10 from U937 nuclear proteins under non-denaturing conditions. We found that both Pbx1a and HDAC2 co-immunoprecipitate with HoxA10 from U937 nuclear proteins (Fig. 1B). No co-immunoprecipitation of HDAC1 was demonstrated (not shown). Together, these results provided initial confirmation of our hypothesis that the HoxA10-Pbx1a complex recruits histone deacetylase activity to myeloid gene promoters. However, interaction of HDAC2 with Pbx1a has not been described. Therefore, we further pursued our hypothesis by investigating the impact of histone deacetylase activity on CYBB and NCF2 transcription.

**Inhibition of Histone Deacetylase Activity Abrogates HoxA10 Transcriptional Repression Activity**—Histone deacetylase inhibitors, such as TSA, induce some differentiation in myeloid leukemia cell lines and normal myeloid progenitors (28). Therefore, it would be reasonable to expect that histone deacetylase inhibition might increase transcription of genes characteristic of mature myeloid cells. We treated U937 cells with TSA, at various doses, and investigated the effect of histone deacetylase inhibition on myeloid-specific gene expression. In initial investigations, we determined the dose of TSA required for histone deacetylase inhibition in U937 cells. Consistent with the reports of other investigators (29), we found greater than 80% of total histone deacetylase activity was inhibited by 24 h of treatment with 200 ng/ml TSA (84 ± 6%, n = 4). However, HDAC2 is relative refractory to most histone deacetylase inhibitors (29). Therefore, we determined the dose of TSA required for specific HDAC2 inhibition. U937 cells were treated with 24 h with various amounts of TSA, HDAC2 was immunoprecipitated from U937 cell lysates, and HDAC activity of HDAC2 (or sham immunoprecipitated proteins) was determined. We found that 200 ng/ml TSA had no effect on HDAC2 activity, 500 ng/ml inhibited HDAC2 activity minimally (23 ± 5%, n = 2), and that HDAC2-specific inhibition required 1000 ng/ml of TSA (74 ± 9%, n = 2). Therefore, we used this higher dose to treat U937 cells, for analysis of gene expression. In control experiments, 200 ng/ml TSA inhibited HDAC1, immunoprecipitated from U937 cells.

Total RNA from control and TSA-treated U937 cells was analyzed by Northern blot (Fig. 2A). The experiment was repeated three times, and mRNA abundance was quantitated by densitometry. For each message, mRNA abundance was normalized to the sample without TSA (Fig. 2A is a representative blot). We found TSA treatment significantly increases mRNA abundance of gp91phox and p67phox (fold increase of 2.44 ± 0.23, p = 0.026, n = 3, and 2.62 ± 0.11, p = 0.004, n = 3, respectively). In contrast, TSA treatment did not significantly increase CD18 mRNA abundance (fold increase of 1.26 ± 0.10, p = 0.133, n = 3), although this gene is also expressed in late myeloid differentiation. This result suggests that TSA treatment induces a gene-specific effect. TSA treatment had no significant effect on abundance of mRNA for the AML1 transcription factor (expressed early in myeloid differentiation) or for γ-actin (used as a loading control in these experiments).

Next, we specifically investigated the effect of histone deacetylase inhibition on the CYBB and NCF2 promoters. U937 cells were transfected with a reporter gene construct, containing either the proximal 450 bp of the CYBB promoter, 400 bp of NCF2 5’ flanking, or empty control vector. Previous investigations demonstrated that these CYBB and NCF2 gene fragments include sequences necessary for differentiation stage-specific expression in myeloid cell lines (30, 31). Reporter
gene activity was assayed with or without 24 h of treatment with 500 or 1000 ng/ml TSA (see above). Consistent with the endogenous messages, 1000 ng/ml TSA significantly increased expression from the CYBB promoter-containing construct (CAT activity: 450cybb untreated, 233 ± 43, 1000 ng/ml TSA, 521 ± 68, n = 6, p = 0.011; -fold increase = 2.23) (Fig. 2B) and the NCF2 5'-flank-containing construct (400ncf2 untreated, 367 ± 32, 1000 ng/ml TSA-treated 1313 ± 335, n = 4, p = 0.04; -fold increase = 3.57) (Fig. 2C). The magnitude of TSA-induced reporter activity is similar to the increase in endogenous gp91PHOX and p67PHOX mRNA abundance in TSA-treated U937 cells discussed above. Although this increase in reporter gene transcription is statistically significant, it is less than the increase in activity of these constructs in response to IFNγ treatment of U937 cells (see Refs. 31 and 32). In contrast, 500 ng/ml TSA did not significantly increase reporter activity of the CYBB or NCF2 promoter/reporter constructs (p = 0.34, n = 6; and p = 0.29, n = 4, respectively). TSA (1000 ng/ml) had no effect on the empty vector in U937 cells (untreated, 88 ± 15 versus 70 ± 17, n = 6, p = 0.28).

The HoxA10 Repression Activity Is Pbx1a-independent—In order to determine whether endogenous HoxA10 repression activity requires functional interaction with HDAC2, we next performed investigations to identify the repression domain. In previous investigations, we expressed HoxA10 as a fusion protein with the DNA-binding domain of the yeast GAL4 transcription factor in U937 cells (HoxA10/GAL4) (13). We determined that HoxA10/GAL4 represses reporter expression from an reporter construct containing multiple copies of the GAL4 DNA-binding domain, linked to a minimal promoter (pgal4TKCAT) (13). In the current investigations, we generated a series of HoxA10 truncation mutants, expressed these mutants as GAL4 fusion proteins in U937 cells, and determined the influence of the fusion proteins on p-gal4TKCAT reporter expression. In initial experiments, HoxA10 amino acids 1–213 was compared with HoxA10 207–393 (Fig. 3A). We found that transcriptional repression by 207–393 aa HoxA10/GAL4 was not significantly different from wild type HoxA10/GAL4 (CAT activity = 52.3 ± 4.7% of control vector expression for HoxA10/GAL4; 49.1 ± 6.9% of control vector expression for 207–393 HoxA10/GAL4, p = 0.20, n = 8). We also determined that HoxA10 aa 1–207 includes a cryptic transcriptional activation domain. This activation domain was not further investigated in the current studies.

Previously, other investigators determined that HoxA10 amino acids 310–313 constitute the Pbx1a interaction domain. These amino acids are included in the 207–393 aa HoxA10 truncation mutant with repression activity. Since Pbx1a interacts directly with nuclear co-repressor proteins (10), we hypothesized that endogenous HoxA10 transcriptional repression activity involves Pbx1a recruitment of HDAC2. The corollary to this hypothesis is that the HoxA10 repression domain would be identical to the Pbx1a interaction domain. However, this would not be consistent with our previous investigations of an alternatively spliced form of HoxA10, referred to as “short A10” (18). Short A10, which is expressed in myeloid leukemia cell lines, includes HoxA10 amino acids 278–393, encompassing the DNA-binding homeodomain and the Pbx1a interaction domain. In our previous investigations, short A10/GAL4 fusion protein did not have endogenous repression activity, suggesting that...
HoxA10 repression activity is Pbx1a-independent. A, HoxA10 contains a cryptic transcriptional activation domain and an endogenous repression domain. U937 cells were co-transfected with a vector to express HoxA10, 1–213 aa HoxA10, or 207–393 aa HoxA10 as fusion proteins with the GAL4 DNA-binding domain or empty vector control and p-gal4TKCAT reporter plasmid. Transcriptional repression activities of wild type and 207–393 aa HoxA10 fusion proteins were not significantly different. In contrast, 1–213 aa HoxA10 activated transcription of the reporter construct. B, mutation of HoxA10 amino acids 312 and 313 abolishes interaction of DNA-bound HoxA10 with Pbx1a. EMSA were performed with a labeled DNA probe representing the HoxA10-Pbx1 binding sequence from the CYBB promoter and in vitro translated wild type HoxA10, N312A/W313T HoxA10, and Pbx1a. Some in vitro translated proteins were treated with Yop tyrosine phosphatase, as indicated. N312A/W313T mutation abolishes interaction of DNA-bound HoxA10 with Pbx1a. The single arrow indicates the specific HoxA10 DNA-bound complex, and the double arrow shows the HoxA10-Pbx1a DNA-bound complex. C, mutation of the HoxA10 Pbx1a interaction domain does not alter endogenous transcriptional repression activity. U937 cells were co-transfected with a vector to express HoxA10, N312A/W313T HoxA10, 207–393 aa HoxA10, or empty vector control and an artificial promoter construct with multiple copies of the GAL4 DNA-binding consensus sequence (p-gal4TKCAT). Mutation of amino acids 312 and 313 did not significantly alter transcriptional repression activity of HoxA10. D, transcriptional repression of the proximal repressor element in the CYBB promoter is mediated by HoxA10 amino acids 207–269 and does not require the HoxA10 Pbx1a interaction domain. U937 cells were co-transfected with a vector to express HoxA10, N312A/W313T HoxA10, 207–393 aa HoxA10 (including the transcriptional repression domain, identified above), 269–393 aa HoxA10 (including the Pbx1a-interaction domain), 314–393 aa HoxA10 (without the Pbx1a interaction domain), or empty vector control and an artificial promoter construct with four copies of the proximal repressor element from the CYBB promoter (p-cybbTATACAT) or empty p-TATACAT control vector. Overexpression of HoxA10 significantly repressed reporter expression from the p-cybbTATACAT vector, as previously described (13, 16). Transcriptional repression of this reporter construct was not significantly different with overexpression of wild type HoxA10, N312A/W313T HoxA10, or 207–393 aa HoxA10. In contrast, 269–393 aa HoxA10 and 314–393 HoxA10 did not significantly repress p-cybbTATACAT. None of the overexpressed proteins significantly altered reporter expression from control p-TATACAT vector. E, TSA treatment abrogates Pbx1a-independent, HoxA10-mediated transcriptional repression of the proximal repressor element in the CYBB promoter. U937 cells were co-transfected with a vector to express HoxA10, N312A/W313T HoxA10, 207–393 aa HoxA10, or empty vector control and p-gal4TKCAT reporter plasmid. TSA treatment abrogates Pbx1a-independent, HoxA10-mediated transcriptional repression of the proximal repressor element in the CYBB promoter. U937 cells were co-transfected with a vector to express HoxA10, N312A/W313T HoxA10, 207–393 aa HoxA10, or empty vector control and p-gal4TKCAT reporter plasmid. TSA treatment abrogates Pbx1a-independent, HoxA10-mediated transcriptional repression of the proximal repressor element in the CYBB promoter. U937 cells were co-transfected with a vector to express HoxA10, N312A/W313T HoxA10, 207–393 aa HoxA10, or empty vector control and p-gal4TKCAT reporter plasmid. TSA treatment abrogates Pbx1a-independent, HoxA10-mediated transcriptional repression of the proximal repressor element in the CYBB promoter. U937 cells were co-transfected with a vector to express HoxA10, N312A/W313T HoxA10, 207–393 aa HoxA10, or empty vector control and p-gal4TKCAT reporter plasmid. TSA treatment abrogates Pbx1a-independent, HoxA10-mediated transcriptional repression of the proximal repressor element in the CYBB promoter. U937 cells were co-transfected with a vector to express HoxA10, N312A/W313T HoxA10, 207–393 aa HoxA10, or empty vector control and p-gal4TKCAT reporter plasmid. TSA treatment abrogates Pbx1a-independent, HoxA10-mediated transcriptional repression of the proximal repressor element in the CYBB promoter.
HoxA10 Interacts with HDAC2

The cells were co-transfected with a vector to overexpress HoxA10, N312A/W313T HoxA10, 207–393 aa HoxA10 (with repression activity as a GAL4 fusion protein), and N312A/W313T HoxA10 (with the Phx1a interaction domain), or 314–393 aa HoxA10 (without the Phx1a interaction domain) (15). Consistent with our previous investigations, overexpressed HoxA10 represses p-cybbTATACAT expression, in undifferentiated U937 cells (CAT activity without HoxA10 = 4229 ± 324; activity with HoxA10 = 1833 ± 27, p = 0.002, n = 3) (Fig. 3D). Consistent with the GAL4 fusion protein experiments, repression activities of HoxA10 and N312A/W313T HoxA10 were not significantly different (reporter activity for N312A/W313T HoxA10 = 1759 ± 228, p = 0.761, n = 3). Also consistent with GAL4 fusion protein results, transcriptional repression by overexpressed 207–393 aa HoxA10 was not significantly different from wild type HoxA10 repression in this assay (reporter activity for 207–393 aa HoxA10 = 1823 ± 50, p = 0.0018, n = 3) (Fig. 3D). In contrast, overexpression of a truncation mutant with the Phx1a interaction domain, but without amino acids 207–269, did not significantly repress reporter expression from the p-cybbTATACAT construct (reporter activity with 269–293 aa HoxA10 = 3461 ± 200, p = 0.116, n = 3). Consistent with this, reporter expression was not significantly repressed by a truncation mutant consisting of the homeodomain but not the Phx1a-interaction domain (reporter activity with 313–393 aa HoxA10 = 3646 ± 273, p = 0.24, n = 3). The minor repression activity observed with these truncation mutants is similar to that observed previously with “short A10.” This repression activity is due to HoxA10 competition for DNA binding with transcriptional activators that interact with overlapping, positive cis elements in the CYBB gene (13, 16).

We next tested whether Phx1a-independent, HoxA10 repression of a genuine target gene requires HDAC activity. U937 cells were transfected, as above, and reporter gene assays were performed after 24 h of TSA treatment (1000 mg/ml). We found that HoxA10 repression is significantly decreased by TSA treatment (1000 mg/ml) of the transfectants (CAT activity with HoxA10 plus TSA = 3421 ± 450, p = 0.024, n = 3). Indeed, CAT activity of the p-cybbTATACAT reporter construct, in TSA-treated transfectants, is not significantly different with and without HoxA10 (CAT activity without HoxA10 and HoxA10 + TSA = 0.943, p = 0.043, n = 3) (Fig. 3E). TSA treatment abolished repression activity of the Phx1a-binding mutant HoxA10 to the same extent as wild type HoxA10 (CAT activity with N312A/W313T HoxA10 = 3568 ± 314, p = 0.81, n = 3) (Fig. 3E). Similar to wild type HoxA10, TSA treatment abolished transcriptional repression by 207–393 aa HoxA10 (Fig. 2E). As might be anticipated from these investigations, TSA treatment does not significantly alter the minimal transcriptional repression of p-cybbTATACAT by 269–393 aa HoxA10, or 313–393 aa HoxA10 (p = 0.776 and 0.809, respectively).

However, it is possible that the inability of overexpressed 269–393 aa HoxA10 to repress transcription in this assay is due to instability of the protein, relative to overexpressed 207–393 aa HoxA10. To investigate this, U937 cells were transfected with the same vectors used in the experiments above to

were co-transfected with a vector to express HoxA10, N312A/W313T HoxA10, 207–393 aa HoxA10, 269–393 aa HoxA10, 314–393 aa HoxA10, or empty vector control and an artificial promoter construct with four copies of the proximal repressor element from the CYBB promoter (p-cybbTATACAT) or empty p-TATACAT control vector. Transfectants were harvested after 24 h of TSA incubation, and reporter gene assays were performed. In TSA-treated transfectants, reporter expression from p-cybbTATACAT was not significantly different with and without overexpression of HoxA10, N312A/W313T HoxA10, 207–393 aa HoxA10, 269–393 aa HoxA10, or 314–393 aa HoxA10 in contrast to non-TSA-treated transfectants (see above). Neither TSA treatment nor HoxA10 protein expression influenced reporter expression from control p-TATACAT vector. F, overexpressed 207–393 aa HoxA10 is not more abundant than overexpressed 269–393 aa HoxA10, in U937 transfectants. U937 cells were transfected with a vector to overexpress 207–393 aa HoxA10, 269–393 aa HoxA10, or empty vector control. Transfectants were harvested after 48 h, and cell lysate proteins were separated by SDS-PAGE. Western blots were probed with an antibody to HoxA10 that recognizes a peptide C-terminal to the homeodomain. Despite differences in transcriptional repression activity of the two HoxA10 truncation mutants, expression of these two proteins is approximately equivalent in U937 cells.
overexpress 207–393 aa HoxA10, 269–393 aa HoxA10, or empty vector control. Cell lysates were investigated for abundance of the overexpressed proteins by Western blot with an antibody that recognizes a unique sequence, C-terminal to the homeodomain (Fig. 3f). These investigations demonstrate approximately equivalent abundance of overexpressed 207–393 aa HoxA10 and 269–393 aa HoxA10, despite differences in repression activity.

In these studies, neither overexpression of HoxA10 nor TSA treatment significantly influenced reporter activity from the control p-TATACAT vector. Similar results were obtained in additional experiments with U937 transfectants, overexpressing both HoxA10 and Pbx1a (not shown). In contrast to these results, treatment with 500 ng/ml TSA did not significantly influence HoxA10 transcriptional repression (not shown).

**HoxA10-HDAC2 Interaction Is Pbx1a-independent**—These results support the hypothesis that interaction with HDAC2 is necessary for endogenous HoxA10 transcriptional repression activity. Although endogenous HoxA10 repression activity does not require interaction with Pbx1a, we have not specifically demonstrated Pbx1a independence of HoxA10-HDAC2 interaction. To approach this directly, we expressed 207–393 aa HoxA10 and 207–393 aa N312A/W313T HoxA10 as GST fusion proteins in *E. coli*, as described previously (13). These proteins (and control GST) were affinity-purified and tested for interaction with *in vitro* translated Pbx1a. We found that 207–293 HoxA10/GST is able to pull down *in vitro* translated Pbx1a (Fig. 4a). In contrast, neither 207–393 N312A/W313T HoxA10/GST nor control GST interact significantly with *in vitro* translated Pbx1a.

We investigated the affinity of these proteins for *in vitro* translated HDAC2. Consistent with our transfection experiments, we found that in *in vitro* interaction between HoxA10 and HDAC2 is not dependent on the HoxA10 Pbx1a interaction domain (Fig. 4a).

However, these results do not exclude the possibility that Pbx1a increases HoxA10 affinity for HDAC2. To investigate this, we determined whether *in vitro* translated Pbx1a increases the affinity of HoxA10/GST for *in vitro* translated HDAC2 (Fig. 4b). We found that inclusion of Pbx1a in the binding reaction did not increase the affinity of HoxA10/GST for *in vitro* translated HDAC2. Indeed, there was a reproducible, slight decrease in the affinity of HDAC2 for HoxA10 in the presence of Pbx1a.

**HoxA10 Amino Acids 207–269 Contain an Endogenous Repression Domain**—The results of our investigations suggest that the HoxA10 repression domain is found between amino acids 207 and 269. We further analyzed 207–393 aa HoxA10 to confirm the identity of this repression domain. We first analyzed HoxA10 truncation mutants containing amino acids 313–393 (DNA-binding domain) or 289–393 (including the Pbx1a interaction domain), expressed as fusion proteins with the GAL4 DNA-binding domain (Fig. 5a). We found that neither of these HoxA10 truncation mutants repress p-gal4TKCAT (CAT activity = 122.0 ± 18.6% of control vector for 313–393 aa HoxA10/GAL4, p = 0.44, n = 5; CAT activity = 97.6 ± 11.0% of control vector for 289–393 aa HoxA10/GAL4, p = 0.90, n = 9), suggesting that the repression domain is between amino acids 207 and 269. Therefore, we expressed HoxA10 amino acids 207–275 and 269–297 as GAL4 DNA-binding domain fusion proteins. We found that 269–297 as HoxA10/GAL4 does not influence p-gal4TKCAT reporter gene expression (CAT activity = 110.3 ± 6.8% control vector with 269–297, p = 0.35, n = 12). In contrast, 207–275 aa HoxA10/GAL4 significantly represses reporter gene expression (CAT activity = 60.3 ± 4.5%, p = 0.0002, n = 19). This transcriptional repression is not significantly different from that seen with 207–393 aa HoxA10/GAL4 (p = 0.45, n = 19).

Based on the experiments above, we anticipate that repression activity of HoxA10 amino acids 207–269 would require histone deacetylase activity. To test this, HoxA10 and 207–275 aa HoxA10 were overexpressed in U937 cells as fusion proteins with the GAL4 DNA-binding domain. Repression p-gal4TKCAT was assayed, with and without 24 h of TSA treatment (Fig. 5b). Consistent with our hypothesis, TSA treatment abrogates transcriptional repression activity by both wild type HoxA10/GAL4 (CAT activity in HoxA10/GAL4-expressing, TSA-treated transfectants = 107.8 ± 15.7% of control vector, p = 0.93, n = 6), and the putative HoxA10 repression domain (CAT activity in 207–275 aa HoxA10/GAL4-expressing, TSA-treated transfectants = 162.0 ± 14.0% of control vector, p = 0.14, n = 3). TSA treatment of empty vector control transfectants did not significantly alter reporter expression from p-gal4TKCAT (p = 0.92).

To verify identification of the endogenous repression domain as the HDAC2-interaction domain, we expressed HoxA10 amino acids 207–275 as a GST fusion protein in *E. coli*. Similar to the experiments above, we investigated interaction between
The HoxA10 Repression Domain Contains ResiduesConserved with Other Transcription Factors—These investigations indicate endogenous HoxA10 transcriptional repression activity is found in the 62 amino acids between 207 and 269. However, we found no matches with previously identified transcriptional repression domains or other conserved protein domains by a GenBankTM search of the Swissprot data base. Therefore, we searched the data base for similarity to short sequences in other proteins (34) and identified a region of homology between the putative HoxA10 repression domain and domains in other transcription factors. We identified several proteins that share a short, putative consensus sequence with HoxA10 aa 235–245; LLASGSXDX<var>AX</var> (Table 1). Consistent with our hypothesis that aa 207–269 contains the HoxA10 transcriptional repression domain, many of these homologous proteins have transcriptional repression activity. However, this putative consensus sequence does not fall within previously described functional domains in any of these proteins.

To determine whether this putative consensus sequence includes the HoxA10 transcriptional repression domain, we generated truncation mutants of HoxA10 207–269 aa, with and without amino acids 235–245. These HoxA10 sequences were expressed in U937 cells as fusion proteins with the yeast GAL4 DNA-binding domain and assayed for transcriptional repression activity, as above. We found that transcriptional repression activity of 207–275 aa HoxA10/GAL4 is not significantly different from 207–249 aa HoxA10/GAL4 (52.0 ± 8.1% versus 43.2 ± 16.7%, respectively, p = 0.38, n = 7) or 224–249 aa HoxA10/GAL4 (49.6 ± 10.9%, p = 0.89, n = 7) (Fig. 6A). In contrast, reporter activity for U937 transfectants overexpressing 207–233 aa HoxA10/GAL4 is not significantly different from empty vector in this assay (105.1 ± 6.2%, p = 0.44, n = 7). These results indicate that the 11 putative, conserved HoxA10 residues are within the 15 amino acids that function as a transcriptional repression domain (aa 234–249).

These U937 transfection experiments and the in vitro experiments described above suggest HDAC2 interaction in vivo with HoxA10 amino acids 234–249. To test this association, we investigated co-immunoprecipitation of HDAC2 with various HoxA10/GAL4 fusion proteins. We transfected U937 cells with vectors to express GAL4 fusion proteins that either repress transcription (HoxA10/GAL4 or 224–249 aa HoxA10/GAL4) or proteins without repression activity (207–233 aa HoxA10/GAL4 or GAL4 DNA-binding domain control). Lysate proteins from these transfectants and sham (no plasmid) transfectants were immunoprecipitated with an antibody to the GAL4 DNA-binding domain (rabbit polyclonal anti-GAL4 DBD antibody). Immunoprecipitated proteins were separated by SDS-PAGE, and Western blots were probed for expression of the various GAL4 fusion proteins (mouse monoclonal anti-GAL4 DBD antibody) (Fig. 6B). U937 cells overexpressing the GAL4 DNA-binding domain, 207–233 aa HoxA10/GAL4, or 224–249 aa HoxA10/GAL4 demonstrate a dominant immunoreactive protein of the appropriate size by anti-GAL4 Western blot (20, 24, and 22 kDa, respectively). In contrast, U947 lysates from cells overexpressing HoxA10/GAL4 demonstrate a dominant band at ~24 kDa and a significantly less intense band at 80 kDa, the anticipated size of the fusion protein. The vector used in these investigations adds the GAL4 DNA-binding domain to the amino-terminal end of the tagged sequence. Therefore, this result might indicate the presence of a proteolytic cleavage site near the beginning of the HoxA10 protein. Consistent with this, our

207–275 aa HoxA10/GST and in vitro translated HDAC2 (Fig. 5C). Consistent with these results, we found that HDAC2 interacts specifically with the functional HoxA10 repression domain. Additionally, these experiments demonstrate that HDAC2 interaction with 207–275 aa HoxA10 is not significantly different from interaction with 207–393 aa HoxA10. Combined with the transfection experiments above, these results suggest the HDAC2 interaction domain is restricted to HoxA10 amino acids 207–269.

The endogenous HoxA10 repression domain is located between amino acids 207 and 269. A, the HoxA10 amino acids 207–267 are sufficient for endogenous repression activity. U937 cells were co-transfected with vectors to express various truncation mutants of HoxA10 as fusion proteins with the GAL4 DNA-binding domain; and p-gal4TKCAT reporter plasmid. Transcriptional repression was only observed with constructs including HoxA10 amino acids 207–269. B, transcriptional repression activity of HoxA10 amino acids 207–275 is abolished by histone deacetylase inhibition. U937 cells were co-transfected with a vector to express HoxA10 or 207–275 aa HoxA10 as fusion proteins with the GAL4 DNA-binding domain or empty vector control and p-gal4TKCAT reporter plasmid. Reporter gene assays were performed with and without 24 h of TSA treatment. Repression activity by both HoxA10/GAL4 and 207–275 HoxA10/GAL4 is abolished in TSA-treated transfectants. C, the HoxA10 endogenous repression domain interacts with HDAC2 in vitro. HoxA10 amino acids 207–383 and 207–275 were expressed in E. coli as a GST fusion protein and evaluated for interaction with in vitro translated, [35S]methionine-labeled HDAC2. HDAC2 interacts specifically with both of these fusion proteins but not with control GST. No other bands are present in regions of the gel not shown in the figure.
HoxA10 Interacts with HDAC2

GenBank™ search demonstrates homology between a sequence in the HoxA10 repression domain and domains present in other transcription factors. This domain has the loose consensus sequence LLASGSXDXXXAR, which is shared with other transcriptional repressors including the forkhead-like protein C (FHKL7), histone deacetylase 3, and the ONECUT2 transcription factor. The putative consensus sequence is in boldface type, and amino acids 224–249, containing the functional repression domain, are underlined.

| 220 | GAGPFPAQGPGRGF | DLLASSDADA | ARKERAL | 250 | HoxA10 |
| 450 | HHEPAARGGRLTSWLNQAGGDLG | DLLASAAA | A | 493 | FHKL7 |
| 182 | DLLASGSBDSTAR | 194 | HDAC3 |
| 299 | DLLASGSBDSTAR | 250 | HDAC3 |
| 33 | GGGPGRGF | ELLASPSBP | ARGP | 55 | ONECUT2 |

Consensus: LLASGSXDXXAR

experiments with various HoxA10/GST fusion proteins identified a proteolytic cleavage site at approximately HoxA10 amino acid 50 (not shown). Cleavage at this site would result in a 26-kDa GAL4 fusion protein.

We analyzed these precipitated fusion proteins for co-immunoprecipitation of HDAC2 by Western blot (Fig. 6B). We found specific co-immunoprecipitation of HDAC2 with HoxA10/GAL4 and 224–249 aa HoxA10/GAL4 but not 207–233 aa HoxA10/GAL4 or the GAL4 DNA-binding domain. In contrast, HDAC1 did not co-immunoprecipitate with these HoxA10 fusion proteins (data not shown). Immunoprecipitates from U937 sham transfecants demonstrated no immunoreactive protein in either anti-GAL4 or anti-HDAC2 Western blots.

**DISCUSSION**

Our previous studies identified the CYBB and NCF2 genes as HoxA10 repression targets in undifferentiated myeloid cell lines. We found that HoxA10 represses transcription of these genes by two mechanisms: competition with transcriptional activators for binding to adjacent cis elements and endogenous transcriptional repression activity. In these investigations, we take advantage of our knowledge of HoxA10 target genes to address the mechanism of HoxA10-mediated transcriptional repression. We demonstrate that, unlike previously described Hox proteins, HoxA10-mediated endogenous transcriptional repression activity is Pbx1a-independent. We also identify a HoxA10 domain that interacts with the transcriptional corepressor protein HDAC2, thereby identifying a novel mechanism for Hox-mediated repression. These studies represent the first demonstration of interaction between a histone deacetylase and an Abd Hox protein. This finding has implications for understanding the various roles different Hox proteins play in differentiating myeloid cells.

Although our previous investigations identified two mechanisms of HoxA10-mediated transcriptional repression, we did not determine the relative significance of these mechanisms to CYBB and NCF2 transcription. The current investigations determine that the major mechanism of HoxA10-mediated repression, in undifferentiated myeloid cells, is TSA-dependent endogenous repression activity. We found that HoxA10-Pbx1a DNA-binding site competition is of minor functional significance in undifferentiated cells. It is possible that function of the adjacent positive cis elements, in the CYBB and NCF2 promoters, requires differentiation-induced modification of trans-activating proteins. In this case, HoxA10-Pbx1a binding site competition might be more significant as differentiation proceeds and activating proteins become functionally competent.

Previously, other investigators found that HoxB1 and D4-mediated transcriptional repression depends on Pbx1a interaction with HDAC1 (12). Consistent with this, induction of leukemia in murine bone marrow transplantation experiments, by overexpression of HoxB3, B4, and A9, requires co-overexpression of Pbx1a (3, 6). These results suggest that Pbx1a plays a pivotal role in Hox repression function. In contrast, we find that the HoxA10-Pbx1a complex recruits HDAC2, not HDAC1, to the negative cis elements in the CYBB and NCF2 genes. Consistent with this, HoxA10-Pbx1a repression activity is not abolished by doses of TSA that inhibit HDAC1; rather, it requires higher doses. In further investigations, we found that
endogenous HoxA10-transcriptional repression activity and HDAC2 interaction are Pbx1a-independent. These results suggest that one mechanism for functional differences between HoxA10 and Pbx1a-dependent Hox proteins might involve different histone deacetylases. However, our investigations do not indicate the role of Pbx1a in CYBB or NCF2 transcription. Previous studies indicate that Pbx1a increases DNA-binding affinity of various Hox proteins. However, our studies indicate that not only is Pbx1a interaction dispensable for HoxA10 endogenous repression activity, but Pbx1a is also dispensable for repression in an assay requiring HoxA10 binding to the CYBB-negative cis element. It is possible that Pbx1a increases affinity of HoxA10 for the CYBB gene, but our transfection assays are not sensitive enough to detect this effect. Alternatively, Pbx1a may participate in HoxA10 binding site selection. Further investigation will be necessary to determine the role of Pbx1a in CYBB and NCF2 transcription.

Although TSA treatment of U937 cells increases transcription of the endogenous CYBB and NCF2 genes, and CYBB and NCF2 promoter/reporter constructs, the increase is less than with IFN-α differentiation. This is despite the fact that TSA abolishes HoxA10 transcriptional repression of an artificial promoter construct with multiple copies of the CYBB cis element. This result is consistent with our previous investigations of CYBB and NCF2 regulation. Those investigations indicate that events in addition to decreased repressor binding to negative cis elements regulate transcription of these genes during differentiation. Specifically, we identified homologous cis elements in the CYBB and NCF2 genes, activated by a multiprotein complex that includes PU.1, interferon regulatory factor 1, and the interferon consensus sequence-binding protein (32). Assembly of this complex requires phosphorylation of component proteins in response to IFN-γ treatment of U937 cells (24, 31). TSA therefore would not be expected to induce maximal CYBB or NCF2 transcription.

In these investigations, we identified a cryptic HoxA10 transcriptional activation domain. However, we have not observed transcriptional activation via HoxA10-Pbx1a-binding cis elements in the CYBB gene, even after differentiation of U937 transfectants. This is not surprising, because U937 differentiation results in HoxA10 tyrosine phosphorylation, which decreases HoxA10-Pbx1a binding affinity to the CYBB cis elements (13, 16). These results suggest that HoxA10 transcriptional activation might be relevant to unidentified target genes, in which HoxA10 participates in protein-DNA complexes not similarly influenced by HoxA10 tyrosine phosphorylation (16). However, in an assay that does not require HoxA10-DNA binding, HoxA10/GAL4 fusion protein also does not activate reporter gene transcription in IFN-γ-treated (13) or TSA-treated transfectants. Since isolated HoxA10 amino acids 1–213 activate transcription in this assay, these results suggest the cryptic activation domain is masked in U937 cells, under the conditions of both assays. One possible explanation would be that HoxA10 interaction with an unidentified partner protein induces a conformational change, unmasking the HoxA10 activation domain. It is of interest that TSA treatment results in some transcriptional activation by 207–275 aa HoxA10/GAL4 fusion protein, although significantly less than activation by 1–213 aa HoxA10/GAL4. This suggests the HoxA10 activation domain may include residues common to the two mutants. The significance of the HoxA10 activation domain is the subject of ongoing investigations in the laboratory.

Our functional studies narrowed the repression domain to 62 HoxA10 amino acids that interact with HDAC2 but not Pbx1a. This HoxA10 domain is not homologous to the HDAC interac-

REFERENCES
1. Acampora, D., D’Esposito, M., Failla, A., Panese, M., Migliaccio, E., Morelli, F., Stornaiuolo, A., Nitro, V., Simone, A., and Boncinelli, A. (1989) *Nucleic Acids Res.* 17, 10385–10400
2. Sauvageau, G., Lansdorf, P. M., Eavee, C. J., Hogge, D. E., Dragowska, W. H., Reid, D. S., and Largman, C. (1994) *Proc. Natl. Acad. Sci. U. S. A.* 91, 12223–12227
3. Sauvageau, G., Thorsteinottir, U., Eavee, C. J., Lawrence, H. J., Largman, C., Lansdorf, P. M., and Humphries, R. R. (1995) *Genes Dev.* 9, 1753–1765
4. Krosl, J., Banban, S., Krosl, G., Rosenfeld, S., Largman, C., and Sauvageau, G. (1998) *Oncogene* 16, 3403–3412
5. Claas, K. R., Sykes, D. B., Pasillas, M., and Kamps, M. P. (2000) *Mol. Cell. Biol.* 20, 3274–3285
6. Kasper, L. H., Brindle, P. K., Schnabel, C. A., Pritchard, C. E., Cleary, M. L., and Van Deursen, J. M. (1989) *Mol. Cell. Biol.* 19, 764–776
7. Krosl, E., Krosl, J., Thorsteinottir, U., Banban, S., Buchberg, A. M., and Sauvageau, G. (1998) *EMBO J.* 17, 3714–3725
8. Thorsteinottir, U., Sauvageau, G., Hough, M. R., Dragowska, W., Lansdorf, P. M., Lawrence, H. J., Largman, C., and Humphries, R. R. (1997) *Mol. Cell. Biol.* 17, 495–505
9. Chang, C. P., Brochieri, L., Shen, W. F., Largman, C., and Cleary, M. L. (1996)
HoxA10 Interacts with HDAC2