MOLECULAR AND CELLULAR BIOLOGY, Apr. 2001, p. 2259–2268 Vol. 21, No. 7
0270-7306/01/$04.00+0 DOI: 10.1128/MCB.21.7.2259–2268.2001


# The Growth Suppressor PML Represses Transcription by Functionally and Physically Interacting with Histone Deacetylases

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Received 22 September 2000/Returned for modification 2 November 2000/Accepted 9 January 2001

**The growth suppressor promyelocytic leukemia protein (PML) is disrupted by the chromosomal translocation t(15;17) in acute promyelocytic leukemia (APL). PML plays a key role in multiple pathways of apoptosis and regulates cell cycle progression. The present study demonstrates that PML represses transcription by functionally and physically interacting with histone deacetylase (HDAC). Transcriptional repression mediated by PML can be inhibited by trichostatin A, a specific inhibitor of HDAC. PML coimmunoprecipitates a significant level of HDAC activity in several cell lines. PML is associated with HDAC in vivo and directly interacts with HDAC in vitro. The fusion protein PML-RARα encoded by the t(15;17) breakpoint interacts with HDAC poorly. PML interacts with all three isoforms of HDAC through specific domains, and its expression deacetylates histone H3 in vivo. Together, the results of our study show that PML modulates histone deacetylation and that loss of this function in APL alters chromatin remodeling and gene expression. This event may contribute to the development of leukemia.**

The nonrandom chromosomal translocation t(15;17), a cytogenetic hallmark of acute promyelocytic leukemia (APL), fuses the retinoic acid receptor α gene (*RAR*α) and the promyelocytic leukemia gene (*PML*) (8, 17, 34). The fusion gene *PML-RAR*α encodes a fusion protein that has been shown to interfere with leukemia cell differentiation (25, 26) and to cause leukemia in animal models (11, 27, 32, 33). Disruption of PML's growth suppressor function in APL is also believed to play a role in leukemogenesis (51). PML is a nuclear-matrix-associated protein localized in the nucleus in a distinct nuclear speckled pattern designated the PML nuclear body (NB), which is disrupted in the leukemic blasts of APL (14, 15, 20, 75). A significant number (>90%) of APL patients can be induced to complete clinical remission by high-dose all-*trans*-retinoic acid (ATRA) or arsenic trioxide ($As_2O_3$) therapy (16, 59, 60, 72, 74). Retinoic acid (RA) treatment induces differentiation of the leukemic blasts, rapid degradation of the fusion protein PML-RARα, and restoration of a normal PML NB (20, 75). Recent studies demonstrated that PML-RARα recruits histone deacetylase (HDAC) by directly interacting with the N-CoR–Sin3 complex through the RARα portion of the fusion protein, turning the fusion protein into a strong transcription repressor for RA-responsive genes. Treating APL cells with high-dose ATRA reverses the binding of PML-RARα to the N-CoR–Sin3 corepressor complex and reactivates RA-responsive genes (24, 32, 45).

PML belongs to a family of nuclear proteins consisting of the RING finger motif and two other Cys-His domains designated the B-box motif. The region following is the α-helical domain, which is responsible for dimerization (57). PML is the major component of this novel NB, and many proteins associated with PML have been identified. For example, the ubiquitin-like protein modifier SUMO-1 (PIC-1 or sentrin) (7, 35, 36, 53, 62), interferon-induced protein ISG20 (23), the immediate-early viral proteins IE1 and IE4 (2, 3), and the Tax-associated protein int-6 (18) have been found to interact directly or indirectly with PML. SUMO1-conjugated PML is exclusively localized to the PML NB (53, 62), indicating that linking of the SUMO1 modifier is important for assembly of the PML NB. PML also interacts with PLZF, a protein fused with RARα in the t(11;17) translocation that occurs in a rare form of APL (38).

PML NB is a frequent target of viral oncoproteins such as the herpes simplex virus type 1 gene product Vmw110 (22), the adenovirus proteins E1A and E4-ORF3, the Epstein-Barr virus-encoded nuclear antigen EBNA-5 (64), and the cytomegalovirus (CMV) major immediate-early proteins IE1 and IE2 (2, 3). After adenovirus infection, the viral protein (e.g., E4-ORF) targeted to PML NB disrupts its organization and recruits its components (e.g., SP100 and NDP55) to the viral replication domain (19). PML NB has been found to be the site of viral DNA replication and transcription. Also, nascent RNA polymerase II transcripts have been found within the PML NB, and PML has colocalized with the transcription coregulator CBP (CREB-binding protein) (40). These findings support the notion that PML may be involved in transcription regulation.

The transcription regulatory function of PML has been demonstrated in several of our previous studies (51, 66–68) and others (29). PML plays a role in regulating transcription by activating transcription of steroid hormone receptors (29) and

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transcription mediated by Fos–AP-1 (67). Also, when fused to the GAL4 DNA-binding domain (DBD), PML acts as a transcription suppressor, inhibiting transcription from the GAL4-responsive promoter (68). Recently, we showed that PML suppresses the promoter of epidermal growth factor receptor (EGFR) by inhibiting EGFR's Sp1-dependent activity (66).

Our previous studies showed that PML is a growth and transformation suppressor (31, 41, 42, 47, 51). The number of PML NB is regulated during progression of the cell cycle, and the highest number is found during the $G_1$ phase (13, 14). Also, PML was found to induce $G_1$ arrest and apoptosis in MCF-7 and normal human lung fibroblasts (41; unpublished results). In HeLa cells, PML induces growth inhibition by lengthening the $G_1$ phase (52). PML affects cell cycle progression by modulating the expression of several key proteins involved in the $G_1$/S checkpoint, and it also causes a dephosphorylation of Rb. Results from a *PML* gene knockout study (71) strongly support a crucial role for PML in the control of cell growth. This study also showed that PML$^{-/-}$ mouse embryo fibroblasts (MEF) grow faster and have a lower number of cells at $G_0/G_1$ phase and a higher number at S phase than normal MEF. PML also plays an essential role in multiple pathways of programmed cell death. Using PML$^{-/-}$ mice and cells overexpressing PML, it was reported that PML is essential for apoptosis induction by Fas, tumor necrosis factor, ceramide, ionizing radiation, and interferons (58, 73). In addition, overexpression of PML from a recombinant adenovirus induced a significant degree of apoptosis in vivo and in tumors induced by MCF-7 cells (41).

We present here compelling evidence that PML functionally and physically interacts with HDAC in vivo and silences transcription by deacetylation of histones associated with the target promoter. This finding raises the possibility that disruption of PML function by t(15;17) in APL may alter the gene expression pattern normally targeted by PML and may influence its growth suppressor functions by redistributing HDAC activities.

## MATERIALS AND METHODS

**Plasmids.** Jalila Adnane (1) supplied the reporter plasmid G5-Sp1-CAT, which contains the GAL4 binding site and the Sp1 binding site linked to the *CAT* gene. E2F1(Gal4)LUC, which has GAL4 binding sites in place of the E2F sites, was kindly provided by David Johnson. The plasmids pCMV/PML, GAL4/PML, 17mer-tkCAT, and GST-PML were constructed as described in our previous report (66). The UAS-TATA-Luc plasmid, containing multiple GAL4 binding sites, was kindly provided by Ming-Jer Tsai. The mutant fusion plasmids encoding GST-HDAC1, GST-HDAC2, GST-HDAC3, and GST-HDAC2 were constructed as described previously (76). The His-tagged PML expression plasmid (pAcSG-HisNT-B/PML) was created by subcloning full-length *PML* cDNA into the *Nco*I/*Sma*I sites of pAcSG-HisNT-B expression plasmid (PharMingen, San Diego, Calif.). To create the expression plasmid pcDNA3His-PML, the *PML* cDNA fragment containing the His-tagged sequence was excised by *Bam*HI/*Bg*III digestion and linked to the *Bam*HI site of the pcDNA3 vector. The hemagglutinin (HA)-tagged HDAC1 expression plasmid was kindly provided by Harel-Bellan (49). Plasmid pHK3NVP16-PML was constructed by cloning the *Nco*I/*Eco*RI fragment of pCDNA3/PML into the *Bam*HI/*Eco*RI sites of the pHK3NVP16 vector. The *Nco*I and *Bam*HI sites were blunt ended before ligation. The plasmid pM2-HDAC1 was constructed by linking the *Bam*HI/*Eco*RI fragment from GST-HDAC1 into the *Bam*HI/*Hin*dIII sites of the pM2 vector. Both the *Eco*RI and the *Hin*dIII ends were blunt ended before ligation. The GAL4-PML(1–216) plasmid was created by deleting the *Bss*HII/*Xba*I fragment of pM2-PML. The plasmid pCDNA3/HDAC1 was constructed by subcloning the *Bam*HI/*Eco*RI fragment isolated from GST-HDAC1 into the pCDNA3 vector. Gal4-PML(1–305) plasmid was created by deleting the *Kpn*I/*Xba*I fragment of pM2-PML. The PML mutants His-PML(1–555), His-PML(1–447), His-PML(1–305), and His-PML(1–216) were constructed by subcloning the *Bam*H/*Mlu*I, *Bam*HI/*Sma*I, *Bam*HI/*Kpn*I, and *Bam*HI/*Bss*HII fragments of the *PML* cDNA, respectively, into the *Bam*HI/*Eco*RV sites of the pCDNA3.1/HisC vector (Invitrogen, Inc., Carlsbad, Calif.). Mutants His-PML(97–633) and His-PML(331–633) were constructed by subcloning the *Avr*II/*Eco*RI and *Bss*HII/*Eco*RI fragments of the *PML* cDNA into the *Bam*HI/*Eco*RI sites of pCDNA3.1/His vector. His-PML(447–633) was constructed by cloning the *Sma*I/*Xba*I fragment of the *PML* cDNA into the *Xho*I/*Xba*I site of pCNDA3.1/His vector.

**Gene transfer, CAT, and luciferase assays.** Cells were cultured to semiconfluence and transfected with the plasmids using the Superfect reagent (Qiagen, Valencia, Calif.) in 5-cm tissue culture dishes. Plasmid DNA (2.5 μg) containing 0.5 μg of the reporter and 1.5 μg of the expression plasmids was used. The plasmid pCMV-βGal (0.5 μg) was also included as an internal control in all transfection and cotransfection assays. The chloramphenicol acetyltransferase (CAT), luciferase, and β-galactosidase activities were determined as described in our previous report (66).

**In vitro transcription and translation.** In vitro transcription and translation of the HDAC and PML proteins were performed as described in our previous report (42) using the TNT-coupled transcription-translation system from Promega Corp. (Madison, Wis.).

**Immunoprecipitation and deacetylase assay.** Cells were transfected with 5 μg of the indicated plasmids per 10-cm tissue culture dish, lysed in radioimmunoprecipitation assay (RIPA) buffer, and subjected to immunoprecipitation using anti-PML antibody or preimmune serum as described previously (51). The immune complexes were then washed in 1× HAD buffer (75.0 mM Tris-HCl, pH 7.0; 2.0 mM 2-mercaptoethanol; 0.1 mM EDTA) without NaCl and used in deacetylase assays. The assays were performed as follows. Immune complexes were incubated with 100 μg of [$^3$H]acetyl-labeled HeLa histones in 1× HAD buffer with 275 mM NaCl for 2 h at 30°C in a total volume of 200 μl. The reactions were stopped by adding 50 μl of 0.12 N acetic acid–0.72 N HCl. The released acetate was extracted in 0.5 ml of ethyl acetate, mixed in 3 ml of scintillation solution, and counted. All assays were performed in duplicate. The acetylase activity, measured in counts per minute, represented the average of several independent measurements. [$^3$H]acetyl-labeled HeLa histones were prepared as previously described (12).

**Mammalian two-hybrid assay.** The mammalian two-hybrid assay was carried out as described in our previous report (66). Briefly, 1 μg of UAS-TATA-Luc reporter plasmid was cotransfected with 0.2 μg of pM2-HDAC1 and either pHK3NVP16 or pHK3NVP16-PML plasmid in U2OS cells. The plasmid pCMV-βGal was included in all transfection assays to monitor transfection efficiency.

**CHIP assay.** The chromatin immunoprecipitation (CHIP) assay was performed according to the method described previously (48), with some modifications. The plasmid GAL4-PML or GAL4-PML(1–216) or the GAL4 parental vector was cotransfected with UAS-TATA-Luc into Cos-1 cells using Superfect reagent in six-well plates in the presence of 0.2 μg of pCMV-βGal to monitor transfection efficiency. Cells were collected in 10 ml of culture medium 48 h after transfection. Formaldehyde (37% in 10% methanol) was added to a final concentration of 1%, and the mixture was incubated for 10 min at room temperature. The cells were then centrifuged, washed three times in cold phosphate-buffered saline, and resuspended in sodium dodecyl sulfate (SDS) buffer containing 2% SDS, 10 mM EDTA, 50 mM Tris-HCl (pH 8.1), and protease inhibitor cocktails (Boehringer Mannheim Corp., Indianapolis, Ind.). The cell suspension was sonicated briefly and centrifuged (13,000 × *g*, 5 min) at 4°C. In each group, one-third of the lysate was used to precipitate total DNA by adding 2.5 volumes of ethanol. Another one-third of the lysate was diluted 10-fold with dilution buffer containing 1% Triton X-100, 2 mM EDTA, 150 mM NaCl, and 20 mM Tris-HCl (pH 8.0). Anti-acetylated histone H3 antibody (Upstate Biotechnology, Waltham, Mass.) was added, and the mixture was incubated for 2 h. Protein A agarose beads (20 μl) were then added, and the suspension was gently agitated overnight. The remaining one-third of the lysate was treated as described above but without the anti-acetylated histone H3 antibody. The immunoprecipitated complexes were harvested by centrifugation at 4°C and washed three times in TSE (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl [pH 8.0]) containing 150 mM NaCl and one time in TSE containing 500 mM NaCl. The DNA complex was eluted by adding 400 μl of elution buffer (1% SDS and 0.1 mM $NaHCO_3$) and rotated for 20 min. The eluted materials were heated to 65°C for 8 h to reverse formaldehyde cross-linking, and the DNA was ethanol precipitated, dried, and resuspended in 50 μl of Tris-EDTA. PCR was performed for 20, 22, and 25 cycles at 95°C for 30 s, 55°C for 45 s, and 72°C for 45 s using the two primers that hybridize to the 5′ end of the luciferase gene. The DNA sequence of the 5′ primer was 5′-CTGGAGAGCAACTGCATAAGGC-3′ and of the 3′ primer was 5′-TCTCTGGCATGCGAGAATCTCAC-3′ with a predicted 550-bp amplified DNA fragment.

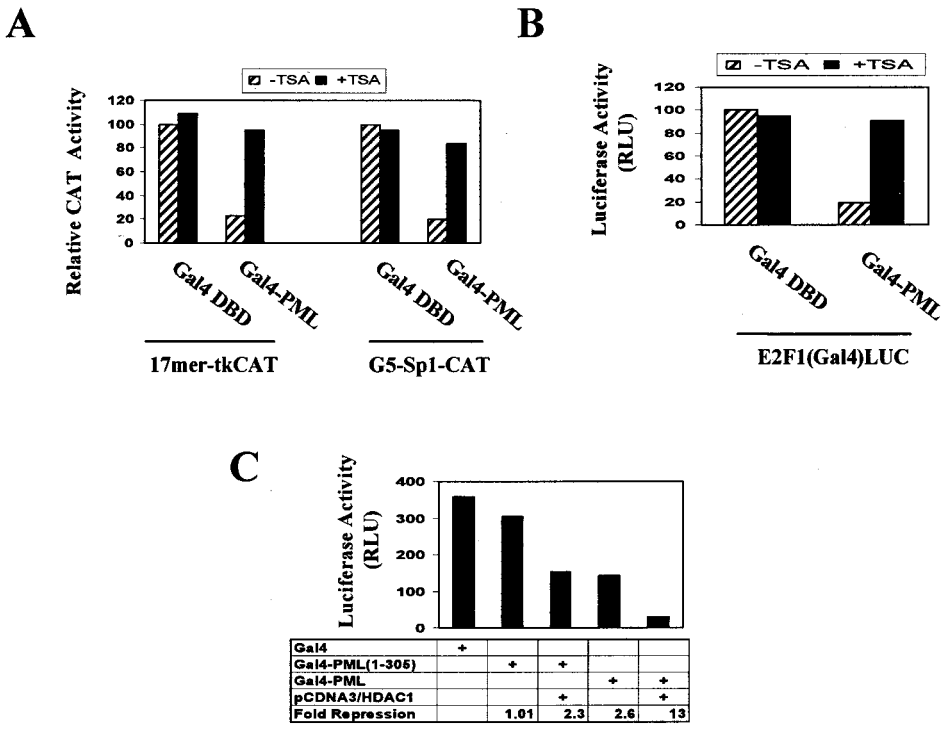


FIG. 1. Evidence that PML represses transcription by association with HDAC. U2OS cells were transfected with the indicated plasmids and incubated for 24 h in the presence or absence of TSA (150 μM). Cells were then lysed and assayed for CAT (A) or luciferase (B) activity. (C) Combinations of the indicated plasmids were cotransfected with the luciferase reporter plasmid UAS-TATA-Luc into Cos-1 cells. The nuclear extract was prepared 24 h after transfection and used for the luciferase assay. In each transfection assay, 100 ng of the β-galactosidase expression plasmid pSV-βGal was included to monitor the transfection efficiency. The level of repression was calculated relative to the luciferase activity in cells transfected with GAL4 alone. All cotransfection assays were repeated at least two times. The data presented here show a typical representative result.

**GST pull-down assay.** The glutathione *S*-transferase (GST) pull-down assay was performed as described in our previous report (66).

**His-tagged pull-down assay and coimmunoprecipitation.** The indicated plasmids were cotransfected into Cos-1 cells using Superfect reagent, and total protein extracts were prepared as described previously (66). His-tagged pull-down assay was performed by adding 20 μl of bovine serum albumin (BSA)-preblocked Ni-nitrilotriacetic acid (NTA) agarose (Qiagen) to the total protein extracts and incubating them for 2 h at 4°C in the presence of 5 mM imidazole to minimize nonspecific binding. The bound proteins were extensively washed in RIPA buffer containing 0.01% SDS and 50 mM imidazole, suspended in 40 μl of 2× SDS loading buffer, and subjected to SDS–10% polyacrylamide gel electrophoresis. Immunoprecipitation was performed as described in our previous report (51).

## RESULTS

**TSA inhibits transcriptional repression mediated by PML.** Our previous study showed that PML could serve as a transcriptional repressor when fused to the GAL4 DNA-binding domain (DBD) (68). To further study the mechanism of PML's effects on transcription, we examined whether repression by PML is related to HDAC activity. We first asked whether HDAC inhibitors such as trichostatin A (TSA) would inhibit repression by GAL4-PML. To test this, a thymidine kinase promoter-CAT reporter containing one GAL4 binding site (17mer-tkCAT) and an Sp1 minimal promoter containing five copies of the GAL4 binding site were cotransfected into Cos-1 cells with plasmids containing the GAL4 DBD (negative control) or GAL4-PML. Transfected cells were then cultured in the presence or absence of TSA. As expected, transcription of both promoter constructs was significantly repressed by GAL4-PML (Fig. 1A). The growth of cells in TSA abolished this inhibitory effect, suggesting that PML-mediated repression of both promoter elements required HDAC activity. Similar experiments were performed using an E2F1 mutant promoter-luciferase construct in which the E2F site was replaced by the GAL4 binding site, E2F1(GAL4)Luc. Again, GAL4-PML significantly repressed transactivation of the luciferase gene, and the presence of TSA abolished transcription repression by GAL4-PML (Fig. 1B).

Because TSA is a specific inhibitor of HDAC, the above results suggested that the transcriptional repression function of PML might be mediated through its association with an HDAC. To further test whether PML-mediated repression involves HDAC activity, we performed another series of transfection experiments in which we overexpressed an HDAC. UAS-TATA-Luc (containing multiple GAL4 binding sites) was cotransfected with GAL4-PML in the presence and absence of the HDAC expression plasmid pCDNA3/HDAC1. Expression of HDAC1 in the transfected cells was confirmed by immunofluorescence staining (data not shown). We found that the presence of HDAC1 and GAL4-PML individually repressed transcription by 2.3- and 2.6-fold, respectively. However, the presence of both HDAC1 and GAL4-PML repressed promoter activity by 13-fold, indicating a synergistic effect be-

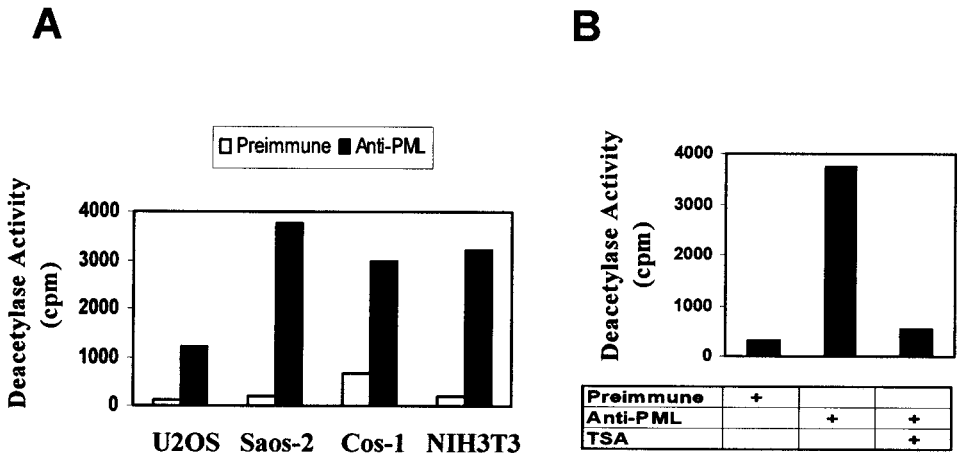


FIG. 2. Coimmunoprecipitation of HDAC activity with PML. (A) The indicated cell lines were infected with Ad-PML as described in our previous report (41). Total cell lysates were prepared 24 h after infection and subjected to immunoprecipitation with an anti-PML antibody or preimmune serum as indicated. The precipitated proteins were absorbed in protein A-agarose, washed extensively, and used for the deacetylase assay. (B) Total protein extracts isolated from Saos-2 cells infected with Ad-PML were subjected to immunoprecipitation as described above. Deacetylase assays were performed using the immunoprecipitated protein in the presence or absence of TSA (400 μM) as shown.

tween GAL4-PML and HDAC (Fig. 1C). These studies indicate a functional association between PML and HDAC.

**PML is associated with HDAC in vivo.** We overexpressed the PML protein in several cell lines by infecting them with a recombinant PML-adenovirus (Ad-PML) as described in our previous reports (31, 41). Total proteins were isolated from these infected cells, and PML was immunoprecipitated using a polyclonal antibody or preimmune control serum. PML coimmunoprecipitated a significant level of HDAC activity in U2OS, Saos-2, Cos-1, and NIH 3T3 cells (Fig. 2A). Interestingly, Saos-2 cells are retinoblastoma protein (Rb) negative, and Rb has been shown to bind HDAC (4), but our results with the Saos-2 cells indicated that PML-associated HDAC activity is Rb independent. Results from the present study further show that the PML-associated HDAC activity was TSA sensitive (Fig. 2B). Together, our results strongly support a role for PML as a transcription repressor that regulates gene expression by association with HDAC activity.

To determine whether PML can associate with specific HDAC proteins, we cotransfected PML with HDAC expression vectors. We first cotransfected a His-tagged PML expression plasmid (His-PML) with an HA-tagged HDAC1 (HA-HDAC1) and assayed the in vivo association between PML and HDAC1 by Ni-NTA agarose binding. The His-tagged plasmid minus an insert was used as a negative control. Western blotting using an anti-HA antibody confirmed that HA-HDAC1 was expressed in the transfected cells. A significant level of HDAC1 protein was associated with His-PML in extracts from cells containing both expression plasmids (Fig. 3A). No detectable level of HDAC1 was associated with the negative control plasmid.

We next determined whether PML coimmunoprecipitated with HA-tagged HDAC1 by using the HA-tagged specific monoclonal antibody. We cotransfected the pcDNA3/PML expression plasmid with HA-tagged HDAC1 into Cos-1 cells. Immunoprecipitation was then performed on cell extracts, and precipitated protein fractions were probed with the PML antibody. As a negative control, cells were transfected with the empty HA-tagged vector and pcDNA3/PML. We found that the anti-HA antibody coprecipitated a significant amount of PML protein, whereas an unrelated anti-E6 control antibody did not (Fig. 3B). No PML protein was immunoprecipitated from extracts from cells transfected with the HA-tagged plasmid alone. Western blot analysis using total protein isolated from the transfected cells showed that PML was expressed well in both experiments. Cotransfection experiments were also performed using an HDAC3 expression plasmid and the His-tagged PML plasmid. Again, we found that HDAC3 associated with PML in Ni-NTA pull-down assays (Fig. 3C). These experiments indicated that PML can associate with specific HDAC proteins in vivo. To further confirm the in vivo association between PML and HDAC1, we performed a mammalian two-hybrid assay by transfecting the expression plasmids GAL4-HDAC1 and VP16-PML into the U2OS cells. Expression of the luciferase activity was enhanced fivefold when cotransfection was done with VP16-PML versus the negative control using VP16 alone. This indicated a physical association in vivo between PML and HDAC1 (Fig. 3D).

Finally, we investigated whether PML is associated with HDAC at the endogenous level in hematopoietic cells. K562 cells were treated with alpha interferon to induce the endogenous level of PML. Coimmunoprecipitation was performed with HDAC1 antibody; the precipitated HDAC1-associated proteins were analyzed by Western blotting with PML antibody. A reverse coimmunoprecipitation assay was also performed using the PML antibody. The results presented in Fig. 3E and F demonstrate that PML is associated with HDAC1 in vivo at the endogenous level.

**PML interacts with HDAC directly in vitro.** The studies presented above demonstrated a functional and physical association in vivo between PML and HDAC. This association may be direct or may involve intermediary proteins. To determine whether PML interacts directly with HDAC in vitro, we tested whether the fusion proteins GST-HDAC1, GST-HDAC2, and

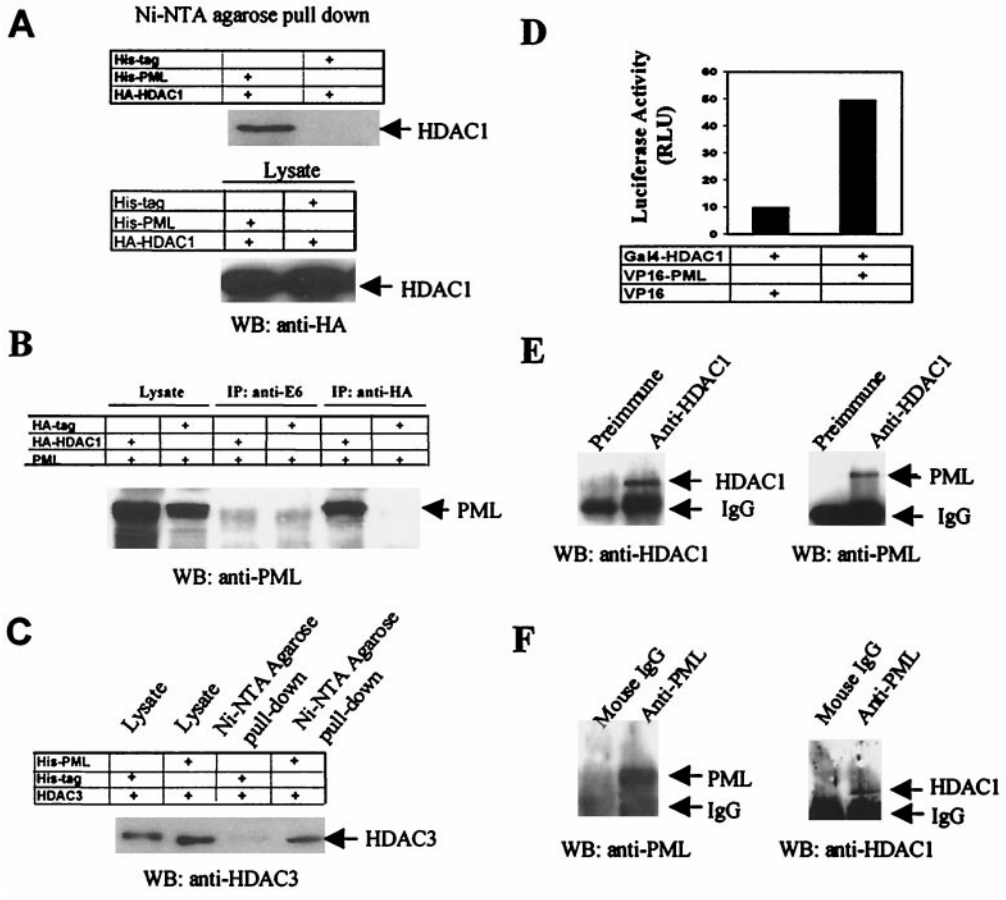


FIG. 3. Association between the PML protein and HDAC in vivo. (A) His-PML and HA-HDAC1 expression plasmids were cotransfected into Cos-1 cells, and His-PML and its associated proteins were pulled down by Ni-NTA agarose. The pull-down protein was detected by Western blotting (WB) using the anti-HA antibody. A cotransfection assay using His-tagged and HA-tagged HDAC1 expression plasmid was used as a negative control. (B) PML coprecipitated HA-HDAC1 using an HA-tagged specific monoclonal antibody. The expression plasmid pcDNA3/PML and HA-HDAC1 were cotransfected into the Cos-1 cells, and total proteins were prepared 24 h after transfection. Immunoprecipitation was performed using the anti-HA specific monoclonal antibody, and the presence of coprecipitated PML protein was detected by Western blotting using our PML antibody. (C) Ni-NTA agarose pulled down His-tagged PML and HDAC3. An experiment similar to that described above was performed except that the His-PML plasmid was cotransfected with HDAC3 expression plasmid. (D) A mammalian two-hybrid assay was used to detect the in vivo association between PML and HDAC. U2OS cells were cotransfected with VP16-PML and GAL4-HDAC1 in the presence of the luciferase reporter plasmid UAS-TATA-Luc. Luciferase activity in each assay was determined and calculated relative to the activity of a control (cotransfected with the empty vector VP16). (E and F) The endogenous association between PML and HDAC1 was tested in K562 cells. K562 cells were pretreated with 1,000 U of alpha interferon per ml to induce a high expression of PML. Total protein was isolated from $3 \times 10^8$ cells, and coimmunoprecipitation was performed. Preimmune serum was included as a negative control.

GST-HDAC3 purified from bacteria were capable of binding to in vitro-translated PML protein. Indeed, all three HDAC isoforms bound to PML in GST pull-down assays (Fig. 4A). However, PML did not bind to GST alone. This experiment was repeated at least two times, and GST-HDAC3 binding to PML was consistently weaker than that of GST-HDAC1 and GST-HDAC2. A similar experiment was performed to assay GST-PML fusion protein binding to in vitro-translated HDAC1 (Fig. 4B). These results suggest that PML directly interacted with all three isoforms of HDAC in vitro.

It has been previously reported that the PML-RARα fusion protein created by fusing the *PML* and *RAR*α genes via t(15;17) in APL interacts with HDAC through the N-CoR–Sin3 corepressor complex. Therefore, we compared PML and PML-RARα binding to GST-HDAC1. The result, presented in Fig. 4C, shows that GST-HDAC1 bound poorly to in vitro translated PML-RARα protein, compared with its strong binding to PML. This study demonstrated that PML-RARα retained poor binding affinity to HDAC1. As expected, the in vitro-translated RARα did not bind HDAC1 (Fig. 4C). This study confirms that PML-RARα requires cofactors for recruitment of HDACs, in contrast to the native PML protein, which interacts with these enzymes directly.

**PML interacts with HDAC through specific domain.** To investigate whether specific domains of PML are involved in its interaction with HDAC1, a series of PML deletion mutants was created (Fig. 5A); these in vitro-translated proteins were used in GST-HDAC1 pull-down assays (Fig. 5B). Like wild-type PML, mutants lacking the proline-rich domain, RING-finger motif, and coiled-coil dimerization domain retained full binding efficiency for HDAC1. Mutant PML(447–633) containing 188 amino acids on the carboxyl-terminal end of PML

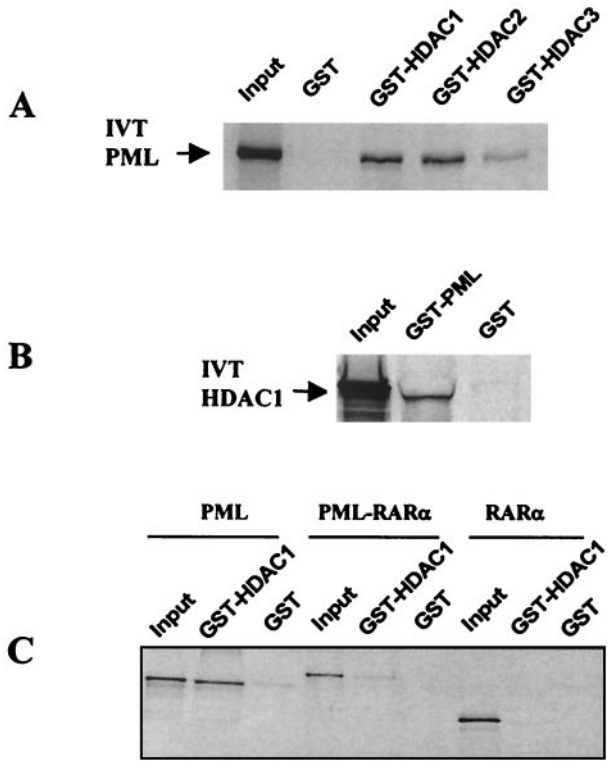


FIG. 4. Analysis of PML and HDAC interaction in vitro by GST pull-down assay. (A) In vitro-translated $^{35}$S-labeled PML protein (IVT PML) was incubated with GST, GST-HDAC1, GST-HDAC2, and GST-HDAC3 immobilized to the glutathione agarose bead. In this assay, GST was used as a negative control. The GST pull-down assay was repeated at least two times, and a weak interaction between HDAC3 and PML was detected consistently. (B) GST-PML pull-down assay of the in vitro-translated $^{35}$S-labeled HDAC1 protein (IVT HDAC1). The experiment was performed as described above. GST was used as a negative control in this study. (C) GST-HDAC pull-down assay for PML-RARα and RARα. In this assay, the in vitro-translated and $^{35}$S-labeled PML, PML-RARα, and RARα were used as described above. GST-HDAC1 was unable to pull down the PML-RARα and RARα proteins.

retain full binding activity. PML mutants lacking the C-terminal domain [PML(1–555), PML(1–447), and PML(1–303)] did not interact with HDAC1 (Fig. 5B). This result suggests that the C-terminal domain (amino acids 555 to 633) is required for interaction with HDAC1. PML's HDAC interacting domain was investigated further by using a His-tag pull-down assay. The results, presented in Fig. 5C, confirm that the C-terminal end of PML is required for interaction with HDAC1.

The results shown in Fig. 1 and 2 demonstrate that PML represses transcription by recruiting HDAC to the target promoter. Therefore, PML mutants unable to interact with HDAC should have lost their ability to repress transcription. To examine this possibility, mutant PML cDNAs were subcloned into the GAL4-DBD vector and tested in a series of cotransfection experiments. The results indeed showed that both PML(1–305) and PML(1–447) had lost their ability to repress transcription (Fig. 1C and data not shown).

To elucidate the domains of HDAC2 involved in interaction with PML, several deletion mutants of HDAC2 in GST fusion vectors were created and used in in vitro pull-down assays. In vitro-translated PML interacted with all mutants except HDAC1(18–488). Deletion of amino acids 1 to 180 significantly reduced HDAC2's ability to bind to PML (Fig. 6). This study demonstrated that PML interacts with the amino-terminal 180 amino acids of HDAC2.

**PML promotes deacetylation of histone H3 on its targeted promoter in vivo.** If the observed physical and functional interactions between the PML and HDAC proteins are important to the function of PML as a transcriptional repressor, then recruitment of PML should result in deacetylation of target promoters in vivo. To test this notion, we used a modified CHIP assay. The CHIP assay has been used to demonstrate that Rb recruits HDAC and deacetylates histone around the E2F promoter in vivo (48). We transfected Cos-1 cells with a UAS-TATA-Luc reporter plasmid together with GAL4, GAL4-PML(1–216), or GAL4-PML. GAL4 and GAL4-PML(1–216) were negative controls because they do not interact with HDAC. Histone-bound DNA fragments immunoprecipitated with anti-acetylated histone H3-specific antibodies were amplified by PCR using primers specific for the UAS-TATA-Luc promoter. Significantly less promoter DNA was precipitated by the anti-acetylated histone H3-specific antibody in the presence of GAL4-PML than in the presence of the two negative controls (Fig. 7). The experiment was repeated at least two times, and in all cases consistent results were obtained. This outcome strongly supports the conclusion that GAL4-directed binding of PML to its target site is associated with deacetylation of the target promoter. This finding also provides strong evidence that PML recruits HDAC and represses transcription by deacetylation of histones associated with target promoters.

## DISCUSSION

**PML is a transcriptional repressor that functionally and physically associates with HDACs.** Our results demonstrate that PML functionally and physically interacts with all three HDAC isoforms through specific domains. Both the C-terminal and the N-terminal regions of the PML protein are necessary for efficient binding to HDAC. Our study also shows that the PML-RARα fusion protein encoded from the t(15;17) breakpoint in APL binds poorly to HDAC.

Modification of the chromatin structure by acetylation or deacetylation plays an important role in the control of gene expression (56, 63). Hyperacetylation and hypoacetylation of histones have been shown to correlate with activation and repression of gene expression (9, 28). Also, acetylation of the lysine residues of core histones neutralizes a positive charge and presumably affects histone-DNA interactions, enabling transcription factors to have access to the promoter regions of their target genes (43, 69). Several transcription coactivators, such as CBP (also called p300), have been found to have histone acetyltransferase activity (63, 70). In addition, transcription activators recruit CBP to target promoters and promote core histone acetylation to achieve transcriptional activation (6, 50, 55, 65). On the other hand, deacetylation of histones promotes nucleosome assembly and renders gene promoters inaccessible by regulatory factors (39, 54). Transcription repressors functionally associate with the corepressor complex and HDAC to achieve transcription silencing of the target promoter by deacetylation of histones (5, 39, 54). The

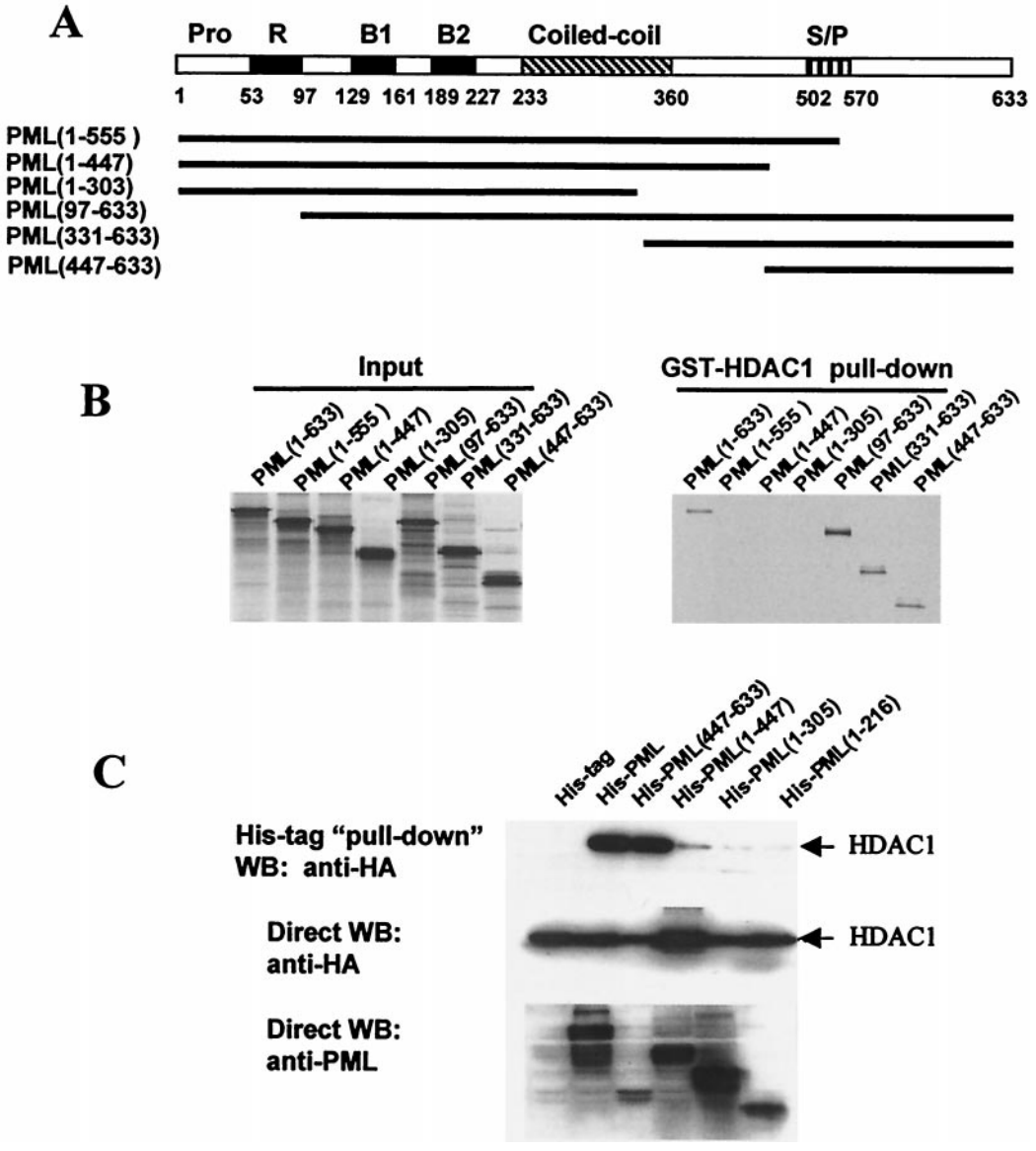


FIG. 5. Interaction of PML and HDAC through specific domains. (A) Schematic illustration of the PML mutants used in this study. Pro, proline-rich domain; R, RING-finger motif; B1 and B2, B boxes; Coiled-coil, dimerization domain; S/P, serine-proline-rich domain. (B) The relative mobility of the in vitro-translated PML and its mutant forms in SDS–10% polyacrylamide gel electrophoresis is shown in the left panel. A GST-HDAC1 pull-down assay of in vitro-translated PML and its mutant proteins is shown in the right panel. Equal quantities of the in vitro-translated PML and mutant proteins were used in the GST pull-down assay in a fixed amount of GST-HDAC1 protein immobilized to the glutathione-agarose bead. The signal intensities shown reflect the relative binding affinities between HDAC1 and PML-PML mutants under various experimental conditions. (C) The interacting domain between PML and HDAC1 was identified by His-tag pull-down assay. His-tagged PML and its mutant expression plasmids were cotransfected with the HA-HDAC1 expression plasmid into U2OS cells. The His-tagged PML and mutants were pulled down by Ni-NTA agarose. The in vivo association between PML or its mutant and HDAC1 was determined by Western blotting using the anti-HA antibody (top panel). The total proteins isolated from each cotransfection assay were used to confirm the expression of HA-HDAC1 (middle panel) and PML (lower panel) by Western blotting.

present study demonstrates that PML also represses transcription by recruiting HDAC to the target gene promoter.

The specific PML domain that interacts with HDAC is different from that which interacts with Rb. A PML mutant unable to interact with Rb is still capable of binding HDAC1 (unpublished result). The N-terminal domain (amino acids 1 to 180) of HDAC2 is responsible for binding PML, and this region is different from the Rb binding domain present in the C-terminal region of HDAC2. The PML binding domain is highly conserved between all three HDAC isoforms (21); this region contains amino acids H141, H174, and D176, which are necessary for the catalytic activity and structural integrity of the proteins (30). Site-directed mutagenesis of any one of these amino acids reduced HDAC catalytic activity by 85 to 100% and prevented efficient binding of mSin3A and RbAp48. The results presented in Fig. 2 and Fig. 6 demonstrated that binding of PML to this region did not affect HDAC catalytic activity. Significant amounts of HDAC were coimmunoprecipitated

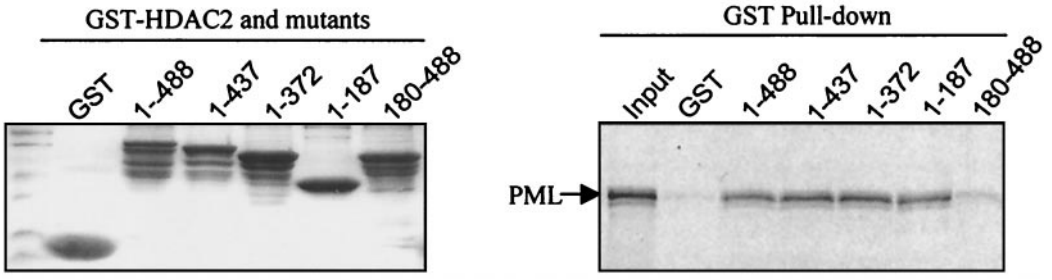


FIG. 6. Analysis of the domain of HDAC2 that interacts with PML. HDAC2 and its mutants containing amino acids 1 to 437, 1 to 372, 1 to 187, and 180 to 488 fused in frame downstream to the GST protein were used in the GST pull-down assay. GST-HDAC2, containing amino acids 1 to 488, represents the wild-type protein. GST protein alone was used as a negative control. Equal quantities of each of the wild-type and mutant GST-HDAC fusion proteins were used in the pull-down assay. Input, a small sample of the in vitro-translated PML protein.

using the PML antibody, and overexpression of PML in a transient-transfection assay significantly reduced the acetylation of histone H3 associated with the target promoter. In addition, PML repression of GAL4-mediated transactivation of a target promoter was TSA sensitive (Fig. 1), supporting the importance of HDAC activity in PML-mediated repression.

A recent report by Li et al. (44) demonstrated that PML inhibited Daxx-mediated transcriptional repression by the sequestration of Daxx to the PML NB. These authors further showed that PML did not interact with all three isoforms of HDAC by far-Western analyses. The PML cDNA used in their study represents the short isoform consisting of amino acids 1 to 560 (34). This finding is in agreement with our result (Fig. 5) that the PML mutant PML(1–555) did not bind HDAC1 in a GST pull-down assay. We obtained the PML cDNA from J. D. Chen (Departments of Pharmacology and Molecular Toxicology, University of Massachusetts Medical School, Worcester), and our results confirmed that this PML isoform does not interact with HDAC1 in a GST pull-down assay (unpublished results).

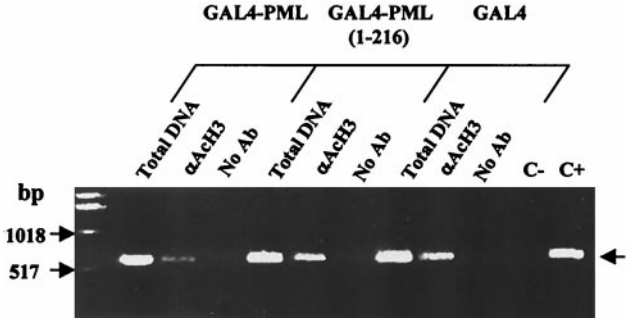


FIG. 7. PML recruits HDAC and deacetylates its target promoter in vivo. Acetylation of plasmid DNA-associated histone H3 in vivo was determined by the CHIP assay. Three groups of experiments were carried out by cotransfection of UAS-TATA-Luc with GAL4-PML, GAL4-PML(1–216), and GAL4 alone into Cos-1 cells. GAL4-PML(1–216) and GAL4 were negative controls. In each group, total DNA was used as a positive control for PCR, and total DNA immunoprecipitation in the absence of antibody served as a negative control. αAcH3, acetylated histone H3-bound DNA amplified by PCR after being immunoprecipitated by the antibody. Primers were designed to amplify a 550-bp fragment of the luciferase gene (arrow). A negative control for PCR without DNA (C−) and a positive control using UAS-TATA-Luc plasmid DNA (C+) were also included.

**A possible role of PML-HDAC association in the regulation of cell cycle progression.** PML interacts with critical cell cycle regulatory proteins, including Rb (4) and Sp1 (65). It is well documented that Rb plays a central role in controlling cell cycle progression by modulating the transcriptional activity of E2F (61). Transcription of many genes involved in the $G_1$-to-S transition is controlled by E2F. At $G_1$, the hypophosphorylated form of Rb recruits HDAC, interacts with E2F, and inactivates its transactivation function (10, 48, 49). During $G_1$/S transition, Rb becomes phosphorylated by the cyclin-dependent kinase cyclin D-Cdk4 or cyclin E-Cdk2 (61). The phosphorylated form of Rb releases E2F and HDAC and reactivates E2F target gene; this enables cell cycle progression from $G_1$ to S. However, the biologic significance of PML interaction with Rb is not clear. Several reports have documented that Sp1 interacts with E2F and synergistically activates transcription of $G_1$/S-phase checkpoint genes (37, 46). Together, these studies suggest that PML could play a role in regulating cell cycle progression by functionally associating with the Rb-E2F complex. PML's role in cell cycle progression is also supported by reports documenting that PML NB is the target of several viral oncoproteins. We hypothesize that interaction of PML with HDACs regulates cell cycle progression by modulating the functional activity of the Rb-E2F complex. Our study shows that PML inhibits Rb-mediated transcriptional repression of the E2F target gene and that this effect can be reversed by an increased HDAC concentration (unpublished observation). This raises the possibility that PML may play a role in regulating Rb-mediated repression of E2F by sequestration of a limited quantity of HDACs.

**Disruption of PML function by t(15;17) and its contribution to the development of APL.** Disruption of PML function by t(15;17) is believed to play a role in the development of APL (51). Differentiation therapy using high-dose ATRA induced a complete clinical remission of APL and then a reorganization of the normal PML NB (14, 20, 75). At least two scenarios may explain this observation: (i) the fusion protein PML-RARα is eliminated as a result of rapid degradation, mainly through a proteasome pathway (37, 77), or (ii) the PML growth suppressor function is reactivated.

Recent studies provide substantial evidence demonstrating that both PML-RARα and PLZF-RARα [encoded by the fusion gene *PLZF-RARα* resulting from t(11;17) in a rare form of APL] form a complex with a transcriptional corepressor and

recruit HDAC to achieve transcriptional silencing of target genes (24, 32, 45). Nonphysiological high-dose ATRA induces PML-RARα to dissociate from the corepressor complex in RA-sensitive APL; this possibly triggers reactivation of RA-responsive myeloid-specific genes and consequently induces differentiation of APL cells. However, in RA-insensitive APL that expresses PLZF-RARα, the corepressor complex is not dissociated by treatment with high-dose ATRA (32). Furthermore, both PML-RARα- and PLZF-RARα-associated corepressor complexes can be dissociated by treatment with RA plus TSA and induced differentiation. This finding indicates that the ability of fusion proteins to recruit a transcriptional corepressor is critical in promoting the development of APL.

The present study demonstrates that PML, but not PML-RARα, interacts directly with HDACs to repress target genes. This finding provides an important implication, that t(15;17) disrupts the transcription-silencing function of PML in APL cells. Such an event may lead to altered chromatin remodeling and an altered pattern of gene expression. It may also contribute to the development of leukemia. PLZF has also been shown to interact with HDAC (38). The domain of PML that interacts with HDAC involves a C-terminal region not included in the PML-RARα fusion protein. However, the domain of PLZF that interacts with HDAC involves the N-terminal domain, and PLZF-RARα retains full HDAC binding activity in addition to its ability to associate with corepressors through RARα. Based on this observation, we hypothesize that in PML-RARα-positive APL, ATRA induces dissociation of the corepressor from RARα and completely disables its ability to act as a transcription silencer. This event leads to RA-induced differentiation of the APL cells. In PLZF-RARα-positive APL, RA does not interfere with HDAC binding to PLZF and is unable to relieve the transcription-silencing effects of the fusion protein on target genes. Therefore, PLZF-RARα-positive APL is insensitive to differentiation therapy using ATRA. This hypothesis explains why RA plus TSA induces differentiation of both types of APL (24, 32, 45).

## ACKNOWLEDGMENTS

We are grateful to Don Norwood for critical reading of the manuscript.

This study was supported by grant CA 55577 from the National Institutes of Health to K.-S.C.

W.-S.W. and S.V. contributed equally to this work.

## REFERENCES

1. **Adnane, J., Z. Shao, and P. D. Robbins.** 1995. The retinoblastoma susceptibility gene product represses transcription when directly bound to the promoter. J. Biol. Chem. **270:**8837–8843.
2. **Ahn, J.-H., and G. S. Hayward.** 1997. The major immediate-early proteins IE1 and IE2 of human cytomegalovirus colocalize with and disrupt PML-associated nuclear bodies at very early times in infected permissive cells. J. Virol. **71:**4599–4613.
3. **Ahn, J. H., E. J. Brignole, and G. S. Hayward.** 1998. Disruption of PML subnuclear domains by the acidic IE1 protein of human cytomegalovirus is mediated through interaction with PML and may modulate a RING finger-dependent cryptic transactivator function of PML. Mol. Cell. Biol. **18:**4899–4913.
4. **Alcalay, M., L. Tomassoni, E. Colombo, S. Stoldt, F. Grignani, M. Fagioli, L. Szekely, K. Helin, and P. G. Pelicci.** 1998. The promyelocytic leukemia gene product (PML) forms stable complexes with the retinoblastoma protein. Mol. Cell. Biol. **18:**1084–1093.
5. **Ayer, D. E., Q. A. Lawrence, and R. N. Eisenman.** 1995. Mad-Max transcriptional repression is mediated by ternary complex formation with mammalian homologs of yeast repressor Sin3. Cell **80:**767–776.
6. **Bannister, A. J., and T. Kouzarides.** 1996. The CBP co-activator is a histone acetyltransferase. Nature **384:**641–643.
7. **Boddy, M. N., K. Howe, L. D. Etkin, E. Solomon, and P. S. Freemont.** 1996. PIC 1, a novel ubiquitin-like protein which interacts with the PML component of a multiprotein complex that is disrupted in acute promyelocytic leukaemia. Oncogene **13:**971–982.
8. **Borrow, J., A. D. Goddard, D. Sheer, and E. Solomon.** 1990. Molecular analysis of acute promyelocytic leukemia breakpoint cluster region on chromosome 17. Science **249:**1577–1580.
9. **Braunstein, M., A. B. Rose, S. G. Holmes, C. D. Allis, and J. R. Broach.** 1993. Transcriptional silencing in yeast is associated with reduced nucleosome acetylation. Genes Dev. **7:**592–604.
10. **Brehm, A., E. A. Miska, D. J. McCance, J. L. Reid, A. J. Bannister, and T. Kouzarides.** 1998. Retinoblastoma protein recruits histone deacetylase to repress transcription. Nature **391:**597–601.
11. **Brown, D., S. Kogan, E. Lagasse, I. Weissman, M. Alcalay, P. G. Pelicci, S. Atwater, and J. M. Bishop.** 1997. A PMLRARalpha transgene initiates murine acute promyelocytic leukemia. Proc. Natl. Acad. Sci. USA **94:**2551–2556.
12. **Carmen, A. A., E. Stephen, S. E. Rundlett, and M. Grunstein.** 1996. HDA1 and HDA3 are components of a yeast histone deacetylase (HDA) complex. J. Biol. Chem. **271:**15837–15844.
13. **Chan, J. Y., L. Li, Y. H. Fan, Z. M. Mu, W. W. Zhang, and K. S. Chang.** 1997. Cell-cycle regulation of DNA damage-induced expression of the suppressor gene PML. Biochem. Biophys. Res. Commun. **240:**640–646.
14. **Chang, K. S., Y. H. Fan, M. Andreeff, J. X. Liu, and Z. M. Mu.** 1995. The *PML* gene encodes a phosphoprotein associated with the nuclear matrix. Blood **85:**3646–3653.
15. **Chang, K. S., S. A. Stass, D. T. Chu, L. L. Deaven, J. M. Trujillo, and E. J. Freireich.** 1992. Characterization of a fusion cDNA (RARA/myl) transcribed from the t(15;17) translocation breakpoint in acute promyelocytic leukemia. Mol. Cell. Biol. **12:**800–810.
16. **Chen, G. Q., X. G. Shi, W. Tang, S. M. Xiong, J. Zhu, X. Cai, Z. G. Han, J. H. Ni, G. Y. Shi, P. M. Jia, M. M. Liu, K. L. He, C. Niu, J. Ma, P. Zhang, T. D. Zhang, P. Paul, T. Naoe, K. Kitamura, W. Miller, S. Waxman, Z. Y. Wang, H. de The, S. J. Chen, and Z. Chen.** 1997. Use of arsenic trioxide ($As_2O_3$) in the treatment of acute promyelocytic leukemia (APL). I. $As_2O_3$ exerts dose-dependent dual effects on APL cells. Blood **89:**3345–3353.
17. **De The, H., C. Lavau, A. Marchio, C. Chomienne, L. Degos, and A. Dejean.** 1991. The PML-RARalpha fusion mRNA generated by the t(15;17) translocation in acute promyelocytic leukemia encodes a functionally altered RAR. Cell **66:**675–684.
18. **Desbois, C., R. Rousset, F. Bantignies, and P. Jalinot.** 1996. Exclusion of Int-6 from PML nuclear bodies by binding to the HTLV-I Tax oncoprotein. Science **273:**951–953.
19. **Doucas, V., A. M. Ishov, A. Romo, H. Juguilon, M. D. Weitzman, R. M. Evans, and G. G. Maul.** 1996. Adenovirus replication is coupled with the dynamic properties of the PML nuclear structure. Genes Dev. **10:**196–207.
20. **Dyck, J. A., G. G. Maul, W. H. Miller, J. D. Chen, A. Kakizuka, and R. M. Evans.** 1994. A novel macromolecular structure is a target of the promyelocyte-retinoic acid receptor oncoprotein. Cell **76:**333–343.
21. **Emiliani, S., W. Fischle, C. Van Lint, Y. Al-Abed, and E. Verdin.** 1998. Characterization of a human RPD3 ortholog, HDAC3. Proc. Natl. Acad. Sci. USA **95:**2795–2800.
22. **Everett, R. D. and G. G. Maul.** 1994. HSV-1 IE protein Vmw110 causes redistribution of PML. EMBO J. **13:**5062–5069.
23. **Gongora, D., G. David, L. Pintard, C. Tissot, T. D. Hua, A. Dejean, and N. Mechti.** 1997. Molecular cloning of a new interferon-induced PML nuclear body-associated protein. J. Biol. Chem. **272:**19457–19463.
24. **Grignani, F., S. De Matteis, C. Nervi, L. Tomassoni, V. Gelmetti, M. Cioce, M. Fanelli, M. Ruthardt, F. F. Ferrara, I. Zamir, C. Seiser, MA Lazar, S. Minucci, and P. G. Pelicci.** 1998. Fusion proteins of the retinoic acid receptor-alpha recruit histone deacetylase in promyelocytic leukaemia. Nature **391:**815–818.
25. **Grignani, F., P. F. Ferrucci, U. Testa, G. Talamo, M. Fagioli, M. Alcalay, A. Mencarelli, C. Peschle, I. Nicoletti, and P. G. Pelicci.** 1993. The acute promyelocytic leukemia-specific PML-RARalpha fusion protein inhibits differentiation and promotes survival of myeloid precursor cells. Cell **74:**423–431.
26. **Grignani, F., U. Testa, M. Fagioli, T. Barberi, R. Masciulli, G. Mariani, C. Peschle, and P. G. Pelicci.** 1995. Promyelocytic leukemia-specific PML-retinoic acid alpha receptor fusion protein interferes with erythroid differentiation of human erythroleukemia K562 cells. Cancer Res. **55:**440–443.
27. **Grisolano, J. L., R. L. Wesselschmidt, P. G. Pelicci, and T. J. Ley.** 1997. Altered myeloid development and acute leukemia in transgenic mice expressing PMLRARa under control of cathepsin G regulatory sequences. Blood **89:**376–387.
28. **Grunstein, M.** 1997. Histone acetylation in chromatin structure and transcription. Nature **389:**349–352.
29. **Guiochon-Mantel, A., F. J. Savouret, F. Quignon, K. Delaber, E. Milgrom, and H. de The.** 1995. Effect of PML and PML-RAR on the transactivation properties and subcellular distribution of steroid hormone receptors. Mol. Endocrinol. **9:**1791–1803.

30. **Hassig, C. A., J. K. Tong, T. C. Fleischer, T. Owa, P. G. Grable, D. E. Ayer, and S. L. Schreiber.** 1998. A role for histone deacetylase activity in HDAC1-mediated transcriptional repression. Proc. Natl. Acad. Sci. USA **95:**3519–3524.
31. **He, D., Z.-M. Mu, X. Le, J.-T. Hsieh, R.-C. Pong, L. W. K. Chung, and K.-S. Chang.** 1997. Adenovirus-mediated expression of PML suppresses growth and tumorigenicity of prostate cancer cells. Cancer Res. **57:**1868–1872.
32. **He, L. Z., F. Guidez, C. Tribioli, D. Peruzzi, M. Ruthardt, A. Zelent, and P. P. Pandolfi.** 1998. Distinct interactions of PML-RARalpha and PLZF-RARalpha with co-repressors determine differential responses to RA in APL. Nat. Genet. **18:**126–135.
33. **He, L. Z., C. Tribioli, R. Rivi, D. Peruzzi, P. G. Pelicci, V. Soares, G. Cattoretti, and P. P. Pandolphi.** 1997. Acute leukemia with promyelocytic features in PMLRARalpha transgenic mice. Proc. Natl. Acad. Sci. USA **94:**5302–5307.
34. **Kakizuka, A., W. H. J. Miller, K. Umesono, R. P. J. Warrell, S. R. Frankel, V. V. V. S. Murty, E. Dmitrovsky, and R. M. Evans.** 1991. Chromosomal translocation t(15;17) in human acute promyelocytic leukemia fuses RARalpha with a novel putative transcription factor, PML. Cell **66:**663–674.
35. **Kamitani, T., K. Kito, H. P. Nguyen, H. Wada, T. Fukuda-Kamitani, and E. T. Yeh.** 1998. Identification of three major sentrinization sites in PML. J. Biol. Chem. **273:**26675–26682.
36. **Kamitani, T., H. P. Nguyen, K. Kito, T. Fukuda-Kamitani, and E. T. Yeh.** 1998. Covalent modification of PML by the sentrin family of ubiquitin-like proteins. J. Biol. Chem. **273:**3117–3120.
37. **Karlseder, J., H. Rotheneder, and E. Wintersberger.** 1996. Interaction of Sp1 with the growth-and cell cycle-regulated transcription factor E2F. Mol. Cell. Biol. **16:**1659–1667.
38. **Koken, M. H. M., A. Reid, F. Quignon, M. K. Chelbi-Alix, J. M. Davies, J. H. S. Kabarowski, J. Zhu, S. Dong, S.-J. Chen, Z. Chen, C. C. Tan, J. Licht, S. Waxman, H. De Thé, and A. Zelent.** 1997. Leukemia-associated retinoic acid receptor alpha fusion partners, PML and PLZF, heterodimerize and colocalize to nuclear bodies. Proc. Natl. Acad. Sci. USA **94:**10255–10260.
39. **Laherty, C. D., W. M. Yang, J. M. Sun, J. R. Davie, E. Seto, and R. N. Eisenman.** 1997. Histone deacetylases associated with the mSin3 corepressor mediate mad transcriptional repression. Cell **89:**349–356.
40. **LaMorte, V. J., J. A. Dyck, R. L. Ochs, and R. M. Evans.** 1998. Localization of nascent RNA and CREB binding protein with the PML-containing nuclear body. Proc. Natl. Acad. Sci. USA **95:**4991–4996.
41. **Le, X.-F., S. Vallian, Z.-M. Mu, M.-C. Hung, and K.-S. Chang.** 1998. Recombinant PML adenovirus suppresses growth and tumorigenicity of human breast cancer cells by inducing $G_1$ cell cycle arrest and apoptosis. Oncogene **16:**1839–1849.
42. **Le, X. F., P. Yang, and K. S. Chang.** 1996. Analysis of the growth and transformation suppressor domains of promyelocytic leukemia gene, PML. J. Biol. Chem. **271:**130–135.
43. **Lee, D. Y., J. J. Hayes, D. Pruss, and A. P. Wolffe.** 1993. A positive role for histone acetylation in transcription factor access to nucleosomal DNA. Cell **72:**73–84.
44. **Li, H., C. Leo, J. Zhu, X. Wu, J. O'Neil, E-J. Park, J. D. Chen.** 2000. Sequestration and inhibition of Daxx-mediated transcription repression by PML. Mol. Cell. Biol. **20:**1784–1796.
45. **Lin, R. J., L. Nagy, S. Inoue, W. Shao, W. H. J. Miller, and R. M. Evans.** 1998. Role of the histone deacetylase complex in acute promyelocytic leukaemia. Nature **391:**811–814.
46. **Lin, S. Y., A. R. Black, D. Kostic, S. Pajovic, C. N. Hoover, and J. C. Azizkhan.** 1996. Cell cycle-regulated association of E2F1 and Sp1 is related to their functional interaction. Mol. Cell. Biol. **16:**1668–1675.
47. **Liu, J. H., Z. M. Mu, and K. S. Chang.** 1995. PML suppresses oncogenic transformation of NIH/3T3 cells by activated neu. J. Exp. Med. **181:**1965–1973.
48. **Luo, R. X., A. A. Postigo, and D. C. Dean.** 1998. Rb interacts with histone deacetylase to repress transcription. Cell **92:**463–473.
49. **Magnaghi-Jaulin, L., R. Groisman, P. Robin, S. Lorain, J. P. Le Villain, F. Troalen, D. Trouche, and B. Harel.** 1998. Retinoblastoma protein represses transcription by recruiting a histone deacetylase. Nature **391:**601–605.
50. **Mizzen, C. A., X. J. Yang, T. Kokubo, J. E. Brownell, A. J. Bannister, T. Owen-Hughes, J. Workman, L. Wang, S. L. Berger, T. Kouzarides, Y. Nakatani, and C. D. Allis.** 1996. The TAF(II)250 subunit of TFIID has histone acetyltransferase activity. Cell **87:**1261–1270.
51. **Mu, Z. M., K. V. Chin, J. H. Liu, G. Lozano, and K. S. Chang.** 1994. PML, a growth suppressor disrupted in acute promyelocytic leukemia. Mol. Cell. Biol. **14:**6858–6867.
52. **Mu, Z.-M., X.-F. Le, S. Vallian, A. B. Glassman, and K.-S. Chang.** 1997. Stable overexpression of PML alters regulation of cell cycle progression in HeLa cells. Carcinogenesis **18:**2063–2069.
53. **Muller, S., M. J. Matunis, and A. Dejean.** 1998. Conjugation with the ubiquitin-related modifier SUMO-1 regulates the partitioning of PML within the nucleus. EMBO J. **17:**61–70.
54. **Nagy, L., H. Y. Kao, D. Chakravarti, R. J. Lin, C. A. Hassig, D. E. Ayer, S. L. Schreiber, and R. M. Evans.** 1997. Nuclear receptor repression mediated by a complex containing SMRT, mSin3A, and histone deacetylase. Cell **89:**373–380.
55. **Ogryzko, V. V., R. L. Schiltz, V. Russanova, B. H. Howard, and Y. Nakatani.** 1996. The transcriptional coactivators p300 and CBP are histone acetyltransferases. Cell **87:**953–959.
56. **Pazin, M. J., and J. T. Kadonaga.** 1997. What's up and down with histone deacetylation and transcription? Cell **89:**325–328.
57. **Perez, A., P. Kastner, S. Sethi, Y. Lutz, C. Reibel, and P. Chambon.** 1993. PMLRAR homodimers: distinct DNA binding properties and heteromeric interactions with RXR. EMBO J. **12:**3171–3182.
58. **Quignon, F., F. De Bels, M. Koken, J. Feunteun, J. C. Ameisen, and H. de The.** 1998. PML induces a novel caspase-independent death process. Nat. Genet. **20:**259–265.
59. **Shao, W., M. Fanelli, F. F. Ferrara, R. Riccioni, A. Rosenauer, K. Davison, W. W. Lamph, S. Waxman, P. G. Pelicci, C. F. Lo, G. Avvisati, U. Testa, C. Peschle, C. Gambacorti-Passerini, C. Nervi, and W. H. Miller, Jr.** 1998. Arsenic trioxide as an inducer of apoptosis and loss of PML/RAR alpha protein in acute promyelocytic leukemia cells. J. Natl. Cancer Inst. **90:**124–133.
60. **Shen, Z. X., G. Q. Chen, J. H. Ni, X. S. Li, S. M. Xiong, Q. Y. Qiu, W. Tang, G. L. Sun, K. Q. Yang, Y. Chen, L. Zhou, Z. W. Fang, Y. T. Wang, J. Ma, P. Zhang, T. D. Zhang, S. J. Chen, Z. Chen, and Z. Y. Wang.** 1997. Use of arsenic trioxide ($As_2O_3$) in the treatment of acute promyelocytic leukemia (APL). II. Clinical efficacy and pharmacokinetics in relapsed patients. Blood **89:**3354–3360.
61. **Sherr, C. J.** 1994. G1 phase progression: cycling on cue. Cell **79:**551–555.
62. **Sternsdorf, T., K. Jensen, and H. Will.** 1997. Evidence for covalent modification of the nuclear dot-associated proteins PML and Sp100 by PIC1/SUMO-1. J. Cell Biol. **139:**1621–1634.
63. **Struhl, K.** 1998. Histone acetylation and transcriptional regulatory mechanisms. Genes Dev. **12:**599–606.
64. **Szekely, L., K. Pokrovskaja, W.-Q. Jiang, H. de Thé, N. Ringertz, and G. Klein.** 1996. The epstein-barr virus-encoded nuclear antigen ebna-5 accumulates in PML-containing bodies. J. Virol. **70:**2562–2568.
65. **Torchia, J., D. W. Rose, J. Inostroza, Y. Kamei, S. Westin, C. K. Glass, and M. G. Rosenfeld.** 1997. The transcriptional co-activator p/CIP binds CBP and mediates nuclear-receptor function. Nature **387:**677–684.
66. **Vallian, S., J. Gaken, E. B. Gingold, T. Kouzarides, K. S. Chang, and F. Farzaneh.** 1998. Regulation of Fos-mediated AP1 transcription by the promyelocytic leukemia protein. Oncogene **16:**2843–2853.
67. **Vallian, S., K. V. Chin, and K. S. Chang.** 1998. The promyelocytic leukemia protein interacts with Sp1 and inhibits its transactivation of the epidermal growth factor receptor promoter. Mol. Cell. Biol. **18:**7147–7156.
68. **Vallian, S., J. A. Gaken, I. D. Trayner, E. B. Gingold, T. Kouzarides, K.-S. Chang, and F. Farzaneh.** 1997. Transcriptional repression by the promyelocytic leukemia protein, PML. Exp. Cell Res. **237:**371–382.
69. **Vettese-Dadey, M., P. A. Grant, T. R. Hebbes, C. Crane, C. D. Allis, and J. L. Workman.** 1996. Acetylation of histone H4 plays a primary role in enhancing transcription factor binding to nucleosomal DNA in vitro. EMBO J. **15**: 2508–2518.
70. **Wade, P. A., and A. P. Wolffe.** 1997. Histone acetyltransferases in control. Curr. Biol. **7:**R82–R84.
71. **Wang, Z. G., L. Delva, M. Gaboli, R. Rivi, M. Giorgio, C. Cordon-Cardo, F. Grosveld, and P. P. Pandolfi.** 1998. Role of PML in cell growth and the retinoic acid pathway. Science **279:**1547–1551.
72. **Wang, Z. G., R. Rivi, L. Delva, A. Konig, D. A. Scheinberg, P. Gambacorti, J. L. Gabrilove, R. P. J. Warrell, and P. P. Pandolfi.** 1998. Arsenic trioxide and melarsoprol induce programmed cell death in myeloid leukemia cell lines and function in a PML and PML-RARalpha independent manner. Blood **92:**1497–1504.
73. **Wang, Z. G., D. Ruggero, S. Ronchetti, S. Zhong, M. Gaboli, R. Rivi, and P. P. Pandolfi.** 1998. PML is essential for multiple apoptotic pathways. Nat. Genet. **20:**266–272.
74. **Warrell, R. P. J., S. R. Frankel, W. H. J. Miller, D. A. Scheinberg, L. M. Itri, W. N. Hittelman, R. Vyas, M. Andreeff, A. Tafuri, A. Jakubowski, et al.** 1991. Differentiation therapy of acute promyelocytic leukemia with tretinoin (all-trans-retinoic acid). N. Engl. J. Med. **324:**1385–1393.
75. **Weis, K., S. Rambaud, C. Lavau, J. Jansen, T. Carvalho, M. Carmo-Fonseca, A. Lamond, and A. Dejean.** 1994. Retinoic acid regulates aberrant nuclear localization of PML-RARalpha in acute promyelocytic leukemia cells. Cell **76:**345–356.
76. **Yang, W. M., Y. L. Yao, J. M. Sun, J. R. Davie, and E. Seto.** 1997. Isolation and characterization of cDNAs corresponding to an additional member of the human histone deacetylase gene family. J. Biol. Chem. **27:**28001–28007.
77. **Yoshida, H., K. Kitamura, K. Tanaka, S. Omura, T. Miyazaki, T. Hachiya, R. Ohno, and T. Naoe.** 1996. Accelerated degradation of PML-retinoic acid receptor alpha (PML-RARA) oncoprotein by all-trans-retinoic acid in acute promyelocytic leukemia: possible role of the proteasome pathway. Cancer Res. **56:**2945–2948.