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The three members of the pocket proteins family share the ability to repress E2F activity through recruitment of a histone deacetylase

ROGER FERREIRA, LAURA MAGNAGHI-JAULIN, PHILIPPE ROBIN, ANNICK HAREL-BELLAN*, AND DIDIER TROUCHE†

Laboratoire "Oncogénèse, Différenciation et Transduction du Signal," Centre National de la Recherche Scientifique Unité Propre de Recherche 9079, IFC-01, 94801 Villejuif, France

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ABSTRACT **The transcription factor E2F plays a major role in cell cycle control in mammalian cells. E2F binding sites, which are present in the promoters of a variety of genes required for S phase, shift from a negative to a positive role in transcription at the commitment point, a crucial point in G_1 that precedes the G_1/S transition. Before the commitment point, E2F activity is repressed by members of the pocket proteins family. This repression is believed to be crucial for the proper control of cell growth. We have previously shown that Rb, the founding member of the pocket proteins family, represses E2F1 activity by recruiting the histone deacetylase HDAC1. Here, we show that the two other members of the pocket proteins family, p107 and p130, also are able to interact physically with HDAC1 in live cells. HDAC1 interacts with p107 and Rb through an "LXCXE"-like motif, similar to that used by viral transforming proteins to bind and inactivate pocket proteins. Indeed, we find that the viral transforming protein E1A competes with HDAC1 for p107 interaction. We also demonstrate that p107 is able to interact simultaneously with HDAC1 and E2F4, suggesting a model in which p107 recruits HDAC1 to repress E2F sites. Indeed, we demonstrate that histone deacetylase activity is involved in the p107- or p130-induced repression of E2F4. Taken together, our data suggest that all members of the E2F family are regulated in early G_1 by similar complexes, containing a pocket protein and the histone deacetylase HDAC1.**

The E2F family of transcription factors includes key regulators of the mammalian cell cycle. E2F binding sites are found in the promoters of genes that are expressed at the G_1/S transition and are required for S phase progression, such as DNA polymerase α (1, 2). The active E2F transcription factor is a heterodimer comprised of one of the five "E2F" proteins and one of the two "DP" proteins characterized to date (3, 4). When bound to E2F sites, the E2F/DP heterodimers activate transcription through a transactivation domain located in the E2F protein.

During the G_1 phase of the cell cycle, E2F activity is repressed by members of the "pocket" proteins family. This family is comprised of the product of the retinoblastoma susceptibility gene (the Rb protein) and the p107 and p130 proteins (5). These three proteins show strong homologies in the A/B pocket domain. The A/B pocket is the target of viral transforming proteins, such as E1A or SV40 T antigen, that inactivate pocket proteins through a direct physical association (5). Pocket proteins interact directly with the E2F activation domain and repress its activity. Rb interacts more specifically with E2F1, E2F2, and E2F3; p107, with E2F4; and p130, with E2F4 and E2F5 (4–6).

Pocket proteins are regulated by phosphorylation. During most of the G_1 phase of the cell cycle, pocket proteins are in a hypophosphorylated and active form. They are phosphorylated at the end of G_1 by cyclin-dependent kinases (cyclin D-cdk4 or cdk6 or cyclin E-cdk2) (7–9). This phosphorylation leads to their inactivation and to the release of "free" E2F, which in turn activates the transcription of its target genes.

The Rb protein is mutated in up to 30% of human tumors. This is not the case for p107 and p130, although recently p130 also has been shown to be mutated in a small cell lung carcinoma (10). In addition, some viral transforming proteins inactivate pocket proteins to induce quiescent cells to enter S phase, thereby enabling viral DNA to be replicated. These data indicate that pocket proteins play a major role as negative regulators of cell progression toward the S phase. Indeed, overexpression of pocket proteins in some Rb-negative cell lines induces an arrest in G_1 (11–13). E2F appears to be a major target for the growth inhibitory functions of pocket proteins because, for example, overexpression of E2F1 relieves Rb induced growth arrest (14).

Repression of E2F by pocket proteins is likely to involve the masking of the E2F activation domain. However, this is clearly not the only mechanism. The E2F/pocket protein complexes bound to E2F sites actively repress transcription (15, 16). Indeed, during the G_1 phase of the cell cycle, E2F sites act as silencing elements (17, 18). This "active" repression is mediated by the pocket protein—indeed, pocket proteins are able to repress transcription when tethered to a promoter through a heterologous DNA binding domain (15, 16, 19). The transcriptional repression domain of pocket proteins corresponds to the A/B pocket (16, 20).

Several results indicate that this active transcriptional repression by pocket proteins plays an important role in cell cycle control (21): inactivation of pocket proteins, whether through phosphorylation by cyclin-cdk or by viral transforming proteins or else by mutations found in tumors, not only results in the loss of E2F binding but also in the loss of active repression (19, 22, 23). Furthermore, a mutant E2F1, deleted for the transactivation domain and that cannot activate E2F site-containing promoters nor recruit Rb to repress transcription, displays a significant transforming potential. This mutant thus seems to act as a transdominant negative mutant for the endogenous repressive E2F/pocket protein complexes (24). Conversely, a chimeric protein in which E2F1 is fused to the transcriptional repressor domain of Rb arrests the growth of SAOS2 cells (19). Active repression by E2F/pocket protein

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Abbreviations: HAT, histone acetyl transferase; TSA, trichostatin A; CMV, cytomegalovirus.

*To whom reprint requests should be addressed. e-mail: ahbellan@vjf.cnrs.fr.
†Present address: LBME, UPR 9006 CNRS, 118 Route de Narbonne, 31 062 Toulouse Cedex, France.

complexes could also explain why, paradoxically, E2F1 can be considered as an oncogene as well as an antioncogene (25).

We and others recently have shown that Rb represses transcription, at least in part, by recruiting a histone deacetylase, the HDAC1 protein (26–28). Histone acetyl transferases (HAT) and histone deacetylases acetylate or deacetylate core histone tails that are protruding out of the nucleosome (29, 30). Histone acetylation is thought to weaken the interaction of histone N-terminal tails with DNA, thus opening up the chromatin and increasing accessibility for activating transcription factors. In line with this model, some transcriptional coactivators possess HAT activity (31–34). Conversely, some corepressors, including Rb, are associated with histone deacetylase activity (26–28, 35).

Transcriptional repression by all three pocket proteins has many features in common (23). Furthermore, Rb interacts with HDAC1 through the A/B pocket (26–28), which largely is conserved in p107 and p130, suggesting that, like Rb, p107 and p130 repress E2F activity by recruiting HDAC1. However, Luo *et al.* (28) reported that p107 was not able to interact with HDAC1, nor was HDAC1 able to cooperate functionally with p107.

In disagreement with this report, we found that, like Rb, both p107 and p130 physically interact with HDAC1. Furthermore, p107 is able to interact with E2F4 and HDAC1 at the same time, suggesting that it can recruit HDAC1 to E2F4-containing heterodimers. In addition, inhibition of cellular histone deacetylases relieved repression of E2F4 by p107 or p130. Taken together, these results suggest that all members of the E2F family of transcription factors are regulated during G_1 by similar complexes, each involving a pocket protein and the histone deacetylase HDAC1.

MATERIALS AND METHODS

Plasmids. pE2Fluc reporter vector and expression vectors for E2F4 (pcDNA3 Myc-E2F4), DP2, p130, and cyclin-cdks [each controlled by a cytomegalovirus (CMV) promoter] were kind gifts from N. La Thangue, C. Sardet, J. Lees, R. Bernards, and S. A. Leibovitch, respectively. pCMV and pGEX2T RB (379–928) were kind gifts from W. Kaelin. p107 expression vector, pGEX2TK p107 (252–816), and pGEX2TK p107 (252–816) C713F were kind gifts from D. Livingston. pGEX 2T E1A13S and pCMV HA were kind gifts from T. Kouzarides. Details of construction of pT7-HDAC1, pT7-HDAC1ΔIACEE, and pCMV HA-HDAC1 are available on request.

Cell Culture, Transfections, and Luciferase Assay. Jurkat and U937 cells were maintained in RPMI medium 1640 and Saos-2 cells in McCOY's 5A medium, each of them supplemented with fetal calf serum and antibiotics. Transfections were carried out by calcium/phosphate coprecipitation. Luciferase activity was assayed by using reagents from Promega according to the manufacturer's instructions, and β-galactosidase activity was quantified by using a kit from Tropix (Bedford, MA).

Immunoprecipitations. For experiments on endogenous proteins, total cell extracts were prepared from 1.3×10^9 Jurkat cells. Cells were resuspended in 6 ml of lysis buffer (50 mM Tris, pH 8.0/300 mM NaCl/10 mM $MgCl_2$/0.4% NP40 and protease inhibitors) and incubated 15 min on ice. The supernatant was cleared by centrifugation, mixed with 6 ml of dilution buffer (50 mM Tris, pH 8.0/0.4% NP40) and immunoprecipitated by using standard procedures. Transfected cells (SAOS) were immunoprecipitated as described (26, 27). Anti-p107 (SD9) and anti p130 (C20) antibodies were purchased from Santa Cruz Biotechnology, anti-HA epitope (12CA5) and anti-myc epitope (9E10) were purchased from Boehringer Mannheim, and anti-Flag (M2) was from Kodak.

Deacetylase Assay. A peptide corresponding to the N-terminal tail of histone H4 (26) was labeled according to the method of Taunton *et al.* (37) and purified by HPLC. Immunoprecipitates or GST pull-downs were washed once with TBS (20 mM Tris, pH 8.0/150 mM NaCl) and were assayed for histone deacetylase activity as described (26). All samples were assayed in duplicate.

GST Pull-Down Assay. GST, GST–E1A 13S, GST-RB, GST-p107, and GST-p107 713C-F coated beads were prepared as reported (38). Purified GST-E1A 13S protein was eluted in TBS (pH 8.0) in the presence of 10 mM reduced glutathione and was dialyzed against TBS supplemented with 10% glycerol.

GST pull-downs with labeled HDAC1 were performed as described (26, 27). GST and GST-p107 coated beads also were used to retain a deacetylase activity from nuclear extracts prepared from Jurkat cells as described (39). In this case, beads were blocked for 10 min with 20 μl of rabbit reticulocyte lysate and then incubated for 1 h at 4°C with 400 μl of nuclear extracts in buffer D from ref. 40. Beads then were washed three times, and deacetylase activity was assayed as described above.

Western Blot Analysis. Immunoprecipitated proteins were loaded onto 8% SDS/PAGE gels before electrophoretic transfer onto nitrocellulose membranes. Western blotting was performed by using an ECL kit (Amersham) according to the manufacturer's instructions.

RESULTS AND DISCUSSION

p107 and p130 Interact with HDAC1 Through the Pocket Domain. We and others have shown that Rb is able to interact physically with HDAC1 through its pocket domain (26–28). The pocket domains of p107 and p130 are highly homologous to that of Rb, suggesting that p107 and p130 could also interact with HDAC1. Indeed, p130 immunoprecipitation from transfected cells resulted in the coretention of a large proportion of cotransfected HA-HDAC1 (Fig. 1*A*, lane 7). The coimmunoprecipitation was specific; HA-HDAC1 was not detected with an irrelevant antibody (Fig. 1, lane 3). Furthermore, when the p130 expression vector was omitted from the transfection, immunoprecipitation using anti-p130 antibody resulted in a weak HA-HDAC1 band (Fig. 1, lane 9), which is likely to be caused by a physical interaction between transfected HA-HDAC1 and endogenous p130. Similarly, immunoprecipitation of transfected p107 resulted in the coretention of coexpressed HA-HDAC1 (Fig. 1, lane 2).

This result stands in contrast to those described by Luo *et al.* (28), who were unable to coimmunoprecipitate p107 and HDAC1. To further demonstrate the interaction between p107 and HDAC1, we performed GST pull-down experiments (Fig. 1*B*). *In vitro*-translated ^{35}S-labeled HDAC1 was incubated with beads coated with bacterially produced GST-p107, GST-Rb fusion proteins, or GST as a control. HDAC1 was retained specifically on p107 beads and not on control GST beads. The amount of HDAC1 retained on p107 beads was similar to that retained on Rb beads (Fig. 1*B*), indicating that HDAC1 can interact with similar affinities with p107 or Rb. Binding of HDAC1 to a mutant of p107 in which the pocket is inactivated (p107 m) was reduced to background levels (Fig. 1*C*), indicating that the integrity of the A/B pocket is required for the interaction between p107 and HDAC1. Taken together, these data indicate that p107 and p130 can interact with HDAC1.

HDAC1 Interacts with p107 Through an LXCXE-Like Motif. The interaction between HDAC1 and p107 could be detected *in vitro* by using recombinant proteins (Fig. 1*B*), strongly suggesting that the interaction is direct. We have shown previously that Rb can interact directly with HDAC1 through an LXCXE-like motif located in the C terminus of HDAC1 (26). The LXCXE motif is a well characterized pocket protein-binding sequence through which viral transforming proteins bind and inactivate the three members of the pocket proteins family. To test whether this motif is involved in the p107/HDAC1 interaction, a GST pull-down analysis was per-

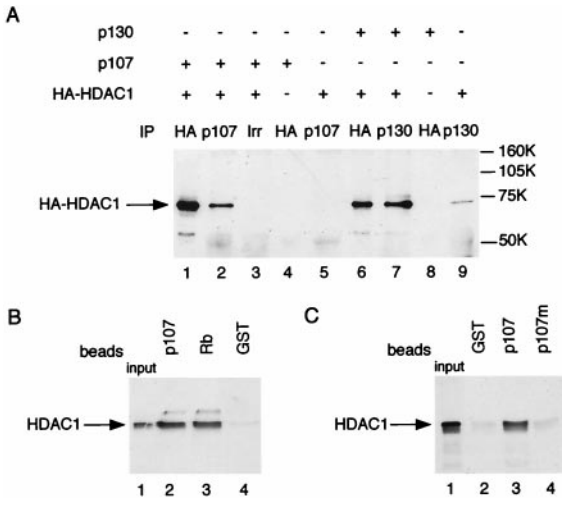

FIG. 1. p107 and p130 interact with HDAC1 (*A*) p107 and p130 interact with HDAC1 in live cells. Total cell extracts of SAOS2 cells transfected with the indicated expression vectors were immunoprecipitated with the indicated antibody (Irr: anti-Flag antibody). The amount of CMV promoter in the transfection was kept constant by using empty vectors. Immunoprecipitates were assayed for the presence of transfected HA-HDAC1 by Western blot by using the anti-HA antibody. In lanes 1, 4, 6, and 8, only one-third of immunoprecipitates was loaded onto the gel. (*B*) p107 interacts with HDAC1 *in vitro*. ^{35}S-labeled, *in vitro*-translated HDAC1 was subjected to a GST pull-down analysis with beads harboring the indicated GST fusion protein. Bound proteins were then separated by SDS/PAGE analysis and visualized by autoradiography. In lane 1, 10% of the input HDAC1 was loaded directly onto the gel. (*C*) The pocket domain of p107 is required for binding to HDAC1. Labeled, *in vitro*-translated HDAC1 was subjected to a GST pull-down analysis with beads harboring either wild-type p107 (lane 3), a point mutant of p107 (p107 m, p107 713 C-F) (lane 4), or GST as a control (lane 2).

formed by using a mutant of HDAC1 in which this motif has been deleted (HDAC1 ΔIACEE; Fig. 2*A*). As expected, this

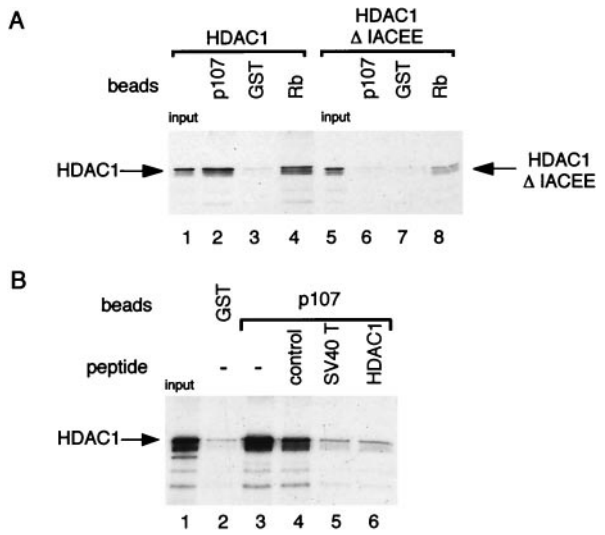

FIG. 2. p107 interacts with the LXCXE-like motif of HDAC1 (A) The IACEE sequence of HDAC1 is required for p107 binding. *In vitro*-translated, labeled HDAC1 (lanes 1–4) or HDAC1 ΔIACEE (lanes 5–8) was subjected to a GST pull-down analysis by using beads covered with the indicated GST fusion protein. (*B*) LXCXE motif-containing peptides inhibit p107/HDAC1 interaction. *In vitro*-translated labeled HDAC1 was subjected to a GST pull-down analysis by using beads covered with GST (lane 2) or GST-p107 fusion protein. Before GST pull-down analysis, beads were incubated without (lane 3) or with 15 μg of control (lane 4), SV40 T (lane 5), or HDAC1 (lane 6) peptide.

mutant showed a reduced ability to interact with GST-Rb beads. When assayed on GST-p107 beads, binding of this mutant was reduced to background levels, indicating that the "IACEE" sequence from HDAC1 is required for binding to p107.

We further investigated the involvement of the LXCXE motif by competition experiments by using synthetic peptides (Fig. 2*B*). An irrelevant peptide did not compete significantly with HDAC1 for binding to p107. Competition with a peptide from the C terminus of HDAC1 containing the IACEE element leads to a decrease of HDAC1 binding, indicating that this sequence of HDAC1 is sufficient for the interaction with p107. Furthermore, a peptide harboring the LXCXE-containing Rb binding site from the SV40 T antigen was also a good competitor. The LXCXE motif in the latter peptide is derived from an unrelated protein, so this result greatly strengthens the conclusion that the C-terminal IACEE sequence of HDAC1 functions like an LXCXE-containing pocket protein-binding motif.

p107 and p130 Associate with a Cellular Histone Deacetylase Activity. The experiments described above indicate that p107 and p130 can interact with HDAC1. We thus reasoned that they could repress E2F activity by recruiting histone deacetylase activity to E2F containing promoters. Histone deacetylase activity is not carried by the HDAC1 protein alone but rather by a complex containing other proteins, including sin3 and p48. To show that p107 and p130 are associated with an histone deacetylase activity, we immunoprecipitated endogenous p107 or p130, and we assayed immunoprecipitates for the presence of deacetylase activity (Fig. 3). Immunoprecipitation of both p107 (Fig. 3*A*) and p130 (Fig. 3*B*) resulted in the coimmunoprecipitation of trichostatin A-sensitive deacetylase activity. This retention was specific; no activity could be detected when extracts were immunoprecipitated with an irrelevant antibody. This result indicates that endogenous p107 and p130 are physically associated with histone deacetylase activity.

In addition, beads covered with GST-p107 fusion proteins were able to retain high levels of deacetylase activity from nuclear extracts (Fig. 3*C*). This retention was specific; no deacetylase activity was observed when control GST beads were used. Preincubation of GST-p107 beads with the LXCXE-containing SV40 T antigen peptide led to a marked decrease in their ability to retain deacetylase activity. Indeed, in the presence of this peptide, deacetylase activity associated with GST-p107 decreased to near background levels, indicating that the SV40 peptide disrupted most, if not all, of the p107/deacetylases complexes. These data indicate that the histone deacetylase activity associates with p107 through a cellular protein harboring an LXCXE-like motif. Results from Fig. 2 suggest that this cellular protein is likely to be HDAC1.

p107 Interacts Simultaneously with HDAC1 and E2F4. p107 is able to bind to E2F4-containing heterodimers and to repress transcription once recruited to promoters (23). Our working hypothesis is that this repression is due to the recruitment of histone deacetylase activity by the E2F4-p107 complex. This model requires that p107 can contact E2F4 and HDAC1 at the same time. E2F4 immunoprecipitates from transfected cells contained a significant proportion of cotransfected HDAC1 in the presence of exogenous p107 (Fig. 4*A*, lane 2). This coretention was specific; no HDAC1 could be detected when E2F4 expression vector was omitted from the transfection (Fig. 4*A*, lane 3). Thus, transfected E2F4 and HDAC1 are part of the same multimolecular complex in live cells. In addition, in the absence of exogenous p107 proteins, the amount of HDAC1 coimmunoprecipitated with E2F4 decreased significantly (Fig. 4*A*, lane 6), a decrease that is not due to a lower expression of the transfected HDAC1 (Fig. 4*A*, compare lanes 1 and 5). This result suggests that the interaction between E2F4 and HDAC1 is mediated through p107. The low level of HDAC1 retained

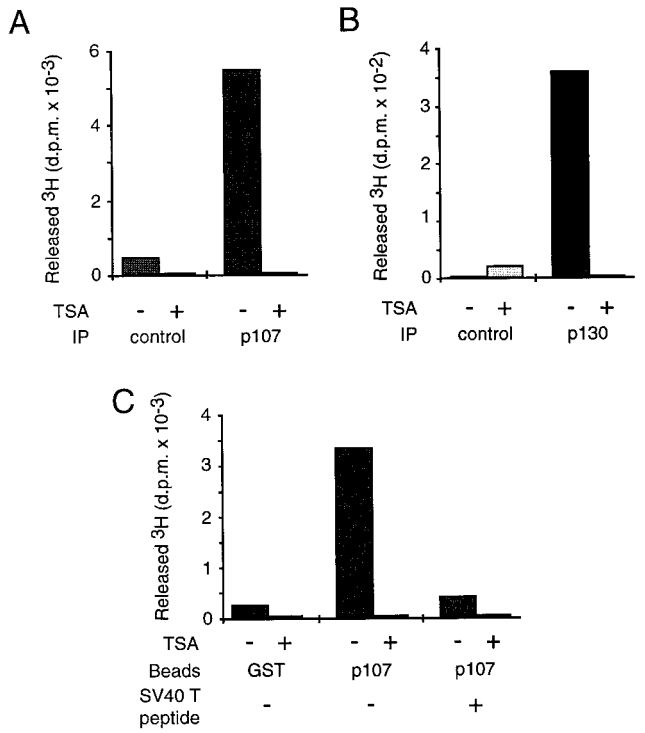

FIG. 3. p107 and p130 interact with a cellular histone deacetylase. (*A*) p107 is associated physically with a histone deacetylase. Nuclear extracts from U937 cells were immunoprecipitated with an anti-p107 (p107) or anti-HA (control) antibodies. Immunoprecipitates then were assayed for the presence of histone deacetylase activity. (*B*) p130 is associated physically with a histone deacetylase. Whole cell extracts from Jurkat cells were immunoprecipitated with anti-p130 (p130) or anti-HA (control) antibodies. Immunoprecipitates then were assayed for the presence of histone deacetylase activity. (*C*) p107 interacts with a cellular histone deacetylase through an LXCXE-like motif. Nuclear extracts from Jurkat cells were subjected to a GST pull-down analysis with beads covered with the indicated GST fusion protein. Before the incubation with nuclear extracts, beads were incubated with 100 μg of SV40 T peptide where indicated or 100 μg of control peptide. Bound proteins were assayed for the presence of deacetylase activity.

in the absence of transfected p107 is likely to be due to endogenous p107. This experiment indicates that p107 is able to bind E2F4 and HDAC1 simultaneously, leading to the formation of a ternary complex.

p107 and p130 Repress Transcription Through a Histone Deacetylase. To test whether the E2F4/p107/HDAC1 ternary

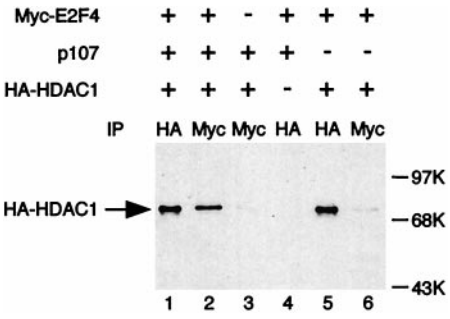

FIG. 4. p107 interacts with E2F4 and HDAC1 simultaneously. Total cell extracts of SAOS2 cells transfected with the indicated expression vectors were immunoprecipitated with the indicated antibody. The amount of CMV promoter was kept constant in the transfection by using empty vectors. Immunoprecipitates were assayed for the presence of transfected HA-HDAC1 by Western blot by using the anti-HA antibody. In lanes 1, 4, and 5, only one-third of the immunoprecipitates was loaded onto the gel.

complex is responsible for the repression of E2F4 activity by p107, we assessed the ability of a histone deacetylase inhibitor, trichostatin A (TSA), to relieve transcriptional repression by p107 in a transient transfection assay (Fig. 5*A*). E2F4 activity was induced slightly by TSA in the absence of exogenous pocket protein expression, likely because TSA relieved repression by endogenous pocket proteins through an histone deacetylase. p107 expression resulted in a dose-dependent repression of E2F4 activity (Fig. 5*A*, black bars). In the presence of TSA, this repression was significantly reduced, indicating that a histone deacetylase is involved. TSA did not completely abolish the repression by p107; p107 thus could repress in part by a histone deacetylase-independent mechanism, such as direct masking of the E2F4 transactivation domain. Similarly, E2F4 repression by p130 was relieved by TSA treatment (Fig. 5*B*), indicating that histone deacetylases also are involved in p130-induced repression of E2F activity.

These results, together with previously published results (26–28), indicate that transcriptional repression by all three pocket proteins is relieved by TSA treatment. Direct comparison of our previous results on repression of E2F1 activity by Rb (26) and results from Fig. 5 for p130 and p107 is difficult because, in our hands, E2F1 activates transcription far more efficiently than E2F4.

There is a significant residual repression by all three pocket proteins in the presence of TSA, indicating that these proteins also repress E2F activity by a histone deacetylase-independent mechanism, such as masking of the transcriptional activation domain; notwithstanding, our results indicate that a histone deacetylase activity is involved in transcriptional repression by all three pocket proteins.

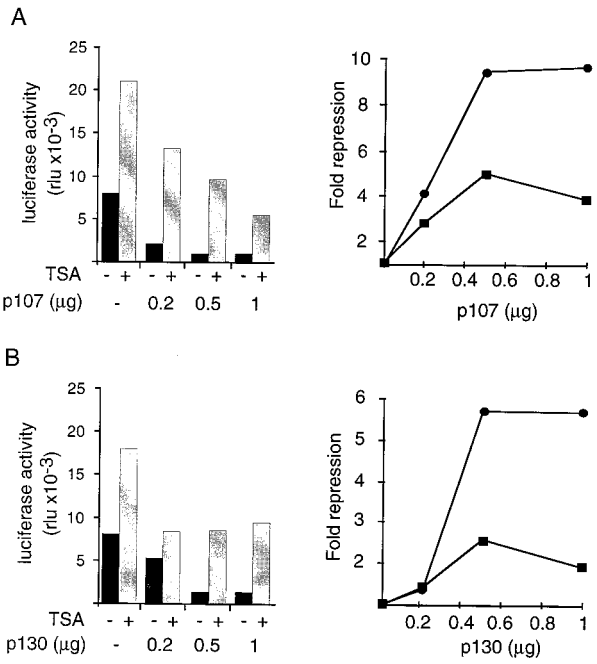

FIG. 5. p107 and p130 repress E2F4 activity through a histone deacetylase. (*A*) SAOS2 cells were transfected transiently with 2 μg of E2F-luc reporter vector, 100 ng of pcDNA3 myc-E2F4, 100 ng of pCMV DP2, and the indicated dose of pCMV p107. The amount of CMV promoter was kept constant by using empty vectors. TSA (100 ng/ml) was added (gray bars) or not (black bars) 8 h later, and luciferase activity was assayed 24 h after transfection. Result of a typical experiment is shown on the *Left*. The mean of fold repression by p107 calculated from various independent experiments in the absence (circles) or in the presence (squares) of TSA is shown on the *Right*. (*B*) SAOS2 cells were transiently transfected and assayed as in *A* with the indicated dose of CMV p130.

Binding to HDAC1 Is Disrupted by Phosphorylation of p107 and by Viral Transforming Proteins. During most of the G_1 phase, pocket proteins keep cells from entering the cell cycle at least in part by repressing E2F activity. Phosphorylation of pocket proteins by cyclin-cdk complexes or expression of viral transforming proteins allows cells to progress toward S phase by relieving this repression. Indeed, the active transcriptional repression by pocket proteins is inhibited by phosphorylation and by viral transforming proteins (19, 22, 23). Results from the above or previously published data (26–28) indicate that the interaction of pocket proteins with the histone deacetylase HDAC1 is involved in this repression, suggesting that it might be disrupted by pocket protein phosphorylation or by viral transforming proteins. During progression in G_1, p107 is phosphorylated mainly by cyclin D-dependent kinases, and this phosphorylation leads to a protein unable to bind E2F4 and to repress E2F activity (8). By coimmunoprecipitation experiments, we found that expression of cyclin D-cdk4 also decreased the physical interaction between transfected p107 and HDAC1 (Fig. 6*A*, *Upper*, compare lanes 1 and 6). This decrease is not due to a lower expression of transfected HDAC1 (Fig. 6*A*, *Lower*) or to a change in the amount of immunoprecipitated p107 (data not shown). Coexpression in cells of cyclin E-cdk2 did not result in any diminution of the p107/HDAC1 interaction (Fig. 6*A*, lanes 3 and 4). However, phosphorylation of recombinant p107 by a large amount of purified cyclin E-cdk2 *in vitro* strongly affected the interaction (data not shown), suggesting that the effect of cyclin-cdks on the p107/HDAC1 interaction is direct.

Similarly, pretreatment of GST-p107 beads or GST-Rb beads with E1A resulted in the abolition of HDAC1 binding in a GST pull-down assay (Fig. 6*B*). Taken together, these data indicate that inactivation of pocket proteins, either by phosphorylation by cyclin D-dependent kinases or by interaction with viral transforming proteins, results in the loss of HDAC1 binding. Moreover, treatment of GST-p107 beads with E1A also diminished their ability to retain histone deacetylase activity from cell extracts (Fig. 6*C*), indicating that the physical interaction between cellular histone deacetylases and p107 also depends on the presence of an active A/B pocket.

Taken together, these results indicate that binding of pocket proteins to the histone deacetylase HDAC1 is disrupted when cells are induced to progress through the commitment point, suggesting that binding to HDAC1 is indeed critical for the ability of pocket proteins to suppress cell proliferation.

Our results show that, like Rb, full length wild-type p107 interacts strongly with HDAC1 both *in vitro* and in transfected cells (Fig. 1). Indeed, HDAC1 contacts p107 and Rb through a $\ll$ LXCXE $\gg$ motif used by viral transforming proteins to interact with all three pocket proteins (Fig. 2; ref. 26). The discrepancy between these results and data reported by Luo *et al.* (28), who could not detect any interaction between p107 and HDAC1 in transfected cells, might be explained by a cell type specificity. Note, however, that those authors used a fusion protein between the Gal4 DNA binding domain and p107. A reasonable hypothesis is thus that this fusion protein is not in the proper conformation to interact with HDAC1. Indeed, transcriptional repression by GAL4-p107 was insensitive to trichostatin treatment, suggesting that p107 represses transcription partly by a deacetylase-independent mechanism (28). In agreement with this interpretation, we found that a significant proportion of transcriptional repression of E2F4 activity by p107 was resistant to trichostatin (Fig. 5). Nevertheless, our results indicate that a histone deacetylase activity

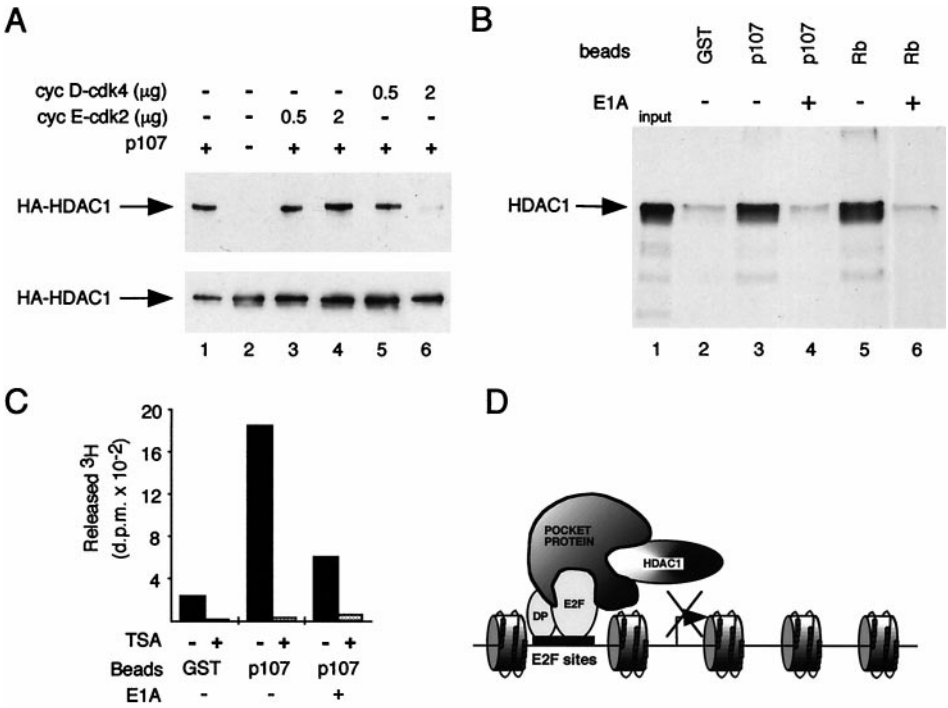

FIG. 6. Inactivation of p107 results in the loss of HDAC1 binding. (*A*) Cyclin D-dependent kinase expression inhibits the p107/HDAC1 interaction. Total cell extracts of SAOS2 cells transfected with the HA-HDAC1 and the p107, cyclin D1, cdk4, cyclin E, or cdk2 expression vectors as indicated were immunoprecipitated with the anti-p107 antibody (*Upper*) or the anti-HA antibody (*Lower*). Immunoprecipitates were assayed for the presence of HA-HDAC1 by Western blot by using the anti-HA antibody. (*B*) E1A competes with HDAC1 for p107 binding. *In vitro*-translated, labeled HDAC1 was subjected to a GST pull-down analysis by using beads covered with the indicated GST fusion protein. Before the GST pull down, p107 beads (lanes 3 and 4) and Rb beads (lanes 5 and 6) were incubated with or without purified recombinant GST-E1A 13S (50 ng) as indicated. (*C*) E1A competes with the cellular histone deacetylase for binding to p107 Jurkat nuclear extracts were subjected to a GST pull-down analysis by using beads covered with the indicated GST fusion protein. Before the GST pull-down, GST-p107 beads were incubated with or without purified GST-E1A 13S protein (300 ng) as indicated. Bound proteins were assayed for the presence of histone deacetylase activity. (*D*) Model of transcriptional repression through E2F sites. A heterodimer comprised of one E2F and one DP protein recognizes the E2F site. The E2F protein binds to a member of the pocket protein family, which recruits the HDAC1 deacetylase to the promoter to block transcription.

is involved in transcriptional repression by p107 and that p107 has the ability to recruit HDAC1 to E2F4 (Figs. 4 and 5).

Our work, together with previously published works (26–28), thus suggests that a common mechanism is used by the cell to repress the activity of all E2F members during most of the G_1 phase of the cell cycle. This repression could involve the formation, on E2F sites, of a multimolecular complex containing an E2F/DP heterodimer, a pocket protein and the histone deacetylase HDAC1 (Fig. 6*D*). Hence, our data suggest that, whatever the precise molecular composition of the E2F heterodimer that forms on a particular E2F site or the identity of the pocket protein that is recruited by the E2F protein, most E2F sites are regulated in similar ways during G_1.

E2F is a major target of pocket proteins, so the interaction of pocket proteins with HDAC1 could be critical for their ability to suppress cell growth. In agreement with this model, inactivation of pocket protein function by mutations (26, 27), viral transforming proteins (ref. 27 and this study) or phosphorylation (ref. 28 and this study) leads to the loss of their ability to bind HDAC1. The loss of the interaction between pocket proteins and HDAC1 thus could be the molecular process corresponding to the commitment point. If this hypothesis is correct, HDAC1 could play a major role in the control of cell growth. Inactivation of HDAC1 function, or of its ability to interact with Rb, could lead to cell transformation and oncogenesis.

Another important question that is raised by these results is how the transcriptional blockage previously induced by HDAC1 is relieved after the commitment point. Of interest, E2F1 is able to interact with the CBP protein (36), which itself possesses HAT activity and which also is associated with other HATs (31, 33, 34). These data suggest a model in which, at the commitment point, the E2F1/Rb/HDAC1 repressor complex would be replaced by an activating complex harboring HAT activity. It is thus tempting to speculate that, after the inactivation of pocket proteins, the other E2Fs are also capable of recruiting proteins possessing HAT activity to promoters. Additional studies will provide new evidence to confirm or invalidate this hypothesis. Whatever the outcome of those experiments, more and more data are emerging that suggest that the equilibrium between histone acetyl transferases and histone deacetylases is critical for proper cell cycle control.

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