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The Bovine Herpesvirus 1 Immediate-Early Protein (bICP0) Associates with Histone Deacetylase 1 To Activate Transcription

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Infected-cell protein 0 encoded by bovine herpesvirus 1 (BHV-1) (bICP0) is necessary for efficient productive infection, in large part, because it activates all 3 classes of BHV-1 genes (U. V. Wirth, C. Fraefel, B. Vogt, C. Vlcek, V. Paces, and M. Schwyzer, J. Virol. 66:2763–2772, 1992). Although bICP0 is believed to be a functional homologue of herpes simplex virus type 1-encoded ICP0, the only well-conserved domain between the proteins is a zinc ring finger located near the amino terminus of both proteins. Our previous studies demonstrated that bICP0 is toxic to transfected cells but does not appear to directly induce apoptosis (Inman, M., Y. Zhang, V. Geiser, and C. Jones, J. Gen. Virol. 82:483–492, 2001). C-terminal sequences in the last 320 amino acids of bICP0 mediate subcellular localization. Mutagenesis of the zinc ring finger within bICP0 revealed that this domain was important for transcriptional activation. In this study, we demonstrate that bICP0 interacts with histone deacetylase 1 (HDAC1), which results in activation of a simple promoter containing four consensus Myc-Max binding sites. The interaction between bICP0 and HDAC1 correlated with inhibition of Mad-dependent transcriptional repression. In resting CV-1 cells, bICP0 relieved HDAC1-mediated transcriptional repression. The zinc ring finger was required for relieving HDAC1-induced repression but not for interacting with HDAC1. In fetal bovine lung cells but not in a human epithelial cell line, bICP0 expression correlated with reduced steady-state levels of HDAC1 in crude cytoplasmic extracts. We hypothesize that the ability of bICP0 to overcome HDAC1-induced repression plays a role in promoting productive infection in highly differentiated cell types.

Bovine herpesvirus 1 (BHV-1) infection can cause conjunctivitis, pneumonia, genital disorders, abortions, and an upper respiratory infection referred to as shipping fever (61). Infection of permissive cells with BHV-1 leads to rapid cell death, in part due to apoptosis (12). Viral gene expression is temporally regulated in three distinct phases: immediate-early (IE), early (E), and late (L). IE transcription unit 1 (IEtu1) encodes a transcriptional activator, bICP0 (65, 66). Although bICP0 is believed to be a functional homologue of herpes simplex virus type 1 (HSV-1)-encoded ICP0, the only well-conserved domain is a C_3HC_4 zinc ring finger located near the N terminus of both proteins (14–17, 46). Most alphaherpesviruses encode a bICP0-like transcriptional activator that contains a zinc ring finger domain (46). These ICP0 homologues transactivate all classes of viral genes (4, 22, 39, 40, 45), demonstrating they are promiscuous *trans*-activators. Mutational analysis has demonstrated the importance of the zinc ring finger domain in HSV-1 ICP0 (14–17, 46), equine herpesvirus 1 ICP0-like protein (EICP0) (4, 5), and BHV-1 bICP0 (35). Zinc ring finger domains are believed to mediate protein-protein interactions (P. S. Freemont, I. M. Hanson, and J. Trowsdale, Letter, Cell **64:**483–484), suggesting that the ability of bICP0 to interact with other proteins is important for efficient productive infection in nondividing cells.

ICP0 (18–20, 51, 52) and bICP0 (35, 55) colocalize with and disrupt the proto-oncogene promyelocytic leukemia protein-containing nuclear domains (ND10 or PODS). ICP0 can regulate the stability of cellular and viral proteins by interacting with the protein degradation machinery (18, 20). For example, the stability of the catalytic subunit of DNA-dependent protein kinase is regulated by ICP0 (44, 56). ICP0 also binds cyclin D3 (38) and elongation factor delta (37). The results of these interactions are perturbation of the cell cycle and altered cellular gene expression (p21, gadd45, and mdm-2, for example) (30). Interestingly, a histone deacetylase (HDAC) inhibitor, trichostatin A, and ICP0 have similar effects on cellular and viral gene expression (30).

Numerous studies have demonstrated that histone acetylases (HAT) and histone deacetylases (HDAC) play an important role in transcriptional regulation by affecting chromatin assembly, transcription factor accessibility, and nucleosome remodeling (24). Several families of acetylases have been identified, including PCAF/GCN5, p300/CBP, TAF250, SRC1, and MOZ (42). HAT can also acetylate other transcription factors (34), including p53 (25), E2F1 (50), EKLF (67), TFIIEa, TFIIF, TCF (64), GATA1 (6), HMGI(Y) (54), ACTR (9), and high-mobility group protein HMG-1 (60). Acetylation regulates many diverse functions, including DNA recognition, protein-protein interaction, and protein stability. To date, six human HDAC have been identified (21, 23, 41, 53, 63). In mammalian cells, HDAC1 and HDAC2 are found in two complexes: the mSinA corepressor complex and the nucleosome-remodeling HDAC complex (NuRD) (28). The mSin3A-HDAC complex is recruited to DNA by Mad1 to repress transcription in an HDAC-dependent manner. HDAC is also recruited to specific promoter regions by other transcription factors such as Rb (48, 49), Sp1 (13), and YY1 (10). These interactions lead to chromatin deacetylation and transcriptional repression.

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A previous study demonstrated that expression of the proto-oncogene *myc* was stimulated following infection of peripheral blood mononuclear cells with BHV-1 (26). The induction of Myc is consistent with cell cycle alterations and apoptosis after infection. The Myc protein dimerizes with Max and binds to a specific *cis*-acting element (CACGTG), which leads to activation of transcription. Myc plays a central role in regulating cell proliferation and apoptosis (58). Conversely, Mad dimerizes with Max, recognizes the same consensus DNA sequence as Myc-Max, and represses transcription. Mad is induced on differentiation of a number of distinct cell types (1, 3, 32, 33). The switch from growth-promoting Myc-Max to growth-inhibiting Mad-Max results in a transition from cellular proliferation to differentiation. Transcriptional repression by Mad-Max heterodimers is mediated by ternary-complex formation with corepressor mSin3 and HDAC activity (27, 43).

In this study, we investigated the ability of bICP0 to activate transcription. We demonstrated that C-terminal sequences and a functional zinc ring finger domain were required for transactivation of a minimal HSV-1 thymidine kinase (TK) promoter. bICP0 interacted with HDAC1, but not with cyclin-dependent kinase 2 (cdk2), in transiently transfected cells. The interaction with HDAC1 correlated with relief of Mad- and HDAC1-mediated transcriptional repression. HDAC1-induced transcriptional repression was observed in serum-arrested cells but not actively growing cells, suggesting that bICP0, in part, stimulates productive infection in differentiated cells by interacting with HDAC1.

MATERIALS AND METHODS

Cells and plasmids. CV-1 cells (African green monkey kidney cells) and human epithelial 293 cells were grown in Earle's modified Eagle's medium supplemented with 5% fetal bovine serum. Bovine fetal lung (BFL) cells were grown in the same medium with 10% fetal bovine serum.

pCMV-bICP0 contains the bICP0 coding sequences under the control of the human cytomegalovirus (CMV) promoter. Mutagenesis of the bICP0 zinc ring finger was described previously (35). The coding regions of the wild-type bICP0 and the zinc ring finger mutant 13G/51A were inserted into Flag-tagged expression vectors pCMV2C (bICP0) or pCMV4B (13G/51A), respectively (Stratagene, San Diego, Calif.). A C-terminal deletion of bICP0 (amino acids 356 to 676; ΔbICP0) was generated by deleting the *Sal*I-*Xho*I fragment from the Flag-tagged bICP0 construct. For a summary of these constructs, see Fig. 6.

pCMV-Mad is a CMV expression plasmid that expresses Mad in mammalian cells and was obtained from D. Ayer (University of Utah, Salt Lake City). pCMV-HDAC1 is a CMV expression plasmid that expresses HDAC1 in mammalian cells and was obtained from T. Kouzarides (Wellcome/CRC Institute, Cambridge, United Kingdom). pSV2cat contains the simian virus 40 early promoter and enhancer and was obtained from B. Howard (National Institutes of Health). pHIVcat contains a minimal human immunodeficiency virus promoter (−29 to +84) and was obtained from C. Wood (Nebraska). pMinCAT contains a minimal HSV-1 TK promoter TATA box (−32 to +51) and was obtained from L. Kretzner (University of South Dakota, Vermillion). pM4minCAT contains four consensus binding sites for Mad-Max or Myc-Max that are upstream of the minimal TK promoter. pM4minCAT was also obtained from L. Kretzner. All promoter constructs were linked to the chloramphenicol acetyltransferase (CAT) gene.

Cytoplasmic and nuclear fractionation. 293 or BFL cells were transfected with 20 μg of the designated bICP0 expression plasmid. At 40 h after transfection, the cultures were rinsed with phosphate-buffered saline PBS and harvested. The cell pellet was gently suspended in hypotonic lysis buffer (10 mM HEPES [pH 7.9], 10 mM KCl, 3 mM $MgCl_2$, 1 mM EDTA, 1 mM dithiothreitol [DTT], 0.05% NP-40, complete proteinase inhibitors [Roche Molecular Biology; 1 tablet/10 ml]). The cell suspension was incubated at 4°C for 30 min and then centrifuged at 2,500 rpm for 5 min at 4°C in a Beckman Avanti 30 centrifuge. The supernatant (cytoplasmic fraction) was transferred to a new tube and stored at −80°C. The nuclear pellet was washed in hypotonic lysis buffer once and centrifuged as above. The crude nuclei were incubated with high-salt buffer (50 mM HEPES [pH 7.9], 250 mM KCl, 0.1 mM EDTA, 5% glycerol, 1 mM DTT, 0.1% NP-40, complete proteinase inhibitors)at 4°C for 30 min. The lysate was then centrifuged at 15,000 rpm for 10 min at 4°C in a Beckman Avanti 30 centrifuge, and the supernatant (nuclear fraction) was stored at −80°C.

Western blot analysis. 293 cells were transfected with 20 μg of the designated bICP0 expression plasmid by calcium phosphate precipitation (68). At 40 h after transfection, cells were collected and lysed in 500 μl of 1× SDS sample buffer (50 mM Tris-HCl [pH 6.8], 10% glycerol, 2% sodium dodecyl sulfate [SDS], 5% β-mercaptoethanol). The cell extract was boiled for 5 min. and the supernatant was used for SDS-polyacrylamide gel electrophoresis. Immunodetection of bICP0 and its mutants was performed with an anti-Flag monoclonal antibody (Stratagene no. 200471-21). HDAC1 protein expression was detected with an anti-HDAC1 antibody (Santa Cruz no. sc-7872).

Analysis of CAT enzymatic activity in transiently transfected cells. Transfection and CAT assays were described previously (68). Briefly, 15 μg of reporter construct and 6 μg of bICP0 expression plasmid were cotransfected into CV-1 cells by the calcium phosphate precipitation method. At 48 h after transfection, the cells were lysed and CAT activity was measured. Chloramphenicol and its acetylated forms were separated by thin-layer chromatography. The amount of acetylated chloramphenicol was measured with a PhosphorImager (Molecular Dynamics, Sunnyvale, Calif.). CV-1 cells were growth arrested by incubating the cultures for 72 h in medium containing 1% serum (31). Growth-arrested CV-1 cells were cotransfected with 15 μg of reporter construct, 1.5 μg of bICP0 expression plasmid, and 0.75 μg of HDAC1 expression vector. At 60 h after transfection, the cells were lysed and CAT activity was measured. All transfection experiments were repeated at least three times to confirm the results.

Coimmunoprecipitation assay. Each Flag-tagged bICP0 expression vector (20 μg) was transfected into 293 cells (100-mm dish) by calcium phosphate precipitation. At 40 h after transfection, the cells were collected and suspended in 250 μl of lysis buffer (20 mM HEPES [pH 7.9], 400 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 20% glycerol, 0.5 mM DTT, 0.5 mM phenylmethylsulfonyl fluoride, 5 μg of leupeptin per ml, 5 μg of pepstatin per ml, 5 μg of antipain per ml). The whole-cell lysate was sonicated and centrifuged for 10 min at 4°C (15,000 rpm) in a Beckman Avanti 30 centrifuge. The supernatant was diluted to 1 ml with the same lysis buffer but containing 20 mM KCl. A 10-μl sample of normal mouse serum and 40 μl of protein A-agarose were then added. The mixture was incubated at 4°C for 1 h and centrifuged at 2,500 rpm in a Beckman Avanti 30 centrifuge for 5 min to pellet the beads. The supernatant was then incubated with 2 μg of the anti-HDAC1 antibody at 4°C for 1 to 3 h. Protein A-agarose beads (40 μl) were added, and the mixture was incubated at 4°C on a rotating device overnight. The beads were collected by centrifugation at 2,500 rpm for 5 min in a Beckman Avanti 30 centrifuge and washed four times with wash buffer (10 mM Tris-HCl [pH 8.0], 50 mM NaCl, 1 mM EDTA, 0.5% NP-40). After a final wash, the beads were suspended in 40 μl of 1× sample buffer and boiled for 3 min. Immunodetection of the precipitated protein was performed using the anti-Flag antibody. Reciprocal immunoprecipitations were also performed. Briefly, 20 μg of each Flag-tagged bICP0 expression vector was transfected into 293 cells. Anti-Flag antibody conjugated to agarose beads (40 μl; Sigma no. A-1205) was used for the immunoprecipitation. Western blot analysis was performed using anti-HDAC antibody.

RESULTS

bICP0 can activate non-BHV-1 promoters. bICP0 can stimulate IE, E, and L BHV-1 promoters, demonstrating that bICP0 is a "promiscuous" *trans*-activator (22, 39, 40). To further evaluate the ability of bICP0 to activate transcription, we tested its ability to activate promoters that are not present in the BHV-1 genome. CV-1 cells were cotransfected with bICP0 expression plasmids and CAT promoter constructs containing the simian virus 40 early promoter-enhancer (pSV2cat), a minimal HIV promoter (−29 to +84; pHIVcat), or a minimal HSV-1 TK promoter (−32 to +51; pMinCAT) (Fig. 1A). bICP0 transactivated pHIVcat and pMinCAT promoter activity more than 10-fold (Fig. 1B). It also stimulated pSV2cat promoter activity, but to a lesser extent. This study demonstrated that bICP0 has the ability to efficiently transactivate promoters that are not derived from BHV-1.

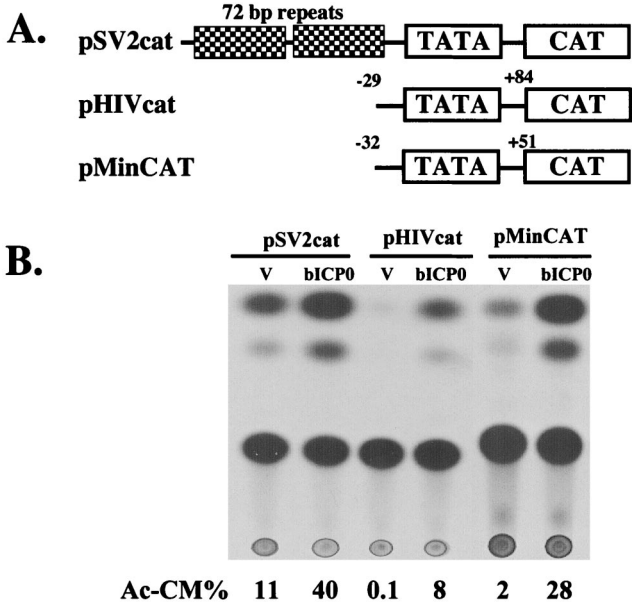

FIG. 1. bICP0 can *trans*-activate several different promoters. The designated CAT reporter constructs (15 μg of DNA) were cotransfected with 6 μg of pCMV-bICP0 or a blank expression vector (V) into CV-1 cells. CAT activity was measured at 48 h after transfection by incubating cell-free lysate with [^{14}C]chloramphenicol (CM) for 3 h. These results are representative of three independent experiments.

bICP0 activates a promoter containing four Myc-Max binding sites. A previous study demonstrated that BHV-1 infection induces c-Myc expression in peripheral blood mononuclear cells (26). Since bICP0 is a promiscuous transcriptional activator, we tested whether bICP0 activated c-Myc-dependent transcription. A reporter construct that contains four consensus binding sites for Myc-Max (or Mad-Max) upstream of a minimal TK promoter was used for this study (pM4minCAT) (Fig. 2A). CV-1 cells were cotransfected with one of the bICP0 expression plasmids and pM4minCAT or, as a control, pMinCAT. CAT activity was measured at 48 h after transfection. bICP0 and ΔbICP0, but not the zinc ring finger mutant protein (13G/51A), stimulated M4 promoter activity (Fig. 2B). Although bICP0 was capable of *trans*-activating the minimal TK promoter (pMinCAT), ΔbICP0 and 13G/51A did not (Fig. 2C). bICP0 *trans*-activated pM4minCAT more efficiently than it *trans*-activated pMinCAT (approximately 35-fold versus 4-fold), suggesting that it had an effect on c-Myc-dependent transcription.

bICP0 relieves Mad-mediated repression. Myc-dependent transcription is regulated by several distinct mechanisms (1, 2). Myc-Max heterodimers bind to consensus Myc binding sites and activate transcription. Conversely, Mad-Max heterodimers bind to the same sequence and repress transcription, in large part, because Mad is associated with HDAC1 (3, 43, 57). Thus, if higher concentrations of Myc or lower concentrations of Mad were present in cells that expressed bICP0, promoter activity of pM4minCAT would be higher. Transient transfection of CV-1 cells with increasing amounts of bICP0 did not dramatically increase c-Myc protein levels or reduce Mad protein levels (data not shown).

To determine whether bICP0 could relieve Mad-mediated transcriptional repression, bICP0, a Mad expression vector, and pM4minCAT were cotransfected into CV-1 cells. CAT ac-

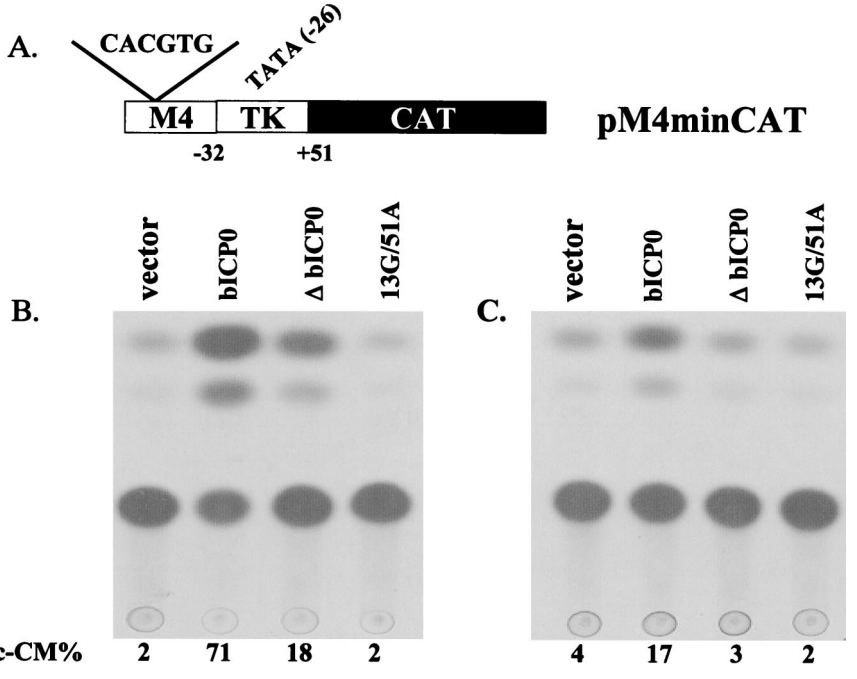

FIG. 2. bICP0 activates a promoter containing four Myc binding sites. (A) pM4minCAT is a plasmid containing four consensus Myc-Max or Mad-Max binding sites upstream of a minimal HSV-1 TK promoter. (B) CV-1 cells were cotransfected with 15 μg of pM4minCAT and 6 μg of vector, bICP0, ΔbICP0, or 13G/51A expression plasmid. (C) CV-1 cells were cotransfected with 15 μg of reporter construct (pMinCAT), which contains the minimal HSV-TK promoter, and 6 μg of vector, bICP0, ΔbICP0, or 13G/51A expression plasmid. At 48 h after transfection, CAT activity was measured by incubating cell-free lysate with [^{14}C]chloramphenicol (CM) for 1 h. The results are representative of three independent experiments.

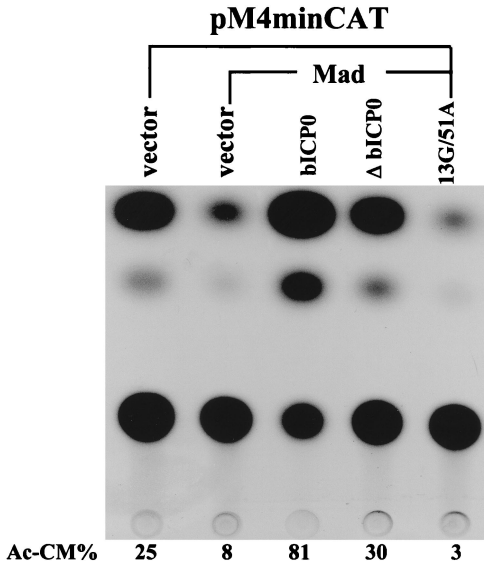

FIG. 3. bICP0 can relieve Mad-mediated transcriptional repression. CV-1 cells were cotransfected with 15 μg of pM4minCAT, 1.5 μg of Mad expression plasmid, and 1.5 μg of vector, bICP0, ΔbICP0, or 13G/51A expression vector. At 48 h after transfection, CV-1 cells were lysed and CAT activity was measured by incubation with [^{14}C]chloramphenicol (CM) for 1 h. The results are representative of three independent experiments.

tivity was measured 48 h after transfection. As expected, introducing Mad into CV-1 cells inhibited pM4minCAT promoter activity approximately threefold (Fig. 3). Wild-type bICP0 and ΔbICP0 relieved Mad-induced repression of pM4minCAT promoter activity. However, the zinc ring finger mutant (13G/51A) was not capable of relieving Mad-dependent repression.

bICP0 relieves HDAC1-mediated repression. To test whether HDAC1 directly repressed gene expression, we cotransfected increasing amounts of HDAC1 expression plasmid with pM4minCAT into CV-1 cells. The M4 promoter was not repressed by HDAC1 in actively growing CV-1 cells (Fig. 4A). Two observations led us to hypothesize that the ability of HDAC1 to function as a transcriptional repressor might be mediated by cell cycle-specific factors. First, growing cells normally express high levels of Myc but lower levels of Mad, suggesting that Myc overcame transcriptional repression of the M4 promoter that was induced by HDAC1. Second, HDAC1 represses cellular TK promoter activity by interacting with Sp1 and consequently interferes with E2F1 and Sp1 association (13). Since the cellular TK promoter is not active in serum-arrested cells (G_0), a model was developed in which HDAC1 is proposed to regulate cell cycle-specific transcription of TK.

We subsequently determined whether HDAC1 was capable of inhibiting pM4minCAT promoter activity in quiescent cells by arresting CV-1 cells in G_0/G_1 by plating them in 1% serum for 72 h. The pM4minCAT promoter construct and increasing amounts of an HDAC1 expression vector were then cotransfected into resting CV-1 cells. At 60 h after transfection, CAT activity was measured. In growth-arrested CV-1 cells, HDAC1 consistently inhibited M4 promoter activity by at least twofold (Fig. 4B).

To determine whether bICP0 relieved HDAC1-dependent repression, bICP0, the HDAC1 expression vectors and pM4minCAT were cotransfected into resting CV-1 cells. Wild-type bICP0 and ΔbICP0 efficiently relieved HDAC1-mediated transcription repression (Fig. 5). In contrast, 13G/51A was not capable of *trans*-activating pM4minCAT in the presence of HDAC1, suggesting that a functional zinc ring finger domain of bICP0 was necessary for relieving repression. In summary, these results demonstrated that bICP0 transactivated the pM4minCAT promoter construct by relieving HDAC1- and Mad-dependent repression.

Analysis of bICP0 protein interaction with HDAC1 in transiently transfected cells. We hypothesized that the ability of bICP0 to relieve HDAC1-mediated repression may be the result of bICP0 interacting with HDAC1. To test whether bICP0 interacted with HDAC1 in transiently transfected cells, we developed Flag-tagged bICP0 expression constructs and performed immunoprecipitation assays. A deletion mutant lacking the C-terminal coding region (amino acids 356 to 676) was prepared from the Flag-tagged construct (ΔC terminus), and this construct designated ΔbICP0. Sequential PCR mutagenesis of

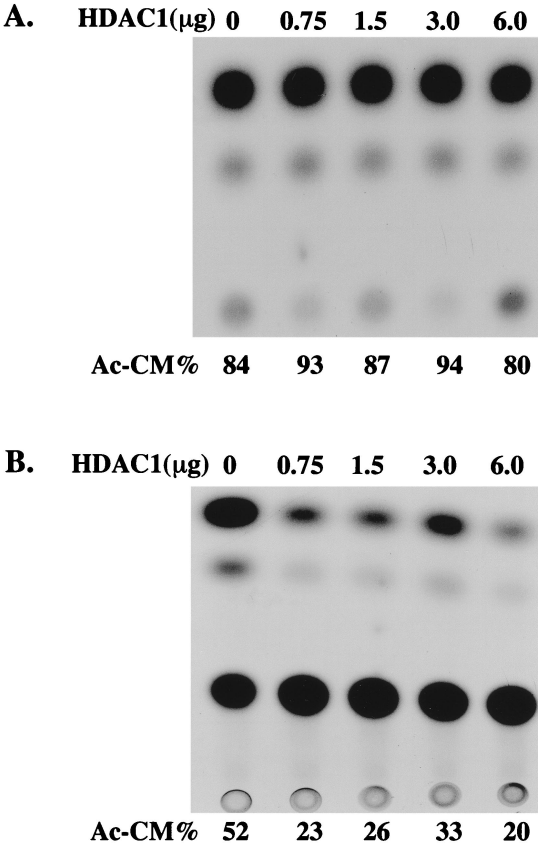

FIG. 4. HDAC1 inhibits pM4minCAT promoter activity in resting CV-1 cells. (A) Actively growing CV-1 cells were cotransfected with 15 μg of pM4minCAT and the designated amounts of HDAC1 expression plasmid. At 48 h after transfection, the cells were lysed and CAT activity was measured by incubation with [^{14}C]chloramphenicol (CM) for 3 h. (B) CV-1 cells were growth arrested in 1% serum culture medium for 72 h. Then 15 μg of pM4minCAT and the designated amounts of HDAC1 expression plasmid were cotransfected into resting CV-1 cells. At 60 h after transfection, cells were lysed and CAT activity was measured by incubation with CM for 3 h. The results are representative of three independent experiments.

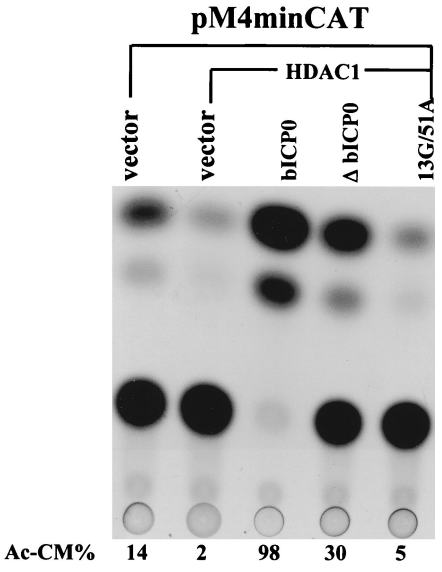

FIG. 5. bICP0 can relieve HDAC1-mediated transcriptional repression in resting CV-1 cells. CV-1 cells were growth arrested as described in the legend to Fig. 4B. pM4minCAT (15 μg), 0.75 μg of HDAC1, and 1.5 μg of vector, bICP0, ΔbICP0, or 13G/51A expression vector were cotransfected into cultures of resting CV-1 cells. At 60 h after transfection, the cells were lysed and CAT activity was measured by incubation with [^{14}C]chloramphenicol (CM) for 3 h. The results are representative of three independent experiments.

the C_3HC_4 zinc ring finger of bICP0 was also performed to change cystine-13 to glycine and cystine-51 to alanine (mbICP0) (35), and this construct is referred to as 13G/51A (Fig. 6B). The mutations in 13G/51A were predicted to disrupt the zinc ring finger (46). The coding region of this double mutant was also cloned into a Flag-tagged expression vector pCMV4B (Fig. 6A). The Flag-tagged expression vectors were transfected into 293 cells. At 40 h after transfection, cells were lysed and expression of Flag-tagged bICP0 or its mutant was detected with an anti-Flag monoclonal antibody. Wild-type bICP0 (Fig. 6C, lane 2) and 13G/51A (lane 4) migrated near 100 kDa when expressed as Flag-tagged fusion proteins. Deletion of 320 amino acids at the C terminus (ΔbICP0) resulted in synthesis of a truncated protein migrating with an apparent molecular mass of 55 kDa (lane 3).

To test whether bICP0 interacted with HDAC1 or a protein complex containing HDAC1, Flag-tagged bICP0 was transfected into 293 cells and HDAC1 was immunoprecipitated at 40 h after transfection. The presence of bICP0 in the immunoprecipitate was tested by Western blot analysis using a monoclonal antibody directed against the flag epitope. bICP0, ΔbICP0, and 13G/51A were all immunoprecipitated by the HDAC1 antibody (Fig. 7A). Reciprocal immunoprecipitation experiments confirmed the interaction between bICP0 and HDAC1. In contrast, the Flag antibody did not coimmunoprecipitate cdk2, nor did cdk2 antibodies coprecipitate the respective bICP0

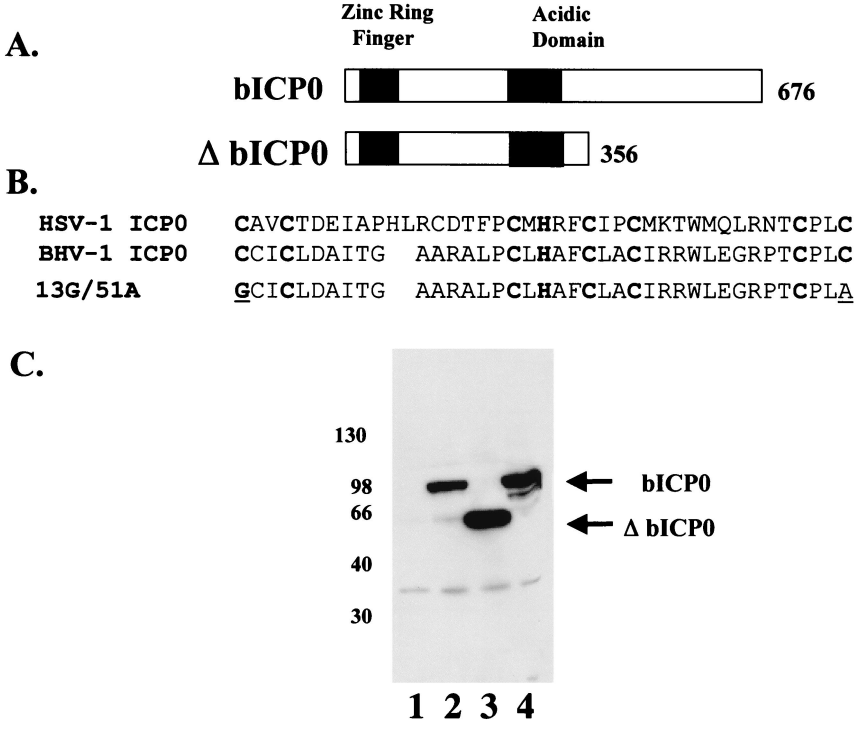

FIG. 6. Expression of bICP0 constructs as Flag-tagged proteins. (A) Schematic of wild-type bICP0 and C-terminal deletion coding sequences. Positions of the zinc ring finger and acidic domain are shown. (B) Amino acid sequences of the C_3HC_4 zinc ring fingers of BHV-1 bICP0 (amino acids 13 to 51) and HSV-1 ICP0 (amino acids 116 to 156). The consensus zinc ring finger sequences are in bold. The mutations in bICP0 are underlined (amino acid 13 was changed from C to G, and amino acid 51 was changed from C to A). The methods used for generating 13G/51A were described previously (35). (C) 293 cells were transfected with 20 μg of each Flag-tagged bICP0 expression vector. At 40 h after transfection, whole-cell lysate was prepared and Western blot analyses were performed with an anti-Flag antibody. Arrows denote the positions of the bICP0 and ΔbICP0 proteins. Lanes: 1, transfected with the blank Flag tag expression vector (pCMV2C); 2, transfected with bICP0; 3, transfected with ΔbICP0; 4, transfected with 13G/51A.

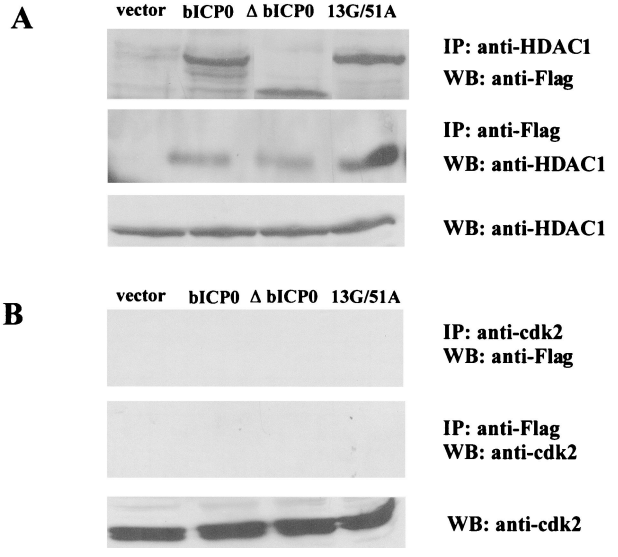

FIG. 7. Interaction between bICP0 and HDAC1. (A) Cultures of 293 cells were transfected with 20 μg of the indicated Flag-tagged bICP0 plasmid. At 40 h after transfection, nuclear extract was prepared. An anti-HDAC1 antibody was used for immunoprecipitation. The presence of bICP0 and the mutant proteins was detected with an anti-Flag antibody. Alternatively, 40 μl of anti-Flag antibody-conjugated agarose was used for immunoprecipitation. The presence of HDAC1 and its expression level in each transfected cells were detected by a polyclonal antibody that specifically recognizes HDAC1. (B) 293 cells were transfected with the designated bICP0 expression vectors, and nuclear extract was prepared as described for panel A. A 40-μl volume of anti-cdk2-conjugated agarose was used for immunoprecipitation (IP). The Western blot analysis (WB) was performed with an anti-Flag antibody. Reciprocal immunoprecipitation was performed with 40 μl of anti-Flag-conjugated agarose. The presence of cdk2 and the protein level of cdk2 in transfected cells were detected with an anti-cdk2 antibody.

proteins (Fig. 7B). In summary, this study demonstrated that bICP0 interacted with HDAC1 or an HDAC1-containing complex in transiently transfected cells.

To examine whether bICP0 had any effect on the subcellular distribution or steady-state levels of HDAC1, 293 or BFL cells were transfected with the various bICP0 Flag-tagged constructs and HDAC1 protein levels were measured by Western blot analysis. This study demonstrated that in BFL cells, bICP0 reduced HDAC1 protein levels in cytoplasmic extracts but not nuclear extracts (Fig. 8). No differences in cdk2 levels were detected in cells transfected with bICP0. In 293 cells, bICP0 did not have any effect on HDAC1 protein levels (data not shown).

DISCUSSION

bICP0 is a promiscuous transcriptional activator because it stimulates all classes of BHV-1 promoters (40, 65) and non-BHV-1 promoters (Fig. 1). The ability of bICP0 to interact with HDAC1 may play an important role in transcriptional activation because HDACs, in general, are transcriptional repressors that deacetylate histones and consequently convert chromatin into a "closed conformation" (41, 59, 62). Our results do not distinguish between bICP0 directly binding to HDAC1 or to a complex containing HDAC1. Regardless of the mechanism, the interaction between bICP0 and HDAC1 may (i) inhibit or alter deacetylase activity, (ii) prevent interaction of HDAC1 with other corepressors, (iii) prevent interaction of HDAC1 with DNA, or (iv) alter the subcellular localization of HDAC1. The finding that bICP0 expression correlated with reduced levels of HDAC1 in crude cytoplasmic extracts prepared from BFL cells suggested this was important in regulating the total HDAC1 activity. Since bICP0 did not apparently reduce the levels of HDAC1 in 293 cells, we suggest that certain cell-type-specific factors play a role in this process.

It is possible that bICP0 overrides or is dominant to the repressor activity of HDAC1 and that the interaction between HDAC1 and bICP0 is not required for relieving repression. However, four lines of evidence support the concept that an interaction between bICP0 and HDAC1 had an effect on transcriptional repression induced by HDAC1. The first was that repression of the M4 promoter construct by Mad was relieved by bICP0 or ΔbICP0 (Fig. 3). The finding that ΔbICP0 was unable to *trans*-activate the minimal TK promoter (Fig. 2) (35) but interacted with HDAC1 (Fig. 7) and relieved Mad induced repression of the M4 construct (Fig. 3) was the second line of evidence. This finding argued against bICP0 merely *trans*-activating the TK promoter in the M4 construct because ΔbICP0 was unable to *trans*-activate the minimal TK promoter (pMinCAT) (Fig. 2C) (35). This study also indicated that indepen-

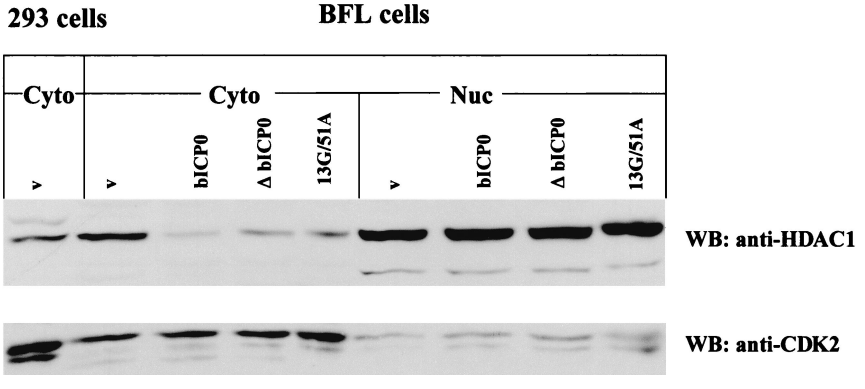

FIG. 8. The cytoplasmic HDAC1 level is reduced in BFL cells transiently transfected with bICP0 expression plasmids. 293 cells and BFL cells were transfected with the designated Flag-tagged bICP0 expression vector. At 40 h after transfection, cytoplasmic and nuclear fractions were prepared and Western blot (WB) analysis was performed using an anti-HDAC1 antibody or an anti-cdk2 antibody.

dent of the ability of bICP0 to interact with HDAC1, the intact zinc ring finger provided a function that was important for relieving Mad-induced repression. Since bICP0 is localized within discrete domains of the nucleus of infected (55) and transfected (35) cells, it is possible that bICP0 can sequester HDAC1 to these domains. The third line of evidence comes from its ability to relieve HDAC1-mediated repression in quiescent cells (Fig. 5). The final line of evidence was the finding that HDAC1 protein levels were reduced in bovine cells transfected with bICP0.

In addition to histones, reversible acetylation regulates a growing number of transcription factors, and thus HDACs are important for this process. For example, E2F family members are differentially regulated by acetylation (50) and acetylation of p53 represses its transcriptional activity (36). E2F (29) and p53 (12) are stimulated as a result of HSV-1 or BHV-1 infection, respectively, suggesting that these proteins are targets for virus-induced changes in acetylation and deacytlation. A recent study has demonstrated that the yeast HDAC1 interacts with two G_2/M checkpoint proteins (7), suggesting that HDAC1 directly influences the cell cycle. This is an intriguing observation because HSV-1-encoded ICP0 inhibits G_2/M cell cycle progression (47) and bICP0 inhibits the growth and survival of transfected cells (35). Considering that acetylation is thought to be as important as phosphorylation with respect to post-translationally modifying proteins (41), it is not surprising that BHV-1 would target this pathway.

Cellular factors in actively growing cells can replace HSV-1-encoded ICP0 (8), suggesting that factors in nondividing cells repress productive infection. HDAC1 repressed pM4minCAT promoter activity in growth-arrested, but not in actively growing, CV-1 cells (Fig. 4), suggesting that an interaction between bICP0 and HDAC1 promotes viral gene expression in growth-restricted cells. This hypothesis is supported by the finding that an HDAC inhibitor (trichostatin A) and ICP0 have similar effects on HSV-1 and cellular gene expression (30). With respect to reactivation from latency, this may be particularly relevant because it is generally accepted that ICP0 or, in the case of BHV-1, bICP0 triggers reactivation (16). Since sensory neurons are terminally differentiated cells and HSV-1 is organized as chromatin in latently infected neurons (11), we hypothesize that HDAC1, in part, maintains the genome in a "repressed transcriptional state". The ability of bICP0 to interact with HDAC1 may be an important step in relieving repression and inducing reactivation.

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