Functional interaction of the human cytomegalovirus IE2 protein with histone deacetylase 2 in infected human fibroblasts

Jung-Jin Park,1† Young-Eui Kim,1† Hong Thanh Pham,1 Eui Tae Kim,1 Young-Hwa Chung2 and Jin-Hyun Ahn1

Correspondence Jin-Hyun Ahn jahn@med.skku.ac.kr

1Department of Molecular Cell Biology, Samsung Biomedical Research Institute, Sungkyunkwan University School of Medicine, Suwon, Republic of Korea
2Department of Nanomedical Engineering, Pusan National University, Miryang, Republic of Korea

In human cytomegalovirus (HCMV)-infected cells, the 86 kDa immediate-early (IE) 2 protein plays a key role in transactivating downstream viral genes. Recently, IE2 has been shown to interact with histone deacetylase 1 (HDAC1) and HDAC3. HDAC1 recruited by IE2 was required for IE2-mediated autorepression of the major IE (MIE) promoter, whereas IE2–HDAC3 interaction was suggested to relieve the repressive effect of HDAC3 on viral early promoters. However, whether IE2 indeed inhibits HDAC’s deacetylation activity on viral promoters and interacts with other HDACs remains unclear. Here, we provide evidence that IE2 functionally interacts with HDAC2 and negates its repressive effect on the viral polymerase promoter. IE2 interacted with HDAC2 in both virus-infected cells and in vitro, and required the conserved C-terminal half for HDAC2 binding. The subcellular localization of HDAC2 was changed in virus-infected cells, showing colocalization with IE2 in viral transcription and replication sites. The overall HDAC2 protein levels and its deacetylation activity slightly increased during the late stages of infection and the IE2-associated deacetylation activity was still sensitive to an HDAC inhibitor, trichostatin A. In transfection assays, however, histone acetylation of the viral polymerase promoter was suppressed by HDAC2, and this was relieved by IE2 binding. Therefore, our data demonstrate that IE2 functionally interacts with HDAC2 and modulates its deacetylation activity on the viral polymerase promoter. Our results also support the idea that interactions of IE2 with several HDACs to modulate the host epigenetic regulation on viral MIE and early promoters are important events in the process of productive infection.

INTRODUCTION

Cellular and viral gene expressions are regulated by changes in chromatin structure and composition, which are induced by the post-translational modifications of core histones. The acetylation levels of core histones are controlled by the activities of histone deacetylases (HDACs) and histone acetyltransferases. In general, HDAC activity is associated with transcriptional repression, whereas histone acetyltransferase activity is related to transcriptional activation (for reviews see Carrozza et al., 2003; Iizuka & Smith, 2003; Thiagalingam et al., 2003).

In human cytomegalovirus (HCMV) infection, two major immediate-early (IE) proteins, 72 kDa IE1 (or IE72) and 86 kDa IE2 (or IE86), are synthesized from a single transcription unit by alternative splicing. These two proteins share the 85 N-terminal amino acids, which are encoded by exons 2 and 3. Genetic studies have shown that IE1 is required for viral growth in cell cultures at a low m.o.i. (Greaves & Mocarski, 1998), and that this growth defect is related with the delayed accumulation of the viral early gene products (Ahn & Hayward, 2000; Gawn & Greaves, 2002). From a study with a recombinant viral genome, IE2 has been shown to be essential for all downstream lytic cycle viral gene expression in cell culture (Marchini et al., 2001). Therefore, they together direct the expressions of downstream lytic genes and are believed to perform key regulatory functions in preparing infected cells for the production of progeny virions (Castillo & Kowalik, 2002; Mocarski & Tan Couecelle, 2001).

Evidence is accumulating to indicate that the regulatory proteins of cytomegalovirus (CMV) target to HDACs and modulate or use their deacetylation activities to control viral and/or cellular gene expression. Mouse CMV (MCMV) IE1 has been shown to form a complex with...
HDAC2, PML and Daxx, and inhibit the deacetylation activity of HDAC2 (Tang & Maul, 2003). For HCMV, IE1 has been suggested to promote viral replication by antagonizing histone deacetylation. This study demonstrated that several HDAC inhibitors rescued the growth defect of IE1-deficient mutant virus at a low m.o.i., and showed that the absence of IE1 in mutant virus was correlated with decreased histone H4 acetylation in the major IE (MIE) and UL44 promoters, and that both IE1 and IE2 interacted with HDAC3 (Nevels et al., 2004). A recent study has shown that HCMV IE2 physically interacts with and recruits HDAC1 to the cis repression sequences (crs) on its own promoter, and uses the associated deacetylation activity for autorepression (Reeves et al., 2006). Therefore, the modes of interaction between viral regulatory proteins and several HDACs and their consequences appear to be divergent.

Although the interactions of IE2 with HDAC1 and HDAC3 have been demonstrated, whether, like IE1, IE2 indeed inhibits HDAC’s deacetylation activity on viral early promoter and whether IE2 interacts with other HDACs remain to be addressed. In this study, we provide evidence that IE2 functionally interacts with HDAC2 and negates its repressive effect on the viral polymerase (UL54) promoter.

**METHODS**

**Cell culture and virus.** Human foreskin fibroblast (HF), U373 and 293T cells were grown in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal calf serum. The HCMV (Towne) virus stocks used in this study were prepared as previously described (Lee et al., 2004).

**Plasmid construction.** Mammalian expression plasmid pSG5-IE2 encoding wild-type IE2(1–579) was described previously (Ahn et al., 2001). Expression plasmids for 5’ haemagglutinin (HA)-tagged wild-type IE2(1–579), IE2(131–579), IE2(290–579), IE2(290–542), IE2(313–579) and IE2(1–290) were generated on a pSG5 (Green et al., 1988) background by using Gateway technology (Invitrogen). Similarly, expression plasmids for 5’ six-myc-tagged wild-type IE2, IE2(1–290) and IE2(290–542) were generated on a pCS3-MT (Turner & Weintraub, 1994) background. Plasmids for HDAC2 were generated on a pCS3-MT (with six-myc tag) or pSPUTK2 (without a tag) (Stratagene) background by using Gateway technology (Invitrogen). These plasmids were also used as template plasmids for in vitro transcription/translation reactions. E. coli expression plasmids for GST alone, GST–IE2(290–542), GST–IE2(313–542), GST–IE2(A313–346) (290–542) background and GST–IE2(346–542) were described previously (Lee et al., 2003). The pLa12 reporter plasmid containing the HCMV UL54(Pol)-luciferase reporter gene was described previously (Ahn et al., 2001).

**Transient DNA transfection.** High efficiency electroporation of HF cells was performed with the Microporator system (Digital Bio Technology). 293T cells were transfected using the Calcium phosphate method, as described previously ([Lee et al.](http://vir.sgmjournals.org), 2003). The pLa12 reporter plasmid containing the HCMV UL54(Pol)-luciferase reporter gene was described previously (Ahn et al., 2001).

**Antibodies.** Mouse monoclonal antibodies (mAbs) against IE1 (6E1) or IE2 (12E2) were purchased from Vancouver Biotech. Mouse mAb 8131, which detects epitopes present in both IE1 and IE2 (exons 2 and 3), was obtained from Chemicon. Anti-HDAC2 rabbit polyclonal antibody (PAb) and anti-α-tubulin mouse mAbs were purchased from Zymed and Sigma, respectively. The anti-HA rat mAbs (3F10), which were either conjugated with peroxidase or labelled with fluorescein, and anti-myc mouse mAb 9E10, were purchased from Roche.

**Indirect immunofluorescence assay (IFA).** For IFA, cells were fixed by either the methanol or the paraformaldehyde procedure. For the methanol procedure, the cells were washed in Tris-buffered saline (TBS), permeabilized with absolute methanol at 20 °C for 10 min and rehydrated in ice-cold TBS for 5 min. For the paraformaldehyde procedure, the cells were washed in phosphate-buffered saline (PBS), fixed with 1% paraformaldehyde solution in PBS at room temperature for 5 min, and then permeabilized in ice-cold 0.2% Triton X-100 solution in PBS for 20 min. All of the subsequent procedures have been described previously ([Lee et al.](http://vir.sgmjournals.org), 2004). Slides were examined and photographed with a Zeiss Axiohot microscope. For confocal microscopy, a Carl Zeiss Axioplan 2 confocal microscope system with LSM510 software (Carl Zeiss) was used.

**In vitro binding assay with GST fusion proteins.** Plasmid DNAs encoding GST fusion proteins were transformed into *E. coli* BL21, and extracts from the bacterial cultures expressing the GST fusion proteins were prepared by standard procedures. These extracts were incubated with glutathione-Sepharose 4B beads (Pharmacia) for 3 h at 4 °C. After three washes with lysis buffer, the beads were resuspended in EBC buffer [140 mM NaCl, 50 mM Tris (pH 8.0), 0.5% Triton X-100, 100 mM NaF, 200 μM Na3VO4]. The HDAC2 protein was synthesized in vitro by using the TNT Quick Coupled Transcription/Translation System (Promega). Aliquots of in vitro-translated (IVT) HDAC2 (5 μl) were mixed with appropriate amounts of GST fusion protein beads (containing ~5 μg of the GST fusion protein, with unbound glutathione beads added for a total bead volume of 20 μl) and resuspended in 500 μl of EBC buffer supplemented with BSA at 1 mg ml−1. The mixtures were then incubated for 2 h at 4 °C with gentle stirring. After binding, the beads were pelleted, washed five times with 1 ml NETT buffer [100 mM NaCl, 20 mM Tris (pH 8.0), 0.5% Triton X-100, 1 mM EDTA], resuspended in 15 μl of 2× SDS sample buffer, boiled for 5 min and loaded together with the IVT-HDAC2 input onto a 10% polyacrylamide SDS gel. After electrophoresis, the amount of the IVT-HDAC2 bound to a given GST fusion protein was determined by immunoblotting with anti-HDAC2 antibody.

**Coimmunoprecipitation assay.** Cells were harvested and sonicated in coimmunoprecipitation (CoIP) buffer (50 mM Tris–Cl, pH 7.4, 50 mM NaF, 5 mM sodium phosphate and 0.1% Triton X-100) supplemented with protease inhibitor cocktail (Sigma). Cell lysates were then incubated with the appropriate antibody. After 2 h incubation at 4 °C, 30 μl of a 50% slurry of protein A- and G-Sepharose (Amersham) was added, and was then absorbed for 16 h at 4 °C. The mixture was pelleted and washed seven times with CoIP buffer. The beads were then resuspended in loading buffer and boiled for 5 min. The samples were analysed by immunoblotting.

**Immunoblot analysis.** Cells were washed with PBS and the total extracts were prepared by incubation of the cells with RIPA buffer. The clarified cell extracts were separated on 8% SDS-PAGE gels, followed by the performance of the standard procedure using an enhanced chemiluminescence system (Amersham).

**Luciferase reporter assay.** Cells were collected and lysed using three freeze–thaw steps in 200 μl of 0.25 M Tris/HCl (pH 7.9) plus 1 mM dithiothreitol. Subsequent procedures were performed as previously described ([Ahn et al.](http://vir.sgmjournals.org), 2001). A TD-20/20 luminometer was used to measure the relative light units (RLU) emitted by the reporter constructs.
(Turner Designs) was used for the 10 s assay of the photons produced (measured in relative light units).

**HDAC activity assay.** The HDAC activity assay was performed using a HDAC Colorimetric Activity Assay kit (BIOMOL International) according to the protocol of the manufacturer. The assay is based on cleavage of an acetylated lysine side chain in the Colorimetric Histone deAcetylase Lysyl Substrate by HDAC. This cleavage sensitizes the substrate to react with a developer, thus producing a yellow colour that is captured at an absorbance of 405 nm. In brief, total cell lysates (about 100 μg) or immunoprecipitated samples were incubated with 2.5 mM Colorimetric Histone deAcetylase Lysyl Substrate for 4 h at 37 °C with or without 1 μM trichostatin A (TSA) in a total volume of 50 μL. After the incubation, 50 μL developer was added to the sample, and the reaction mixture was further incubated at 37 °C for 15 min. Absorbance was measured at 405 nm using a colorimetric plate reader.

**Chromatin immunoprecipitation (ChIP) assay.** ChIP assays were performed using a ChIP assay kit (Upstate Biotechnology) with minor modification. In brief, virus-infected (1.8 × 10^6 cells for each ChIP) or DNA-transfected HF cells (1.6 × 10^6) were fixed with 1 % formaldehyde for 10 min at different time points after infection, and were then lysed with lysis buffer provided in the kit. ChIP assays were performed with anti-acetylated histone H4 antibody (Upstate) or control IgG. One-fourth of the lysate was kept to facilitate quantification of the amount of DNA present in different samples prior to immunoprecipitation. For detection of the MIE or UL54 promoter of HCMV, DNA purified from immunocomplex was amplified by PCR using the following primers for the MIE promoter, 5′-TGGGACTTCTCATTCTGG-3′ (sense) and 5′-CCAGGCATCTGACGGTT-3′ (anti-sense); for the UL54 promoter, 5′-TCTGCAGAAGGAGACTTGTGATA-3′ (sense) and 5′-TCTGCAGAAGGAGACTTGTGATA-3′ (anti-sense for infection experiments) or 5′-TCAGATACGGGTGGAAA-3′ (anti-sense for reporter assays). The program used was 95 °C for 5 min, 28 (for MIE promoter) or 30 (for UL54 promoter) amplification cycles (94 °C for 40 s, 50 °C for 40 s and 72 °C for 72 s) and a final step at 72 °C for 10 min.

**RESULTS**

**IE2 interacts with HDAC2**

To investigate whether IE2 interacts with HDAC2 in virus-infected cells, coimmunoprecipitation assays were carried out in HCMV-infected fully permissive HF cells. Cells were mock-infected or infected with HCMV and harvested at 24 h for assays. The results of coimmunoprecipitation indicated that immunoprecipitation of cell lysates with anti-HDAC2 antibody specifically coprecipitated IE2 (Fig. 1a) and, in reciprocal experiments, immunoprecipitation with anti-IE2 antibody coprecipitated HDAC2 (Fig. 1b). This result demonstrated that IE2 is associated with HDAC2 in virus-infected cells.

Interaction of HDAC2 with IE2 was also confirmed by coimmunoprecipitation assays in cotransfected cells. When both myc-HDAC2 and HA-IE2 were coexpressed in 293T cells, immunoprecipitation of myc-HDAC2 coprecipitated IE2 (Fig. 2a). We then attempted to map the domains of IE2 required for HDAC2 binding using several IE2 deletion mutants in similar assays. The results showed that the C-terminal half of IE2, encompassing amino acids from 313 to 579, and a region of IE2 from 290 to 542, were sufficient for HDAC2 binding, whereas the 290 aa N-terminal region of IE2 failed to bind to HDAC2 (Fig. 2a).

We further investigated the interaction of HDAC2 with IE2 in vitro. When coimmunoprecipitation assays were conducted with in vitro-translated IE2 and HDAC2, we found that both wild-type IE2 and IE2(290–542) were coprecipitated with HDAC2, whereas IE2(1–290) was not (Fig. 2b). This result was consistent with the mapping data in cotransfected cells demonstrating that the C-terminal half of IE2 is responsible for HDAC2 binding. Interestingly, the HDAC2 binding strength of IE2(290–542) was much higher than that of wild-type IE2, suggesting a masking effect of the N-terminal region of IE2 for HDAC2 binding. Interaction between HDAC2 and IE2 was also observed in in vitro GST pull-down assays. We found that in vitro-translated HDAC2 physically bound to bacterially purified GST–IE2(290–542) and GST–IE2(313–542), but did not bind to smaller fragments of IE2 such as GST–IE2(Δ313–346) (in 290–542 background) and GST–IE2(346–542) (Fig. 2c). From these results, we concluded that IE2 is able to physically bind to HDAC2, and also determined that the region between 313 and 542 is necessary for HDAC2 binding. The mutant IE2 proteins used in these mapping experiments and the results of the experiments are summarized in Fig. 2(d).

**Colocalization of HDAC2 with IE2 in viral transcription and replication sites in HCMV-infected cells**

We investigated the effects of HCMV infection on the intracellular distribution of HDAC2. To examine the
Fig. 2. Interaction of IE2 with HDAC2 in vitro and mapping of IE2 domains required for HDAC2 binding. (a) Interaction of IE2 with HDAC2 in cotransfected cells. 293T cells were cotransfected with plasmids encoding myc-tagged HDAC2 and HA-tagged wild-type or mutant IE2 as indicated. Immunoprecipitation was performed with anti-myc antibody at 48 h, followed by SDS-PAGE and immunoblotting with anti-HA antibody (top). Immunoblots of the total cell extracts with anti-myc or anti-HA antibodies to correspond to the expression levels are shown (bottom). Three separate experiments with different mutant IE2 constructs are shown. (b) Coimmunoprecipitation of in vitro-cotranslated IE2 and HDAC2. The myc-tagged wild-type IE2(1–579), IE2(1–290) or IE2(290–542) protein was cotranslated with HDAC2 via in vitro transcription/translation reactions. The products were then immunoprecipitated with anti-HDAC2 antibody and subjected to SDS-PAGE and immunoblotting with anti-myc antibody (top). Results of immunoblotting for the input translation products are shown (bottom). The positions of wild-type IE2, IE2(1–290) and IE2(290–542) proteins in the gel are indicated. (c) In vitro binding assay of HDAC2 with GST–IE2 fusion proteins. The GST alone, or GST–IE2(290–542), GST–IE2(313–542), GST–IE2(D313–346) (in 290–542 background), or GST–IE2(346–542) fusion proteins immobilized to glutathione-Sepharose beads were incubated with the in vitro-translated HDAC2 protein. The bound proteins were fractionated in SDS-PAGE and visualized by immunoblotting with anti-HDAC2 antibody (top). One-fifth of the proteins used in each binding reaction were shown by immunoblotting (for HDAC2) and Coomassie blue staining (for GST fusion proteins) as input controls (bottom). The position of a non-specific (NS) band is indicated by an open circle. (d) Structures of wild-type or mutant IE2 constructs used in in vitro binding assays and the summary of the results are shown. The positions of the reported nuclear localization signals (NLS) within exon 5 (between codons 145 and 151 and between codons 321 and 328) for IE2 (Pizzorno et al., 1991) are indicated as open circles. The regions encoded by exons 2 and 3 (black bars) and by exon 5 (grey bars) are indicated. The abilities of each protein to bind to HDAC2 in indicated assays are summarized as (+) or (−).
localization patterns of HDAC2 during the early period of infection, HF cells infected with HCMV were fixed at 6 h and subjected to double-label IFA. We found that HDAC2 was diffusely localized in the nucleoplasm in uninfected cells, but that nuclear foci containing HDAC2 were formed in IE1-positive or PML-displaced infected cells (Fig. 3a–f). Note that, in HCMV-infected cells, IE1 is transiently colocalized with PML in subnuclear structures known as PML oncogenic domains (PODs) or nuclear domain 10 (ND10) and subsequently disrupts PODs by 4 h (Ahn & Hayward, 1997; Korioth et al., 1996; Wilkinson et al., 1998). Similar changes of HDAC2 localization patterns were observed when infected cells were fixed with paraformaldehyde, and the use of control mouse or rabbit antibodies did not produce non-specific cross-staining (data not shown). Since IE2 interacted with HDAC2 in infected cells and HCMV-induced HDAC2 foci observed at 6 h resembled IE2 protein domains, where input viral genomes are found and IE transcription occurs (Ahn et al., 1999; Ishov et al., 1997), we further investigated whether these HDAC2 foci are colocalized with IE2. When we performed confocal double-label IFA, high power magnification images captured at 6 h revealed that IE2 foci were completely colocalized with HDAC2 foci (Fig. 3g–i). Our results demonstrated that HDAC2 was redistributed to and colocalized with IE2 domains at early times in HCMV-infected cells.

IE2 has been shown to accumulate in viral pre-replication foci and in replication compartments during the late stages of infection (Ahn et al., 1999). Therefore, we further examined the localization pattern of HDAC2 at 24 h after infection. Interestingly, HDAC2 was still colocalized with IE2 in small globular structures that represent pre-replication foci or early forms of viral replication compartments (Fig. 4a–c). When infected cells were fixed at 48 h, HDAC2 was found to accumulate in the fully grown viral replication compartments, and was colocalized with IE2 (Fig. 4d–f).

Effects of HCMV infection on HDAC2 protein levels and deacetylation activity

We investigated whether HCMV infection causes changes in the steady-state levels of HDAC2. When HF cells were infected with HCMV at an m.o.i. of 2, immunoblot analysis showed that the HDAC2 levels slightly decreased, to 60% of the level of mock-infected cells at 6 h, but increased to about twofold at 48 h and up to threefold at 72 h as infection progressed (Fig. 5a). A similar increase of the HDAC2 protein levels at late times after infection was observed in virus-infected semi-permissive U373 cells (Fig. 5b). To investigate whether expression of IE2 alone is responsible for this accumulation of HDAC2, IE2 (or IE1 as a control) was expressed in HF cells via a high efficiency electroporation system. In experimental conditions in which the transfection efficiency measured with a control GFP plasmid was about 80%, expression of IE2 or IE1 alone did not increase the HDAC2 protein levels (Fig. 5c),
suggesting that interaction of HDAC2 with IE2 is not sufficient for the increased accumulation of HDAC2 observed in the late stages of HCMV infection.

We next investigated whether deacetylation activity is changed in HCMV-infected HF cells. When the total deacetylation activity in infected-cell lysates was measured, it was also slightly reduced, to 81% of that in mock-infected cell lysates at 6 h, but again increased up to 1.6-fold at 72 h (Fig. 5d). To measure the HDAC2-specific deacetylation activity, cell lysates were immunoprecipitated with anti-HDAC2 antibody and used for the assay. The results showed that HDAC2-associated deacetylation activity also increased almost twofold at 72 h (Fig. 5e, left). These increases of deacetylation activity correlated with the slight increases of the HDAC2 protein levels at the later stages of infection. When infected-cell lysates were immunoprecipitated with anti-IE2 antibody and used for the assays, the IE2-associated HDAC activity at 72 h was still sensitive to an HDAC inhibitor, trichostatin A (TSA) (Fig. 5e, right), suggesting that the IE2-bound HDAC(s) may retain at least TSA-sensitive deacetylation activity in vitro.

IE2 negates the repressive effect of HDAC2 on the viral polymerase promoter

Finally, we investigated whether the IE2-HDAC2 interaction contributes to the IE2-mediated transactivation of the viral polymerase (UL54) promoter. First, we examined whether expression of HDAC2 has a repressive effect on the transactivation of UL54 promoter using luciferase reporter assays. When HF cells were cotransfected with a Pol-LUC plasmid together with increasing amounts of plasmid expressing HDAC2, the UL54 promoter was efficiently repressed by HDAC2 in a dose-dependent manner (data not shown). When similar luciferase reporter assays were carried out with IE2 and HDAC2, it was found that IE2(1–290), which failed to bind to HDAC2, did not affect the HDAC2-mediated repression of UL54 promoter, whereas IE2(290–542), which was able to bind to HDAC2, efficiently reversed the repressive effect of HDAC2 (Fig. 6a). It should be noted that IE2(290–542) lacks the most C-terminal transactivation domain and therefore, the derepression of the UL54 promoter by IE2(290–542) is due to the interaction between IE2 and HDAC2.

To further investigate whether the binding of IE2 to HDAC2 indeed interferes with histone deacetylation activity on the UL54 promoter, ChIP assays were performed in experimental conditions similar to those of the reporter assays. The results showed that HDAC2 expression reduced the acetylated histone levels on the UL54 promoter, whereas coexpression with IE2(290–542), but not IE2(1–290), restored the acetylated histone levels (Fig. 6b). In control experiments, ChIPs with a control IgG yielded no PCR products. Overall, our data from reporter assays and ChIP assays strongly suggest that the histone deacetylase activity of HDAC2 has a repressive effect on the transactivation of UL54 promoter, and that IE2 is able to negate this repressive effect via HDAC2 binding.

We also measured changes of the acetylated histone levels on the UL54 promoter during viral infection by using ChIP assays. HF cells were infected with HCMV, and ChIP assays were performed at 6, 24, 48 and 72 h. The results showed that the levels of acetylated histones on the UL54 promoter increased throughout the progression of infection (Fig. 6c, top). In control experiments, ChIPs with a control IgG yielded no PCR products. Notably, the amount of PCR-amplified total viral DNA also slightly increased during later periods of infection, but the levels of acetylated histones were drastically increased at 48 or 72 h. When we also examined changes of the histone acetylation levels on the MIE promoter in the same experimental set, we found that the levels of acetylated histones were increased by 24 h, but were drastically decreased at 48 and 72 h (Fig. 6c, bottom). This result is generally consistent with a recent
finding that IE2-mediated autorepression of the MIE promoter requires histone deacetylation activity which was recruited to the cis-repression sites by IE2 (Reeves et al., 2006).

**DISCUSSION**

In this study, we demonstrate that IE2 interacts with HDAC2 in both virus-infected cells and in vitro. In in vitro binding assays, we mapped a region of IE2 encompassing amino acids 313 to 542 within the C-terminal half, which is highly conserved among equivalent proteins of β-herpesviruses and essential for viral growth, and is necessary for HDAC2 binding. Although the lack of viable IE2 mutant with a deletion within this region limited our evaluation of IE2–HDAC2 interaction in virus context, our transfection analyses, which were performed in fully permissive HF cells, demonstrate that the histone acetylation levels on the viral polymerase promoter is suppressed by HDAC2, and that IE2 negates the repressive effect of HDAC2 on this promoter via HDAC2 binding. Given that IE2 binds to several HDACs including HDAC1, HDAC3 and HDAC2 (in this study), IE2, like IE1 (Nevels et al.,
may antagonize host deacetylation for activation of early genes in productive infection. Repression of viral promoters by different HDACs appears to be promoter-specific, since, unlike the viral polymerase promoter, the UL112-113 promoter was not repressed by HDAC2 (data not shown).

IE2 has also been shown to interact with components of the basal transcriptional machinery and with numerous transcriptional factors ([Castillo & Kowalik, 2002; Mocarski & Tan Couecelle, 2001] and references therein). A DNA microarray analysis revealed that IE2 pushes the progression of the cell cycle from G0/G1 toward the G1/S transition point by activating the E2F-responsive genes (Song & Stinski, 2002). The modulation of histone acetylation by IE2 may also contribute to its regulation of cellular genes. In this regard, it is notable that human papillomavirus (HPV) E7 activates E2F transcription by interacting with class I HDACs, thereby facilitating viral replication (Longworth et al., 2005).

Recently, Reeves et al. (2006) showed that IE2 physically interacts with both HDAC1 and histone methyltransferases such as G9a and Suvar(3-9)H1, and that IE2-recruited HDAC1 contributes to a reduction of histone acetylation levels on the MIE promoter at late phases of infection. Our observation that the IE2-associated HDACs still retained at least TSA-sensitive deacetylation activity in vitro also
supports this positive role of HDAC in IE2-mediated autorepression. Since IE2-mediated recruitment of HDAC1 occurred only when the crs was present in the MIE promoter (Reeves et al., 2006), it is conceivable that the initiation of viral DNA replication sensitizes IE2 to recruit HDACs to the crs of MIE promoter or modulates the already-associated HDACs to exert their deacetylation activity in this site.

In the present study, we demonstrate that HDAC2 is dramatically redistributed to viral transcription and replication sites. The rapid accumulation of HDAC2 in IE2 domains, where input viral genome deposition and viral early transcription take place (Ishov et al., 1997), may reflect an attempt by the host to silence the viral promoters via histone deacetylation. We also observed similar formation of the HDAC1-containing foci in virus-infected cells with HDAC1-specific antibody (data not shown). Although HDAC2 binds to IE2 and is colocalized in IE2 domains at early times, expression of IE2 alone was not sufficient to induce typical HDAC2 foci that were found in infected cells, and UV-inactivated virus failed to induce these HDAC2 foci (data not shown). Therefore, it is likely that both viral gene expression and input viral genome are responsible for formation of the typical HDAC2 foci found in infected cells. The recruitment of HDAC2 on viral genome in infected cells also appears to be required for its repressive effect on the viral polymerase promoter, since the transfected Pol promoter in the Pol-LUC reporter construct was not sufficiently repressed by the endogenous levels of HDAC2. We also observed that treatment of cells with lipofectamine induced the formation of some HDAC2 foci (data not shown). Considering that treatment of cells with cationic liposomes induces the activation of mitogen-activated protein kinases (MAPKs) (Iwaoka et al., 2006), it is also possible that the cellular signalling events elicited by virus entry may also contribute to triggering relocalization of HDAC2 to IE2 domains early in infection. The slight increase of total HDAC2 levels at late times in virus-infected cells appears to correlate with both the expression of IE2 and the development of viral pre-replication sites and replication compartments in infected cells. HDAC2 was shown to undergo basal turnover via the ubiquitin (Ub)-proteasome pathway (Kramer et al., 2003). Since expression of IE2 alone did not increase HDAC2 levels, it is likely that deposition of HDAC2 in replication sites may interfere with recognition of HDAC2 by the Ub-proteasome system.

Taken together, our data demonstrate that IE2 functionally interacts with HDAC2 and modulates its deacetylation activity on the viral polymerase promoter. Our results also support the idea that interactions of IE2 with several HDACs to modulate the host epigenetic regulation on viral MIE and early promoters are important events in the process of productive infection. It would be tempting to further investigate how IE2 differentially regulates HDAC activity on different viral promoters at the different stages of infection.

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