Arch Virol
DOI 10.1007/s00705-009-0516-4

ORIGINAL ARTICLE

The transactivating effect of HSV-1 ICP0 is enhanced by its interaction with the PCAF component of histone acetyltransferase

Weizhong Li · Wei Cun · Longding Liu · Min Hong · Lei Wang · Lichun Wang · Chenghong Dong · Qihan Li

Received: 9 June 2009 / Accepted: 6 September 2009

Abstract ICP0 is a multifunctional protein that plays diverse roles in herpes simplex virus type 1 (HSV-1) infection. It can promote the lytic replication of HSV-1 and activate a variety of viral or cellular genes when introduced into cells by transfection or infection. However, the exact mechanism of ICP0 action is not fully understood. In the present study, we observed the co-localization of ICP0 and PCAF (P300/CBP-associated factor), a component of histone acetyltransferase (HAT), in the ND10 (nuclear dot 10) nuclear body. We further confirmed the interaction between ICP0 and PCAF via yeast two-hybrid assay, co-immunoprecipitation, and histone acetyltransferase assays. Analysis of the functional significance of this interaction suggested that PCAF improved the ability of ICP0 to activate transcription of viral genes. Using chromatin immunoprecipitation (ChIP) assays, we observed ICP0-enhanced histone acetylation levels in both viral and cellular gene promoters. Our study suggests that ICP0 regulates transcription through specific interaction with PCAF.

Introduction

Herpes simplex virus 1 (HSV-1, genus *Simplexvirus*, family *Herpesviridae*) possesses a set of regulatory proteins that activates and modulates its own and its host's gene expression. These proteins include immediate-early proteins ICP0, ICP22, ICP27, ICP4, and tegument protein VP16 [25]. Among this group of proteins, ICP0 has long been considered a promiscuous and potent transactivator. It not only activates transcription of all classes of HSV-1 genes [5] but also strengthens the expression of a spectrum of heterologous viral or host cell promoters when introduced into cells by transfection or infection [14, 28, 32], without obvious sequence specificity or *cis*-acting element dependence [12].

ICP0 was found to accumulate initially at or near ND10 (nuclear dot 10) structures when it was expressed via either transfection or virus infection [27]. The main organizing component of ND10 is the promyelocytic leukemia (PML) protein, which is capable of recruiting a number of important factors or co-factors essential for the cellular transcriptional process, including CBP [31]. Therefore, clarification of the interaction between ICP0 and the ND10 compartment will greatly aid our understanding of the molecular basis for ICP0's transactivating function. Nevertheless, there is still no evidence thus far that ICP0 interacts directly with the ND10 component.

Histone acetyltransferase (HAT) and histone deacetyltransferase (HDAC) play a vital role in transcriptional regulation. P300/CBP-associated factor (PCAF), a transcriptional co-activator containing intrinsic HAT activity, mediates transcriptional activation by acetylating nucleosomal histones and numerous transcriptional regulatory factors. Many studies have described the influence of a growing number of viral proteins on PCAF function, including human cytomegalovirus IE86 protein [4], adenovirus E1A and E1B 55 KD proteins [16, 22, 35], and human immunodeficiency virus Tat protein [3].

The genome of HSV-1, like its host cells, is subject to chromatin-mediated repression and regulation. Histone modifications during lytic and latent infections by HSV-1 have been reported [2, 17, 19, 21, 37]. Furthermore, other

W. Li · W. Cun · L. Liu · M. Hong · L. Wang · L. Wang · C. Dong · Q. Li (✉)
Department of Viral Immunology, Institute of Medical Biology, Chinese Academy of Medicine Sciences and Peking Union Medical College, 650118 Kunming, Yunnan, China
e-mail: imbcams.lq@gmail.com; liuld@imbcams.com.cn

Published online: 07 October 2009

work has suggested that HSV-1 gene expression is accelerated by two HDAC inhibitors, sodium butyrate and trichostatin A [10]. Additionally, one research group described a functional interaction between class II HDACs and ICP0 [24], while another study reported that ICP0 initiated the infectious process by releasing viral DNA from its tight chromatin structure [33]. These reports support the hypothesis that ICP0 functions, at least partially, through involvement in the HAT/HDAC system, although the mechanism that underlies this process is still not clearly defined.

To investigate the possible role of ICP0 and the HAT/HDAC system in transcriptional regulation, we performed a series of experiments to analyze the physical interaction of ICP0 and PCAF and its functional significance. Our results suggest that the transactivating ability of ICP0 is enhanced by its interaction with PCAF.

Materials and methods

Cell lines, viruses, plasmids and reagents

HeLa, Vero and L-02 cells (human embryo liver cells) were routinely cultured in Eagle's minimal essential medium supplemented with 10% fetal calf serum at 37°C in 5% CO_2. Stocks of wild-type HSV-1 (strain 17) were prepared in Vero cells.

pEGFP-110wt, which encodes GFP-tagged ICP0, and pEGFP-FXE, which encodes a GFP-tagged RING finger mutant of ICP0, were generous gifts from Roger D. Everet. pDR27, containing the full-length coding sequence of ICP0, pEGFP-PML, pCI-Flag-PCAF and pcDNA3/HA-HDAC1 were kindly provided by Peter O'Hare, Avri Ben-Ze'ev, Yoshihiro Nakatani and Tony Kouzarides, respectively. Plasmids pGBK-01, pGBK-02 and pGBK-03 were constructed by inserting the coding sequence for amino acids (aa) 1–110, 111–241 and 242–775 of ICP0, respectively, into the pGBKT7 vector. The full-length coding sequence of PCAF was amplified from pCI-Flag-PCAF by PCR and inserted into pEGFP-N2 or pGADT7 vector to yield plasmid pEGFP-PCAF or pGAD-PCAF. pcDNA3/HA-PCAF was made by deleting the *Bam*HI/*Eco*RI fragment containing the entire HDAC1 cDNA from pcDNA3/HA-HDAC1 and cloning PCAF cDNA in its place. Plasmid pcDNA3-FXE carrying the RING finger mutant of ICP0 was created by sequentially inserting *Hin*dIII/*Bam*HI and *Bam*HI/*Bam*HI fragments of pEGFP-FXE into the pcDNA3 vector. Three reporter plasmids, pICP4-CAT, pTK-CAT and pgC-CAT, were generated by fusing the ICP4 gene promoter (nt −332 to +30), TK gene promoter (nt −250 to +1) and gC gene promoter (nt −124 to +71), respectively, to the chloramphenicol acetyltransferase (CAT) gene within the pCAT3-basic vector. All of the constructs were verified by sequencing.

Rabbit polyclonal antibody against acetyl-histone H3 was purchased from Upstate (Lake Placid, NY, USA), rabbit anti-HA polyclonal antibody and rabbit anti-PML polyclonal antibody from Santa Cruz (Santa Cruz, CA, USA), rabbit anti-PCAF polyclonal antibody from Abcam (Cambridge, MA, USA), mouse anti-HA monoclonal antibody from Tiangen (Beijing, China), mouse anti-β-actin antibody and peroxidase-conjugated goat anti-rabbit antibody from Sigma (St. Louis, Mo, USA), TRITC- and peroxidase-conjugated goat anti-mouse antibody from Dingguo (Beijing, China), Cy3-labeled goat anti-rabbit antibody from Beyotime (Jiangsu, China), and Alexa Fluor 488-conjugated goat anti-mouse antibody from Molecular Probes (Eugene, OR, USA). Mouse polyclonal antibody against the amino-terminal 1–105 aa of ICP0 was produced by our laboratory.

Immunofluorescence microscopy

HeLa cell cultures on coverslips were transfected with the appropriate plasmids using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) or infected with HSV-1 at an MOI of 1. At the indicated time points post-transfection or postinfection, cells were washed with PBS, fixed in 4% paraformaldehyde, and permeabilized with 0.2% Triton X-100. Then, the transfected cells were incubated with mouse anti-ICP0 antibody (1:500) or mouse anti-HA antibody (1:500) for 2 h and further incubated with TRITC-conjugated goat anti-mouse antibody (1:250) for 1 h. The slides were finally examined using a Nikon E600 fluorescence microscope. Infected cells were detected with mouse anti-ICP0 antibody (1:500) and either rabbit anti-PCAF antibody (1:400) or rabbit anti-PML antibody (1:500), followed by staining with Alexa Fluor 488-conjugated goat anti-mouse antibody (1:300) and Cy3-labeled goat anti-rabbit antibody (1:300) for the final confocal microscopy analysis.

Yeast two-hybrid assay

Yeast two-hybrid assay was carried out using the MATCHMAKER GAL4 two-hybrid system 3 (Clontech, Palo Alto, CA, USA) according to the manufacturer's protocols. Briefly, to test the potential interaction between ICP0 and PCAF, yeast strain AH109 or Y187 was transformed with plasmid pGBK-01, pGBK-02 or pGBK-03 along with pGAD-PCAF and plated onto SD/-Leu/-Trp (DDO) and SD/-Ade/-His/-Leu/-Trp medium (QDO). Yeast transformed with plasmids pGBKT7-P53 plus pGADT7-T and pGBKT7-lam plus pGADT7-T was used as positive and negative control, respectively. The plates

were incubated at 30° until the appearance of colonies between 3 and 7 days. Then, the Y187 colonies growing on the DDO agar plates were picked and subjected to β-galactosidase activity analysis following the manufacturer's instructions.

Co-immunoprecipitation and Western blot analysis

L-02 cells were transfected with the indicated plasmid or infected with HSV-1 at an MOI of 1. Forty h post-transfection or 4 h postinfection, cells were washed with PBS and lysed for 30 min on ice in cold NP-40 lysis buffer (50 mM Tris–HCl, pH 8.0, 150 mM NaCl, 1% NP-40, 1 mM phenylmethylsulfonyl fluoride, 1 mg aprotinin/ml, 0.1 mg leupeptin/ml). The cell lysate was centrifuged at 14,000*g* for 10 min, and the supernatant was precleared by incubation with protein A-agarose (Santa Cruz) for 1 h at 4°C. Then, the sample was incubated with the mouse anti-ICP0 antibody for 2 h at 4°C with gentle agitation. Normal mouse IgG was used as a control. Protein A-agarose beads were added to the protein-antibody mixture and incubated overnight at 4°C with rotation. The beads were collected by centrifugation and washed once with RIPA buffer (50 mM Tris–HCl, pH 8.0, 150 mM NaCl, 1% NP-40, 0.1% SDS, 0.5% sodium deoxycholate and a protease inhibitor mixture) and three times with NP-40 lysis buffer. Bound proteins were eluted by boiling in 2× SDS loading buffer for 5 min and then were separated on 10% SDS-PAGE gels. Western blot analysis was carried out using rabbit anti-PCAF antibody and peroxidase-conjugated goat anti-rabbit antibody. Immunoblots were developed using ECL detection reagents (Tiangen, Beijing, China).

Immunoprecipitation and histone acetyltransferase assay

The in vitro acetylation assay was performed as described elsewhere [4]. Briefly, 6×10^6 L-02 cells were transfected with 20 μg of total plasmid DNA or infected with HSV-1 at an MOI of 1. At 48 h post-transfection or 4 h postinfection, cells were resuspended in 0.5 ml IPH buffer [50 mM Tris–HCl (pH 8.0), 150 mM NaCl, 5 mM EDTA, 0.5% NP-40, 0.5 mM phenylmethylsulfonyl fluoride and a protease inhibitor cocktail] and left on ice for 20 min before clearing by centrifugation at 12,000*g* for 10 min. Anti-ICP0 or anti-PCAF antibody was added to the supernatants, which were then rotated for 2 h before protein A-agarose beads were added. The mixtures were then incubated overnight at 4°C with gentle rotation. The immune complexes were pelleted by gentle centrifugation and washed three times with 1 ml of IPH buffer. After the final washing, each precipitate was resuspended in 30 μl of IPH buffer. Following addition of [^{3}H]-labeled acetyl coenzyme A and core histones, complexes were incubated at 30°C for 1 h, and then 10 μl of the sample was spotted onto Whatman P81 filter paper. Filters were washed three times with 10% trichloroacetic acid and once with 95% ethanol and then air-dried prior to counting in a liquid scintillation counter (LS 6000, Beckman Coulter).

Analysis of CAT enzymatic activity

L-02 cells were cultured in six-well plates and grown to 90% confluence. Transient transfections were performed using CAT reporter plasmids along with different combination of effector plasmids (pDR27, pcDNA3-FXE, or pcDNA3/HA-PCAF). pcDNA3 empty vector was added to normalize the total amount of DNA per transfection. Forty-eight hours after transfection, cells were rinsed with PBS and harvested into 200 μl of lysis buffer (Promega, Madison, WI, USA). CAT activity was analyzed by incubation of cell extracts (110 μl) with 10 μl [^{3}H]-chloramphenicol (0.025 mCi/ml) and 5 μl *n*-butyryl coenzyme A (Sigma) at 37°C for 6 h, followed by xylene extraction and liquid scintillation counting. The results were plotted as a mean of three independent experiments.

Chromatin immunoprecipitation

Confluent L-02 cells were transfected with the indicated plasmids and treated 48 h later with formaldehyde (1% final concentration) for 10 min at room temperature to cross-link proteins to DNA. Cells were washed with cold PBS and lysed in SDS lysis buffer (1% SDS, 10 mM EDTA, 50 mM Tris–HCl, pH 8.1, and a protease inhibitor cocktail). The lysate was sonicated to shear DNA to a length between 200 and 1,000 bp. The sonicated supernatant was diluted 10-fold with ChIP dilution buffer [0.01% SDS, 1% Triton X-100, 1.2 mM EDTA, 16.7 mM Tris–HCl (pH 8.1), 167 mM NaCl], precleared with salmon sperm DNA/protein A-agarose (Upstate) for 1 h, and then incubated with rabbit polyclonal antibody against acetyl-histone H3 (Upstate) or preimmune serum overnight at 4°C with rotation. Immune complexes were collected using salmon sperm DNA/protein A-agarose and washed sequentially for 5 min each with low-salt buffer, high-salt buffer, LiCl buffer and TE buffer. The pellet was dissolved in elution buffer (1% SDS and 0.1 M $NaHCO_3$) and centrifuged to remove the agarose. The supernatant was treated with 5 M NaCl and heated to 65°C for 8 h to reverse cross-links. The immunoprecipitated DNA was purified by proteinase K treatment, phenol–chloroform extractions, and ethanol precipitation. Immunoprecipitated DNA and input (non-immunoprecipitated) DNA were analyzed quantitatively by real-time PCR using SYBR green reagent. The specific primer sets used were as follows:

5′-CCCCCTGCCCGTTCCTCGTT-3′ and 5′-GGGGCGTC CTCGGGCTCATAT-3′ for the ICP4 promoter; 5′-AGTG CAGGTGCCAGAACATTTC-3′ and 5′-GGCGGGGTTT GTGTCATCATAG-3′ for the TK promoter; 5′-ATGATTT CGCCATAACACCCAA-3′ and 5′-ATCTAC CTCCACA CGGACCA-3′ for the gC promoter; 5′-ACCGGCTGG CCTGCTGGAACT-3′ and 5′-TCTGCCGCCGCTCTCTC ACCT-3′ for the P21 promoter; 5′-AGCCTGGAAATGCT CTAT-3′ and 5′-TTGAAGGGTACTTGGTGT-3′ for the CGA promoter; 5′-TGCGTCCCTCACTGA AACCT-3′ and 5′-CTCGCTTGCTTCCTCCTCC-3′ for the Bcx-xL promoter; and 5′-CTGCGCATAGCAGACATACAA-3′ and 5′-CTGGGCTTGAGAGGTAGAGTG-3′ for the β-actin promoter. Relative enrichment factors were calculated as $2^{-\Delta\Delta C_t}$, where $\Delta\Delta C_t = \Delta C_t$ (ICP0 or FXE) $- \Delta C_t$ (pcDNA3), ΔC_t (ICP0 or FXE) $= C_t$ (IP) $- C_t$ (input), and ΔC_t (pcDNA3) $= C_t$ (IP) $- C_t$ (input).

Results

Association of ICP0 with PCAF in the nuclei of transfected cells

Using fluorescent microscopy, we observed an overlapping distribution of the GFP-ICP0 fusion protein and PCAF, which indicated that ICP0 was localized to the same location as the PCAF component of HAT in HeLa cells (Fig. 1a, A–C). This co-localization pattern was similar to that of ICP0 and PML (Fig. 1a, D–F). Although localization of PCAF in ND10 has not been reported, some proteins, including another component of HAT, CBP, have been reported to be conditionally recruited to ND10 structures [31]. Upon further evaluation, PCAF was found to be located at the same position as PML in transfected HeLa cells, but with a lower degree of overlapping distribution between them (Fig. 1a, G–I). In addition, confocal immunofluorescence microscopy was used to ascertain whether there was a spatial association of ICP0 with PCAF in the context of infection. Our results clearly showed the apparent co-localization of ICP0 and PCAF in the same subnuclear sites at 4 h postinfection (Fig. 1b).

Physical interaction between ICP0 and PCAF

The observed co-localization of ICP0 and PCAF with PML structures gave rise to a reasonable prediction: ICP0 may interact directly with PCAF. Therefore, the yeast two-hybrid technique was used to investigate this hypothesis. For this study, three bait plasmids containing different coding sequences of ICP0 were designed to avoid the transcriptional auto-activation function in yeast (Fig. 2a). The results showed that, similar to the positive control (T plus p53), the yeast strain AH109 containing ICP0 (111–241 aa) and PCAF was able to grow on QDO plates (data not shown). Additionally, co-expression of ICP0 (111–241 aa) and PCAF led to much higher levels of β-gal activity compared to the negative control (T plus Lam) (Fig. 2b), implying that this RING finger motif-containing region was essential for the interaction between ICP0 and PCAF.

Further investigation to establish whether ICP0 and PCAF interacted was carried out using co-immunoprecipitation assays in transfected or infected mammalian cells. We found that the ICP0 antibody is capable of precipitating a complex containing ICP0 and PCAF from extracts of L-02 cells transfected with pDR27, suggesting a physical interaction between ectopically expressed ICP0 and endogenous PCAF (Fig. 3a). However, the fact that a RING finger motif deletion mutant of ICP0 lost the ability to interact with PCAF indicates that the interaction between these two molecules was probably mediated by the RING finger motif (Fig. 3a). A supporting experiment, in which the extracts from cells infected by HSV-1 were precipitated by ICP0 antibody and detected by anti-PCAF antibody, also verified the interaction of ICP0 and endogenous PCAF during the viral infection process (Fig. 3b).

In view of the intrinsic histone acetyltransferase activity of PCAF, we next examined if the ICP0-containing complex possessed HAT activity by performing immunoprecipitation and histone acetyltransferase (IP-HAT) assays. As shown in Fig. 4, the complex precipitated by the ICP0-specific antibody from cells co-transfected with ICP0 and PCAF plasmids exhibited significantly higher HAT activity than those from cells transfected with the ICP0 or PCAF plasmid alone. Conversely, cellular lysates precipitated by the ICP0 antibody did not display any HAT activity when the FXE plasmid was introduced into cells either alone or in combination with the PCAF plasmid. Meanwhile, the precipitate from ICP0-transfected or HSV-1-infected cells also produced elevated HAT activity relative to the negative control. However, this was due to the binding of native cellular PCAF to ICP0. The above findings further supported the interaction between ICP0 and PCAF via the RING finger motif.

PCAF is able to improve transactivation of ICP0 on viral genes

ICP0 can transactivate the expression of the HSV-1 α, β, and γ-genes; however, the mechanism is not completely understood. Most likely, since ICP0 does not bind a DNA sequence motif, it functions through its interactions with other cellular or viral proteins. In this case, its interactions with other proteins might impact its effect on transactivation. Here, a reporter assay was employed to investigate the combinatorial effect of ICP0 and PCAF on transactivation

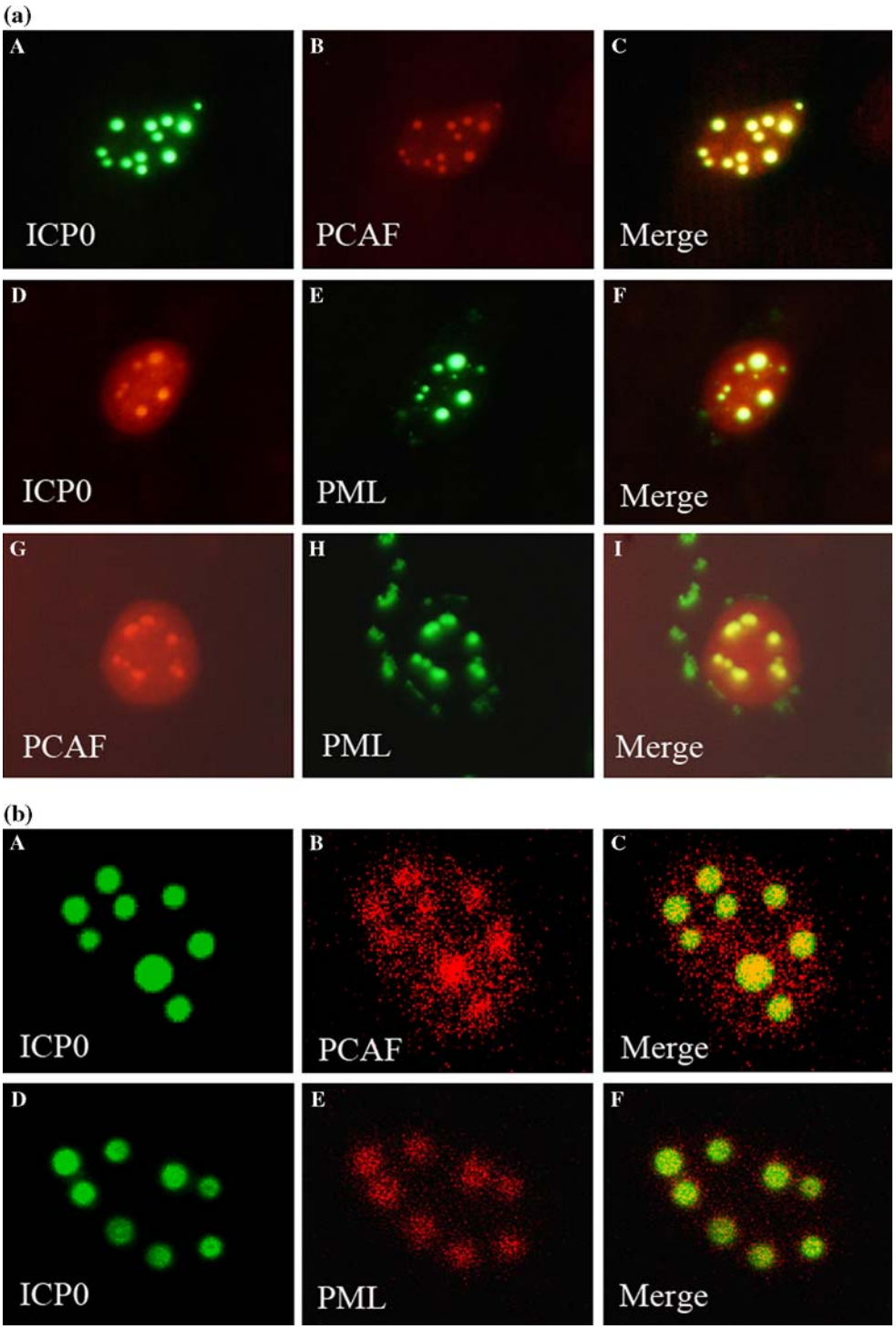

Fig. 1 Association of ICP0 and PCAF in the ND10 nuclear body of HeLa cells. **a** HeLa cells were transfected with the following plasmids: pEGFP-110wt and pcDNA3/HA-PCAF (*A–C*), pDR27 and pEGFP-PML (*D–F*), or pcDNA3/HA-PCAF and pEGFP-PML (*G–I*). At 36 h post-transfection, cells were fixed, permeabilized, incubated with either mouse anti-ICP0 antibody (*D–F*) or mouse anti-HA antibody (*A–C*, *G–I*) for 2 h, and stained with TRITC-conjugated goat anti-mouse IgG for 1 h. Images were viewed using a Nikon E600 fluorescence microscope. **b** HeLa cells were infected with HSV-1 at an MOI of 1 for 4 h, followed by incubation with mouse anti-ICP0 antibody and either rabbit anti-PCAF antibody (*A–C*) or rabbit anti-PML antibody (*D–F*) for 2 h. The cells were then subjected to staining with Alexa Fluor 488-conjugated goat anti-mouse antibody and Cy3-labeled goat anti-rabbit antibody for 1 h. Digitized images were captured with a Zeiss confocal microscope

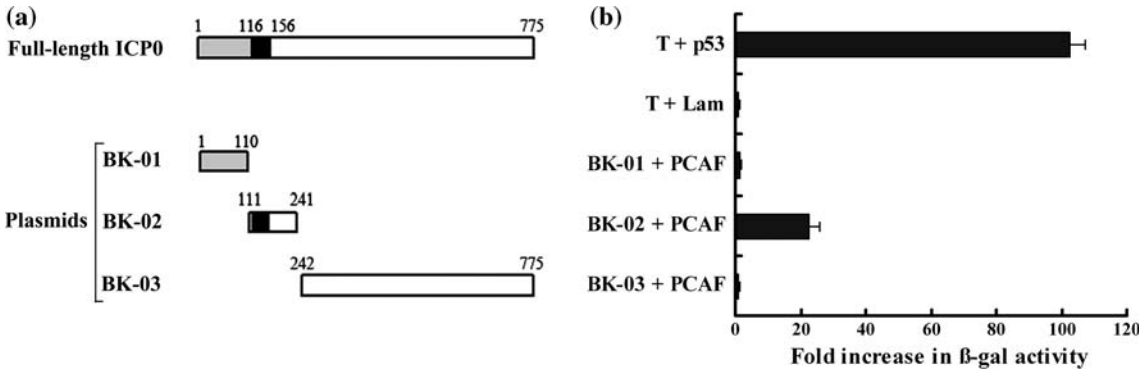

Fig. 2 Physical interaction of ICP0 and PCAF in yeast two-hybrid assay. **a** Schematic diagrams illustrate the full-length ICP0 and its truncated mutants used in this experiment. *Solid black bars* indicate a RING finger domain with positions mapped between amino acids 116–156. The first and last amino acid residues in each construct are also *numbered*. **b** Determination of β-galactosidase activity in yeast. Yeast strain Y187 was transformed with the indicated plasmids, and the activity of β-galactosidase was measured. The data represent fold-increases of β-galactosidase activity relative to the negative control (pGADT7-T plus pGBKT7-Lam) from three independent experiments

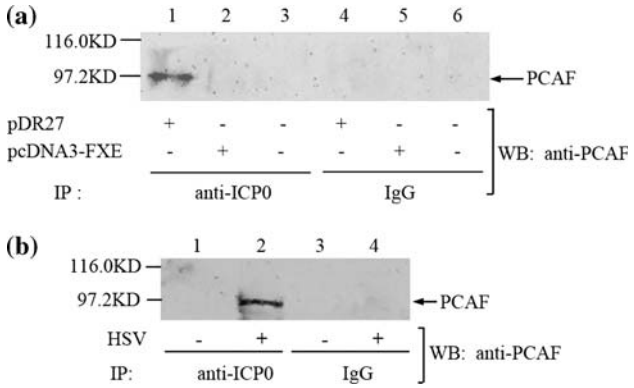

Fig. 3 Examination of the interaction between ICP0 and PCAF using the Co-IP method. After 40 h post-transfection with the plasmid pDR27 or pcDNA3-FXE (**a**), or 4 h post-infection with HSV-1 at an MOI of 1 (**b**), L-02 cells were lysed in NP-40 buffer and precipitated with mouse anti-ICP0 antibody or normal mouse IgG, followed by blotting with rabbit anti-PCAF antibody

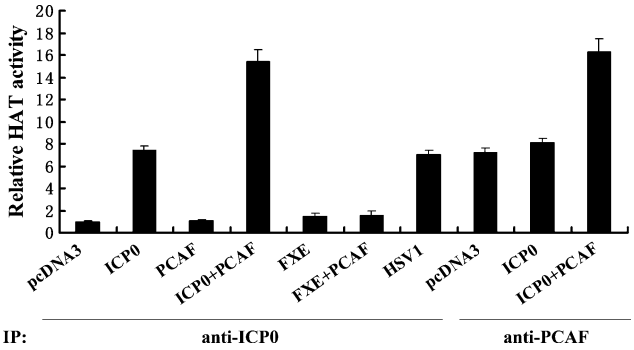

Fig. 4 HAT activity detection in complexes associated with ICP0. L-02 cells were transfected with the indicated plasmids or infected with HSV-1. HAT activity of the complex precipitated with ICP0 antibody or PCAF antibody from cell extracts was determined as described in "Materials and methods". Data represent the average fold-increase in HAT activity over activity detected in the control group (cells transfected with pcDNA3 and precipitated by anti-ICP0 antibody) from three independent experiments

of specific genes. Interestingly, the expression of PCAF obviously increased the transactivation efficiency of ICP0 on promoters of α-4 (ICP4), TK, and gC genes (Fig. 5a). Next, we asked whether the enhanced transactivation of ICP0 in the CAT assay was caused by the increased expression of ICP0 in the presence of PCAF; we found that the expression level of ICP0 and PCAF remained unchanged when they were transfected into cells cooperatively or individually, as shown by western blot analysis (Fig. 5b).

ICP0 can improve acetylation of viral and cellular gene promoters

As shown by the above data, ICP0 regulated viral gene transcription through interaction with or recruitment of the PCAF component of HAT. This finding led to questions regarding the ability of ICP0 to influence the acetylation process. If this is the case, ICP0 should non-specifically impact the acetylation process associated with PCAF, including histone acetylation related to chromosome remodeling. Based on this inference, ChIP analysis was performed to evaluate the impact of ICP0 on the acetylation of histones. Figure 6a shows a marked elevation of acetylated histone levels around the HSV1 α-4, TK, and gC promoters in the presence of ICP0. Moreover, increased histone acetylation of the cellular gene promoters p21 and CGA was also apparent when ICP0 was introduced into cells by transfection (Fig. 6b). Interestingly, ICP0 did not seem to impact acetylation of the BcL-xL promoter or the constitutively active β-actin promoter (Fig. 6b). The reason for this is unclear, but in this experiment, we were not able

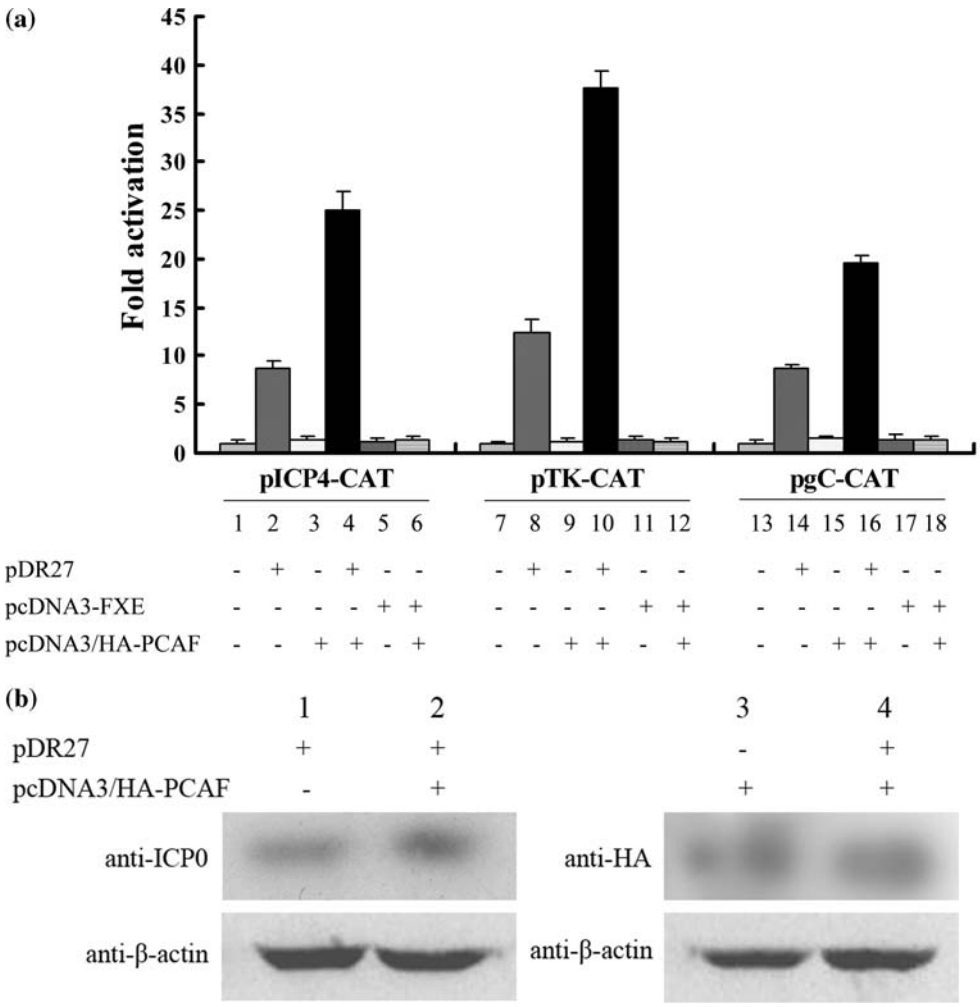

Fig. 5 Enhanced transactivating ability of ICP0 by PCAF in CAT assays. **a** Synergistic effects of ICP0 and PCAF on the transcriptional activation of HSV-1 α, β, and γ promoters. L-02 cells were transfected with reporter plasmids (pICP4-CAT, pTK-CAT, or pgC-CAT) in parallel with different combinations of the expression vectors for ICP0, FXE, and PCAF. pcDNA3 was used to normalize the amount of DNA in each transfection. CAT assays were performed on cellular extracts as described in "Materials and methods". CAT activity of cells transfected with pICP4-CAT, pTK-CAT, or pgC-CAT individually within each reporter group was normalized to one. The average value of three independent experiments is shown. **b** The expression levels of ICP0 and HA-PCAF remain unchanged. Cells were transfected with ICP0- and HA-PCAF-encoding plasmids, either alone or in combination, and western blot assays were performed using anti-ICP0, anti-HA, or anti-β-actin antibodies

to suitably determine that PCAF, as an intermediate, was involved in this process. Moreover, it should be noted that FXE, which cannot interact with PCAF, also failed to promote histone acetylation of all promoters tested (Fig. 6b). Therefore, ICP0 improved the acetylation of histones around viral and some cellular gene promoters by coordination with PCAF.

Discussion

Accumulated data have indicated that the process of acetylation of histone or non-histone proteins by HAT might be influenced by some viral proteins during infection. For example, adenoviral oncoprotein E1A can directly bind and suppress the acetyltransferase activity of P300 and PCAF to facilitate viral replication and cell transformation [6, 16, 35]. Likewise, interaction between the BZLF1 protein of Epstein-Barr virus and CBP is able to promote viral early gene transcription [1].

HSV-1 ICP0, an important regulator of viral gene transcription, interacts with class II HDACs 4, 5, and 7 and overcomes MEF2A repression mediated by the amino terminus of the HDAC [24]. ICP0 can also dissociate HDAC1/2 from the REST/CoREST complex and therefore suppress the inhibitory capacity of this complex [15]. Another study demonstrated that bICP0, the homolog encoded by bovine herpes virus 1, interacted with histone acetyltransferase P300 and augmented a productive infection [40]. In addition, a recent article confirmed that ICP0 promoted both histone removal and acetylation of viral DNA to facilitate the replication of HSV-1 [8]. Therefore, it is evident that ICP0 utilizes cellular acetylation and deacetylation functions to create a favorable host environment for virus growth. However, the exact mechanism by which this occurs is far from being clear.

In the present study, we investigated the interaction between ICP0 and the PCAF component of HAT. Using immunofluorescence microscopy, we found that ICP0 was co-localized with PCAF in the ND10 nuclear body in

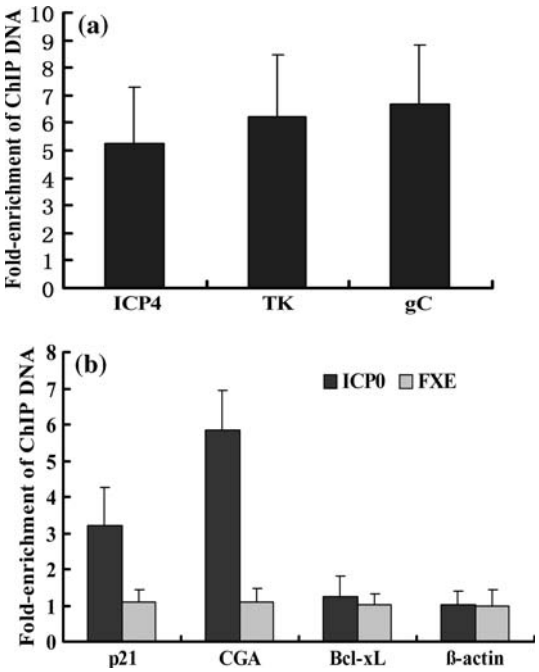

Fig. 6 ICP0 improves the acetylation of histone wrapping in viral or specific cellular promoters. **a** Effect on viral promoters by ectopic expression of ICP0. L-02 cells were transfected with pICP4-CAT, pTK-CAT, or pgC-CAT in combination with the ICP0-expressing plasmid (pDR27) or pcDNA3. Acetylation of plasmid-DNA-associated histone H3 was determined by ChIP assay with anti-acH3 antibody and specific primers complementary to the promoters of HSV-1 α-4, TK, and gC. The data represent the average fold-increase in immunoprecipitated DNA from the effector group (transfected with ICP0) over that from the control group (transfected with pcDNA3). The values were calculated using the formula $2^{-\Delta\Delta C_t}$ as described in "Materials and methods". **b** Influence of over-expressed ICP0 or its RING finger mutant (FXE) on cellular promoters. L-02 cells transfected with pDR27, pcDNA3-FXE, or pcDNA3 plasmid were subjected to ChIP analysis using specific primer sets for p21, CGA, Bcl-Xl, and *β*-actin. Fold-enrichment of immunoprecipitated DNA from ICP0- or FXE-transfected cells compared to DNA from pcDNA3-transfected cells was determined by the $2^{-\Delta\Delta C_t}$ method

transfected or infected cells. ND10 is characterized by the presence of PML, a main organizing component, and many other proteins, including CBP [31]. In our experiments, PCAF was localized to ND10 nuclear bodies to some extent. In view of the well-established interaction between P300/CBP and PCAF [39], we can also speculate that ND10 nuclear bodies serve as storage sites for these important histone acetyltransferases, which can be utilized by ICP0 to suit the needs of viral replication.

Several lines of evidence from the yeast two-hybrid, co-IP, and IP-HAT assays clearly demonstrate a RING-finger-motif-dependent interaction of ICP0 and PCAF, which highlights the important role of this region for the transactivating capability of ICP0. The RING finger domain of ICP0 contains a C3HC4 zinc-binding motif that exists in proteins involved in signal transduction, ubiquitination, gene transcription, differentiation, and morphogenesis [13]. In addition, all members of the alphaherpesvirus family encode a protein with similar function to ICP0 of HSV-1, including BICP0 in bovine herpesvirus 1 [38], Vg61 in varicella-zoster virus [29], Eg63 in equine herpesvirus 1 [36], and EP0 in pseudorabies virus [7]. However, these proteins show little amino acid residue similarity, with the exception of the conserved RING finger domain near their N-termini [11, 13, 23]. If these homologs of ICP0 exert their transcriptional regulatory functions through similar mechanisms, the interaction between these proteins and an unidentified cellular factor via their RING finger domains may play a vital role. Given the results of our experiments, PCAF may be seen as a candidate. Interaction between PCAF and functional molecules usually brings about acetylation of the molecules [26, 34]. However, no evidence for direct acetylation of ICP0 by PCAF was found in our study (data not shown).

To further define whether the physical interaction of ICP0 and PCAF had functional significance, we examined the effect of ICP0 and PCAF on viral promoter activity using CAT reporter assays. For this, promoters of the HSV1 α-4, TK, or gC genes were inserted into the pCAT3-basic vector. The results showed that ICP0 improved transcription of these promoters and, most importantly, that ICP0 cooperated with PCAF to augment the transcriptional efficiency of these promoters to a higher level than ICP0 alone.

A recent study demonstrated ICP0-mediated viral genome de-repression in latent infection through enrichment of acetylated histones at viral promoters [9]. Moreover, another group reported that ICP0 was required for both the reduction of HSV-1 DNA association with histone H3 and the increase in histone acetylation [8]. Combined with our results described above, a reasonable explanation for these findings is that the elevated acetylated histone levels on HSV-1 genes might be at least partly attributed to the interaction between ICP0 with PCAF. To further confirm this hypothesis, we performed ChIP assays using an antibody against acetyl-histone H3. Three viral gene promoters (HSV-1 α-4, TK, and gC) encoded by recombinant plasmids that can be bound by histones and form chromatin-like structures [18, 20, 30], as well as four cellular gene promoters (p21, CGA, BcL-xL and *β*-actin) within native chromatin, were tested. The results showed that the presence of ICP0 increased acetylation of histone H3 wrapping in the promoters of HSV1 α-4, TK, gC, p21, and CGA, probably through recruitment of PCAF. Interestingly, ICP0 did not affect BcL-xL and *β*-actin promoter wrapping by histones. The reason for this is still unclear, but this may represent a differential and promoter-specific influence by ICP0. In addition, a RING finger mutant of ICP0 failed to influence histone acetylation around the promoters due to a lack of interaction with PCAF.

In conclusion, our data suggest that ICP0 indeed targets cellular acetylation signaling functions to create an optimal environment for the initiation of viral gene transcription. To further explore the role of ICP0 in transactivation, it is necessary to extensively investigate and characterize the relationship of ICP0 with various components of the HAT/HDAC system.

Acknowledgments We thank Drs. Roger D. Everet, Peter O'Hare, Yoshihiro Nakatani, Tony Kouzarides, and Avri Ben-Ze'ev for plasmids. This work was supported by the National Science Fund (No. 30670094) and Scientific Research Fund of the Institute of Pathogen Biology (No. 2007IPB10).

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