Cellular and viral chromatin proteins are positive factors in the regulation of adenovirus gene expression

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ABSTRACT

The adenovirus genome forms chromatin-like structure with viral core proteins. This complex supports only a low level of transcription in a *cell-free* system, and thus core proteins have been thought to be negative factors for transcription. The mechanism how the transcription from the viral DNA complexed with core proteins is activated in infected cells remains unclear. Here, we found that both core proteins and histones are bound with the viral DNA in early phases of infection. We also found that acetylation of histone H3 occurs at the promoter regions of viral active genes in a transcription-independent manner. In addition, when a plasmid DNA complexed with core proteins was introduced into cells, core proteins enhanced transcription. Knockdown of TAF-I, a remodeling factor for viral core protein–DNA complexes, reduces the enhancement effect by core proteins, indicating that core proteins positively regulate viral transcription through the interaction with TAF-I. We would propose a possible mechanism that core proteins ensure transcription by regulating viral chromatin structure through the interaction with TAF-I.

INTRODUCTION

The adenovirus (Ad) genome is a linear double-stranded DNA of $\sim$36 000 bp in length and is covalently bound with terminal proteins (TP) at each 5′-terminus. In the virion, it forms a chromatin-like complex, designated Ad-core, with viral basic core proteins VII, V and polypeptide μ. Protein VII is a major component of Ad-core and has limited amino acid sequence homology with protamine, a sperm-specific basic protein (1). In purified Ad-core, protein VII is bound to the viral DNA most tightly and seems to be the only protein component of 'beads-on-a-string' structure (2). Although the precise structure of protein VII–DNA complexes is not clear, protein VII could introduce superhelical turns into DNA, as do cellular histones (3). Protein V is less tightly associated with protein VII–DNA complexes and seems to tether them to the capsid structure (4,5). Polypeptide μ has a strong ability to condense DNA (6).

The nuclear import of the viral genome occurs through nuclear pore complexes (NPC), concomitantly with disassembly of the virion (7). After the entry of the viral genome into the nucleus, protein V seems to be dissociated from viral chromatin (8). On the other hand, protein VII remains associated with the viral DNA during early phases of infection, suggesting that at least protein VII is a component of viral template for early transcription and DNA replication in infected cells (9,10). However, core proteins function as repressors for transcription and replication in 'cell-free' systems (11,12). It is also reported that the expression of protein VII leads to condensation of DNA and appears to repress transcription in *Xenopus* oocytes (13). Thus, protein VII is thought to be a negative factor for genome functions. However, the exact role of protein VII in infected cells remains to be determined. Viral genes are expressed with the temporal regulation and divided into two major groups, early and late genes. In early phase of infection, viral early genes (e.g. E1A, E3 and E4 genes) are actively transcribed, whereas late genes (e.g. MLP, IVa2 genes) are kept silent in that time, and then activated concomitantly with the onset of viral DNA replication.

We have identified Template Activating Factor (TAF)-I, TAF-II/NAP-1 and TAF-III/nucleophosmin/B23 from uninfected HeLa cell extracts as stimulatory factors in 'cell-free' Ad DNA replication and transcription systems using Ad-core as a template (11,14–17). Biochemical analyses revealed that TAF-I forms a stoichiometric complex with protein VII–DNA complexes and enhances the nuclease sensitivity of Ad-core, suggesting that TAF-I remodels Ad-core by formation of ternary complexes,

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thereby facilitating transcription and replication from Ad-core (10,18). In good agreement with the biochemical results, TAF-I is bound to viral chromatin through the interaction with protein VII during early phases of infection in infected cells (10). Knockdown (KD) of TAF-I expression results in reduction of the expression level of viral early genes, suggesting that TAF-I plays an important role in early phases of infection in infected cells (19). In addition to its role in Ad life cycle, it is shown that TAF-I regulates the cellular gene expression through its histone chaperone activity. TAF-I binds to histones directly and remodels chromatin template in a 'cell-free' system (20). It is shown that TAF-I stimulates a subset of genes in a histone acetylation-independent manner (21). TAF-I is also reported as a component of an inhibitor of the histone acetyltransferase complex (INHAT) (22). TAF-III/nucleophosmin/B23 is a nucleolar protein and functions as a histone chaperone for the regulation of rRNA gene expression (23). Recently, we have reported that B23 interacts with core protein V and a precursor protein of protein VII in late phases of infection, and has a potential role for viral chromatin assembly (24).

Association of cellular histones with the viral genome DNA in infected cells remains controversial. It was shown that the nucleosome-like ladder of intranuclear viral DNA is generated by micrococcal nuclease (MNase) digestion, indicating the possibility that core proteins are replaced with cellular histones during early phases of infection (25–27). On the other hand, in the UV cross-linking experiments, no interaction between viral DNA and cellular histones was observed during early phases of infection (9). To clarify the structure of viral chromatin in infected cells, we performed chromatin immunoprecipitation (ChIP) assays and found that viral chromatin is associated with both viral protein VII and cellular histones with post-translational modification (acetylation of histone H3) in infected cells. In addition, by introducing purified Ad-core or reconstituted protein VII–DNA complexes into cells, we found that protein VII enhances transcription and TAF-I is required for this effect of protein VII, but not for the binding or acetylation of histone H3. Taken together, the results obtained here suggest that viral transcription is positively regulated by protein VII and a host factor. We would propose a possible mechanism that core proteins ensure transcription by regulating viral chromatin structure through the interaction with TAF-I.

MATERIALS AND METHODS

Cells and viruses

The monolayer cultures of HeLa cells and TAF-I KD HeLa cell lines (clones 4 and 7) (19) were maintained at $37°C$ in minimal essential medium (MEM; Nissui) containing 10% fetal calf serum and used for all experiments in this study. Purification and infection of human adenovirus type 5 (HAdV5) were carried out essentially as described earlier (19). Hydroxyurea and α-amanitin (Sigma) were added at the final concentration of 2 mM and 25 μg/ml right after infection when DNA replication and transcription were to be blocked, respectively.

siRNA-mediated knockdown of TAF-I expression

TAF-I Stealth siRNA and negative control Stealth siRNA (Invitrogen) were introduced into HeLa cells with Lipofectamine 2000 (Invitrogen) according to the manufacturer's protocol. After incubation for 3 days, the reduction of the expression level of TAF-I was confirmed by western blot analysis using rabbit anti-TAF-Iα/β antibody (see below).

Antibodies

Antibodies used in this study are as follows: rat antiprotein VII (10), mouse anti-TAF-Iβ (KM1720) (28), rabbit antihistone H3 (catalog no. ab1791; abcam), antiacetylated histone H3 (catalog no. 06-599; Millipore), mouse anti-FLAG M2 (catalog no. F3165; Sigma), mouse anti-β-actin (Sigma), rabbit anti-Hsp90 [kindly provided by Drs Y. Miyata and E. Nishida (Kyoto University)], rabbit anti-RNA polymerase II (N-20, catalog no. sc-899; santa cruz biotechnology) and rabbit anti-Sp1 (catalog no. 07-645; Millipore) antibodies. Rabbit anti-TAF-Iα/β antibody was raised against His-tagged TAF-IβΔC3 (29) and purified with antigen-coupled affinity column according to standard protocols.

Recombinant proteins

Preparation of recombinant protein VII was carried out as described earlier (10,18).

Transfection of Ad-core, TP-DNA and reconstituted complexes

Ad-core and TP-DNA were purified from virions as described earlier (11). Briefly, Ad virions were purified by centrifugation in CsCl solution, followed by dialysis against 10 mM Tris–HCl (pH 7.9) and 1 mM EDTA as described earlier. For the purification of Ad-core, virions were heated at $56°C$ for 60 s in the presence of 0.5% sodium deoxycholate and immediately chilled on ice, and then Ad-core was purified by centrifugation in a 15–60% glycerol density gradient in 10 mM Tris–HCl (pH 7.9) and 1 mM EDTA. For the purification of TP-DNA, virions were denatured with guanidine hydrochloride (GuHCl) at the final concentration of 4 M. TP-DNA was purified by centrifugation in a 5–20% sucrose gradient in 10 mM Tris–HCl (pH 7.9), 1 mM EDTA, 4 M GuHCl and 2 mM β-mercaptoethanol and dialyzed against 10 mM Tris–HCl (pH 7.9), 1 mM EDTA and 20% glycerol. For reconstitution of protein VII–DNA complexes, pSV-Luc (PGV-C, TOYO INK, 100 ng) DNA and recombinant proteins were incubated in a buffer containing 40 mM Tris–HCl (pH 7.9), 150 mM NaCl and 12.5% glycerol at $37°C$ for 15 min, and formed complexes were confirmed by a 1% agarose gel electrophoresis in $0.5 \times$ TBE (Tris–borate/EDTA). HeLa cells were transfected with Ad-core, TP-DNA, or the reconstituted complexes (100 ng DNA) using GeneJuice (Novagen) according to the manufacturer's protocol. For separation of cytoplasmic and nuclear fractions, transfected cells were lysed with a buffer containing 10 mM Tris–HCl (pH 7.9), 100 mM NaCl, 3 mM $MgCl_2$

and 0.5% NP-40. After centrifugation, the supernatant fraction was recovered as a cytoplasmic fraction, while the pellet was resuspended in the same buffer and used as a nuclear fraction. To confirm the separation procedure, each fraction was subjected to a 12.5% SDS–PAGE, followed by western blot analyses using anti-Hsp90 and antihistone H3 antibodies. Cytoplasmic or nuclear DNA was recovered from each fraction and subjected to PCR using the specific primers for the MLP or SV40 promoter regions where described.

ChIP assays

ChIP assays were carried out according to the protocol from Chromatin Immunoprecipitation Assay Kit (catalog no. 17-295; Millipore) with minor modification. Elution buffer used in this study contains 1% SDS, 100 mM NaHCO$_3$ and 10 mM DTT. The recovered DNA was amplified by PCR using Paq5000 DNA polymerase (Agilent Technologies) or KOD plus DNA polymerase (Toyobo) with the primer sets according to the manufacturers' protocols. The primers used here were as follows: 5′-GGGTCAAAGTTGGCGTTTTA-3′ and 5′-CAAAATGGCTAGGAGGTGGA-3′ for the E1A promoter region, 5′-AAGTTCAGATGACTAACTCAGGGG-3′ and 5′-AGAGTTAGGATTGCCTGACGAG-3′ for the E3 promoter region, 5′-CCATAACAGTCAGCCTTACCAGT-3′ and 5′-GTGACGATTTGAGGAAGTTGTG-3′ for the E4 promoter region, 5′-AGGTGATTGGTTTGTAGGTGTAGG-3′ and 5′-CTCCTCGTTTTTGGAAACTGAC-3′ for the MLP region, 5′-CGCAGTGGTCTTACATGCAC-3′ and 5′-CACACGGTTATCACCCACAG-3′ for the hexon ORF region, 5′-ATTCTCCGCCCCATGGCTGAC-3′ and 5′-GGCGTCTTCCATTTTACCAACAGTACC-3′ for the SV40 promoter region, and 5′-CTGGCGAACTACTTACTCTAGCTT-3′ and 5′-GTGTAGATAACTACGATACGGGAGG-3′ for the β-lactamase (Amp^r) ORF region. PCR products were separated on a 7% PAGE gel and visualized by staining with EtBr. The quantitative PCR (qPCR) was performed using FastStart SYBR Green Master (Roche) and Thermal Cycler Dice Real Time System (Takara) according to the manufacturer's protocol. The reaction condition was as follows: polymerase-activating step (95°C for 10 min), followed by amplification step (95°C for 10 s, 56°C for 20 s, and 72°C for 20 s) × 40 cycles. The results were analyzed using Thermal Cycler Dice Real Time System Software Ver. 2.10 (Takara). In all the experiments with qPCR, mean values with SD were obtained from three independent experiments. Re-ChIP assays were performed by the same protocol, except that the primary immunocomplex obtained using the first antibody was diluted 25-fold with ChIP dilution buffer (16.7 mM Tris–HCl (pH 7.9), 1.2 mM EDTA, 167 mM NaCl, 1.1% Triton X-100 and 0.01% SDS), and then subjected to immunoprecipitation with the second antibody.

RT–PCR

Cells were collected into tubes, quickly frozen by liquid N$_2$ and stored at −80°C just before RNA extraction. RNA extraction was performed according to the standard protocol for the acid guanidium thiocyanate-phenol chloroform method within 12 h after sample collection. RNAs were treated with DNase I (Invitrogen) at 37°C for 30 min, followed by phenol/chloroform extraction. After ethanol precipitation, the concentration and the A260/A280 ratio of RNA samples were measured using NanoDrop (Thermo Scientific). cDNA was synthesized from total RNA (1 μg) using ReverTraAce (Toyobo) and oligo-dT primer according to the manufacturer's protocol, and stored at −30°C. RT–PCR was performed using specific primer sets as follows: 5′-ATGGGTCAGAAGGATTCCTATGT-3′ and 5′-GGTCATCTTCTCGCGGTT-3′ for β-actin, 5′-GAGACATATTATCTGCCACGGAG-3′ and 5′-AGTGAGTAAGTCAATCCCTTCCTG-3′ for E1A, 5′-AATCCTAGGTTTACTCACCCTTGC-3′ and 5′-TATACTCTGTAGTGTCACCTGGCTG-3′ for E3, 5′-ACAGAACCCTAGTATTCAACCTGC-3′ and 5′-GACAGCGACATGAACTTAAGTGAG-3′ for E4 and 5′-ACTCTCTTCCGCATCGCTGT-3′ and 5′-GTGACTGGTTAGACGCCTTTCT-3′ for MLP. PCR products were analyzed as described earlier.

Luciferase assays

Cells were lysed with Luc lysis buffer (25 mM Tris–HCl [pH 7.9], 1% Triton X-100 and 10% glycerol). The cell lysates and a firefly luciferase substrate (Promega) were mixed, and the luciferase activity was measured by Lumat LB9506 (BERTHOLD). The protein concentration of cell lysates was determined by the Bradford method, and the luciferase activity was normalized by the protein concentration. Mean values with SD were obtained from three independent experiments.

Western blotting

For checking the expression level of TAF-I and β-actin, cell lysates were prepared using lysis buffer for ChIP assays. Nuclear/cytoplasmic fractions were prepared as described earlier. Cell lysates or nuclear/cytoplasmic fractions were subjected to SDS–PAGE, and then proteins were transferred to a PVDF membrane (Millipore). The membranes were blocked with 5% skim milk/TBST (Tris-buffered saline containing 0.1% Tween-20) at room temperature, and then incubated with indicated primary antibodies at room temperature. After washing with TBST, the membranes were incubated with antimouse or rabbit IgG antibodies conjugated with horseradish peroxidase (GE healthcare) at room temperature, followed by washing. The blots were developed using Chemi-Lumi One (Nacalai tesque).

RESULTS

The structure of viral chromatin in infected cells

The Ad DNA forms a chromatin-like complex with viral core proteins in virions and remains associated with protein VII in the nucleus during early phases of infection (9,10). Several lines of evidence suggest that cellular histones are incorporated into viral chromatin in early phases of infection (25–27), but the actual structure of

viral chromatin in infected cells remains unclear. To clarify how viral chromatin structure changes to be a template for viral early transcription after the entry into the nucleus, we first performed ChIP assays with antiprotein VII, antihistone H3, antiacetylated H3 (AcH3) and anti-FLAG (for negative control) antibodies (Figure 1). The antibody against AcH3 used in this study specifically recognizes acetylated K9 and/or K14 of histone H3, both of which are markers for active chromatin (30). HeLa cells were infected with HAdV5 at an MOI (multiplicity of infection) of 100. Infected cells were cross-linked with formaldehyde and then sonicated to release chromatin fragments. The average size of DNA

purified from chromatin fragments was <1 kb (data not shown). Viral DNA replication occurs ~8 hpi (hours post infection) (data not shown) (10). Viral core proteins are encoded by late genes, which are expressed only after viral DNA replication, and newly synthesized core proteins are likely to be localized throughout the nucleus in late phases of infection (31). Therefore, to eliminate the effect of viral DNA replication and/or late gene expression on the structure of viral (and/or cellular) chromatin, hydroxyurea (HU), a replication inhibitor, was added in the case of 10 hpi. We confirmed that the addition of HU gives no effect on the structure of viral chromatin in the earlier time of infection (Supplementary Figure S1A).
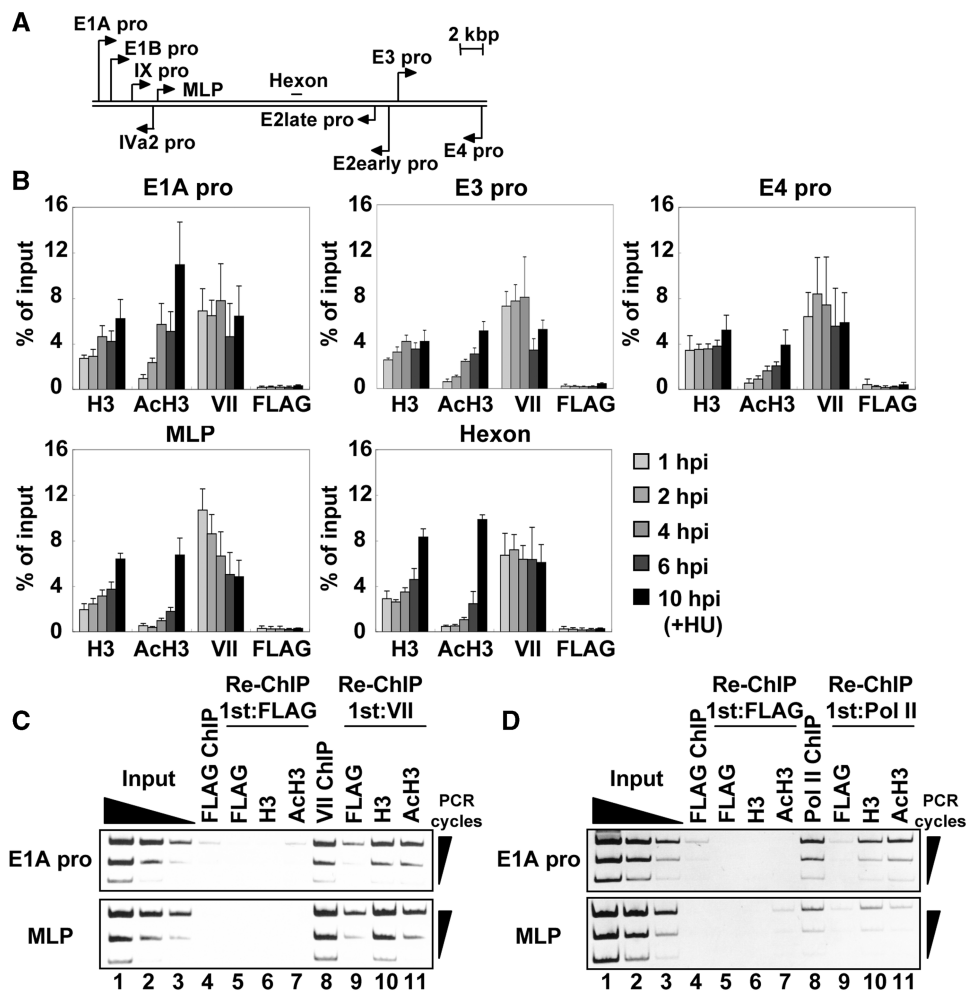

Figure 1. Viral chromatin structure in early phases of infection. (**A**) The illustration of the structure of Ad genome. Arrows represent each promoter region of viral genes. Target region of primer set for Hexon is indicated as a bar. (**B**) ChIP assays using quantitative PCR (qPCR). HeLa cells were infected with HAdV5 at an MOI of 100 and harvested at 1, 2, 4, 6 or 10 hpi. In the case of 10 hpi, HU was added right after infection to prevent viral DNA replication. The cells were cross-linked by formaldehyde and lysed. The lysates were subjected to immunoprecipitation with antibodies against histone H3, acetylated histone H3 (AcH3), protein VII and FLAG. qPCR was performed with primers for five regions of the viral genome (the E1A promoter region, E1A pro; the E3 promoter region, E3 pro; the E4 promoter region, E4 pro; major late promoter region, MLP; and hexon ORF region, Hexon) and immunoprecipitated DNA as templates. (**C**) Re-ChIP assays with antiprotein VII antibody. HeLa cells were infected with HAdV5 at an MOI of 100 and harvested at 6 hpi. The cells were subjected to first ChIP using anti-FLAG (lanes 4–7) or antiprotein VII (lanes 8–11) antibodies, and the immunoprecipitated complex was then subjected to the second ChIP with anti-FLAG (lanes 5 and 9), antihistone H3 (lanes 6 and 10) and anti-AcH3 (lanes 7 and 11) antibodies. For lanes 4 and 8, DNAs purified from 10% of the first immunocomplexes were used as template. Immunoprecipitated DNA was amplified by semi-quantitative PCR using primer sets for E1A pro (upper panel) and MLP (lower panel). PCR products were separated on a 7% polyacrylamide gel and visualized by staining with EtBr. Input DNAs (lanes 1–3) were purified from 0.2, 0.05 and 0.0125% of cell lysates used for first ChIP. (**D**) Re-ChIP assays with anti-RNA polymerase II (pol II) antibody. Re-ChIP assays were performed as described in (**C**), except that anti-pol II antibody was used for the first immunoprecipitation (lanes 8–11).

We selected five regions for ChIP assays, three early gene promoters (E1A pro, E3 pro and E4 pro), one late gene promoter (MLP), and the central region of the viral genome (ORF region of hexon, Hexon, Figure 1A). MLP and Hexon regions are included in the transcription unit of E2 gene, one of viral early genes, on the opposite strand (Figure 1A). However, MLP was not fully activated in early phases of infection under the condition employed here. By RT–qPCR assays with a primer set for amplification of the region just downstream of the transcription start site, we could not detect mRNAs transcribed from MLP in early phases of infection (unpublished data). In addition, USF1 and USF2, transcription factors known to regulate the transcription from MLP (32), were not recruited to this region in early phases of infection. (unpublished data). As reported earlier (10), protein VII was bound throughout the viral genome so far tested (Figure 1B, VII). The binding level of protein VII was slightly changed in the course of infection, and the binding of protein VII was observed up to 10 hpi at least. These results suggest that protein VII is a component of viral chromatin during early phases of infection. It was also shown that histone H3 is bound throughout the viral genome with a gradual increment (Figure 1B, H3). The binding of histone H2A-H2B and H4 to viral chromatin was also detected by ChIP assays using anti-H2A-H2B and anti-H4 antibodies, and the ratio of each core histone bound on viral chromatin was comparable to that on cellular chromatin, although the binding levels of core histones on viral chromatin were lower than those on cellular chromatin (Supplementary Figure S1B and C, and see 'Discussion' section). These results indicate that viral chromatin contains, at least partly, the canonical nucleosomes in infected cells. In ChIP assays with antiprotein VII antibody, no signals were detected by PCR using primer sets for cellular chromatin (Supplementary Figure S1C), reflecting the specificity of this antibody. The binding of histones and protein VII to viral chromatin was also detected by ChIP assays using cells infected at lower MOI (MOI of 5, data not shown). These results suggest that not only viral protein VII, but also cellular histones are associated with the viral genome DNA in early phases of infection. The binding level of AcH3 was also increased as infection proceeded (Figure 1B, AcH3). The binding of histone H3 and acetylation of histone H3 were also observed at other regions of the viral genome (Supplementary Figure S1D).

Although our ChIP assays clearly indicate that both protein VII and histones are bound with viral chromatin in early phases of infection, it is also possible that protein VII and histones are bound to two different chromatin templates independently. To examine whether both protein VII and histones are components of the same viral chromatin in infected cells, we performed re-ChIP assays (Figure 1C). At 6 hpi, infected HeLa cells were fixed and first immunoprecipitated using anti-FLAG or antiprotein VII antibodies. Then, the immunocomplexes were released from the first antibody and subjected to the second immunoprecipitation using anti-FLAG, anti-H3 or anti-AcH3 antibodies. Histone H3 was detected on the protein VII-bound viral chromatin (Figure 1C, lane 10), indicating that both protein VII and histones are associated with the same viral genome DNA in early phases of infection. In addition, the binding of AcH3 was detected on the protein VII-bound viral chromatin at the high level around the E1A promoter, but at the low level around MLP (Figure 1C, lane 11). This pattern was in parallel with that of normal ChIP assays (Figure 1B), and acetylation of histone H3 is highly correlated to viral transcription (Figure 2B, see below). To examine more directly whether AcH3 is bound with active viral chromatin, we performed re-ChIP assays using anti-RNA polymerase II (pol II) antibody (Figure 1D and Supplementary Figure S1E). The binding of AcH3 was also observed on the pol II-bound viral chromatin as was the case for re-ChIP assays using antiprotein VII antibody, indicating that AcH3 is indeed a component of active viral chromatin in infected cells. Taken together, we conclude that in early phases of infection, viral chromatin is associated with both viral protein VII and cellular histones, and this 'chimeric' chromatin functions as a template for viral early transcription.

Acetylation of histone H3 occurs in the absence of transcription at active promoter regions

Using ChIP assays with anti-AcH3 antibody, we observed the gradual increment of the binding of AcH3 on viral chromatin (Figure 1B). This acetylation pattern could be correlated with the transcription state, because the acetylation level of histone H3 at the E1A promoter, which is the first activated promoter, was higher than that of other regions (Figure 1B, also see Figure 2B). The binding level of AcH3 at the promoter regions of other early genes, which are active in early phases of infection, was also higher in earlier infection periods than that at inactive (late) gene promoters (Supplementary Figure S1D). This gives rise to the possibility that acetylation of histone H3 has a role in viral early transcription. Alternatively, this acetylation might be a consequence of ongoing transcription because AcH3 was detected even at inactive promoters and other regions in the later time of infection (Figure 1B, MLP and Hexon, and Supplementary Figure S1D), all of which are present in the transcription units of other viral active genes (Figure 1A). Therefore, to examine the relationship between ongoing transcription and acetylation of histone H3, we performed ChIP assays using cells cultured in the presence of α-amanitin, which blocks pol II-mediated transcription (Figure 2A). Histone H3 as well as protein VII were found associated with the viral DNA in the presence of α-amanitin (Figure 2A, H3 and VII). We also observed the efficient acetylation of histone H3 at early gene promoters in the presence of α-amanitin (Figure 2A and B, E1A pro, E3 pro and E4 pro and Supplementary Figure S1D). However, in the presence of α-amanitin, the acetylation level of H3 at regions other than early gene promoters was much less than that in the absence of α-amanitin (Figure 2A and B, MLP and Hexon, and Supplementary Figure S1D). These results suggest that acetylation of histone H3 efficiently occurs at the promoter regions of early genes even in the absence of ongoing transcription,
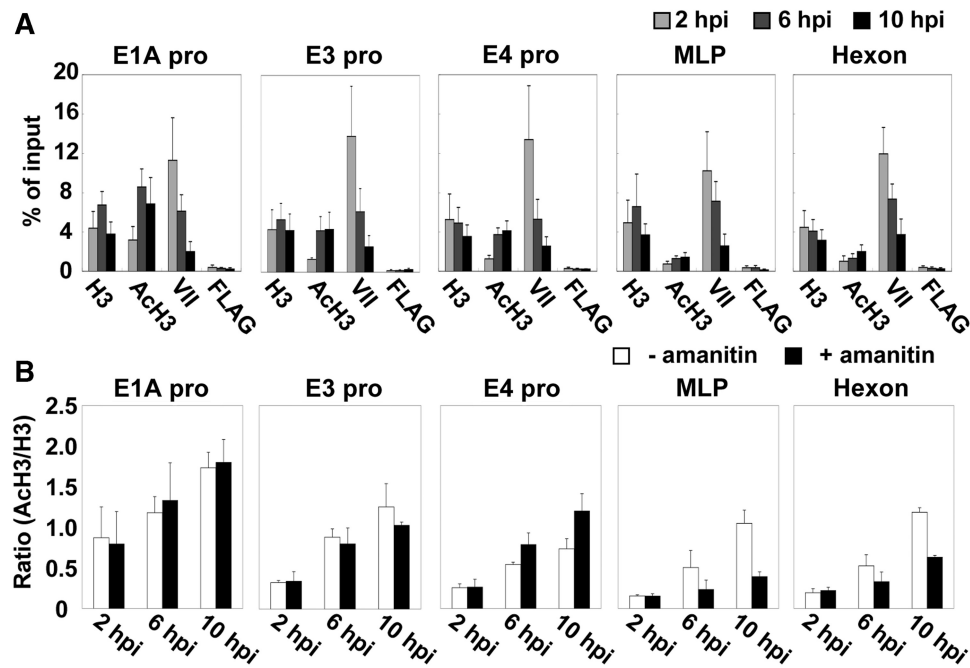
Figure 2. Effect of transcription on viral chromatin. (**A**) ChIP assays in the presence of α-amanitin. HeLa cells were infected with HAdV5 at an MOI of 100 and incubated in the presence of 25 μg/ml of α-amanitin. The cells were harvested at 2, 6 or 10 hpi, and cell lysates were subjected to ChIP assays as described earlier. (**B**) Relative acetylation levels of histone H3. The level of AcH3 in the absence (Figure 1B) or presence of α-amanitin (2A) was normalized by that of H3 [ratio (AcH3/H3)].

whereas the acetylation is coupled with ongoing transcription at regions other than early gene promoters. It is noted that the binding of protein VII was decreased gradually in the presence of α-amanitin (Figure 2A, VII). Recently, a microscopic analysis suggested that the addition of α-amanitin inhibits the release of protein VII (33). The results obtained here differ somehow from those from the previous report, but the exact reason for this contradiction is unclear at present ('Discussion').

TAF-I is required for viral transcription, but not for the binding of histone H3 and AcH3

TAF-I is one of the remodeling factors for core protein–DNA complexes and forms ternary complexes on the viral DNA through the interaction with protein VII *in vitro* and *in vivo* (10). TAF-I plays an important role in viral gene expression during early phases of infection because KD of TAF-I expression results in the reduction of viral mRNA levels (19). TAF-I is also reported to regulate cellular gene expression through its histone chaperone activity or as a component of INHAT (21,22). Therefore, it is possible that TAF-I contributes to viral transcription through the regulation of the binding and/or acetylation of histones on viral chromatin. To test this possibility, we used TAF-I KD HeLa cell lines we had established previously (19). The expression level of TAF-I in KD cell lines (clone 4) was reduced to ∼10% relative to that in HeLa cells, whereas WT cell lines (clone 7) showed the almost same expression level of TAF-I compared with HeLa cells (Figure 3A). These cell lines were infected with HAdV5 at an MOI of 100 and subjected to RT–qPCR assays at 4 hpi (Figure 3B and Supplementary Figure S2A). The

mRNA levels of viral early genes in KD cell lines were ∼50% of that in WT cell lines, as reported earlier (19). At the same time point, we also performed ChIP assays (Figure 3C). However, the binding level of histone H3, AcH3 and protein VII was the almost same in two cell lines (Figure 3C, H3, AcH3 and VII). These results indicate that TAF-I is required for viral early gene transcription but not for the binding of histone H3 or acetylation of histone H3 K9/14 on viral chromatin. It is also suggested that TAF-I does not strip protein VII of viral chromatin, but rather stably forms ternary complexes on viral chromatin to regulate viral transcription (10). By re-ChIP assays, we found that AcH3 is also bound to the TAF-I-bound viral chromatin at the high level around the E1A promoter, but not around MLP (Supplementary Figures S1E and 2B), confirming that TAF-I functions on the 'chimeric' viral chromatin.

Positive role of protein VII in infected cells

It was shown that protein VII–viral DNA complex supports only a low level of transcription in cell-free systems (11,12). In addition, protein VII was suggested to function as a repressive factor in *Xenopus* oocytes (13). However, as described earlier, protein VII remains bound with viral chromatin in infected cells. Therefore, we hypothesized that protein VII may have a potential role for viral transcription and TAF-I is required for its process. To test this, we purified TP-DNA (naked viral DNA covalently bound with TP) and Ad-core (TP-DNA with core proteins) from virions and used for transfection of HeLa cells. The quality of Ad-core and TP-DNA was confirmed by silver staining of proteins in samples and
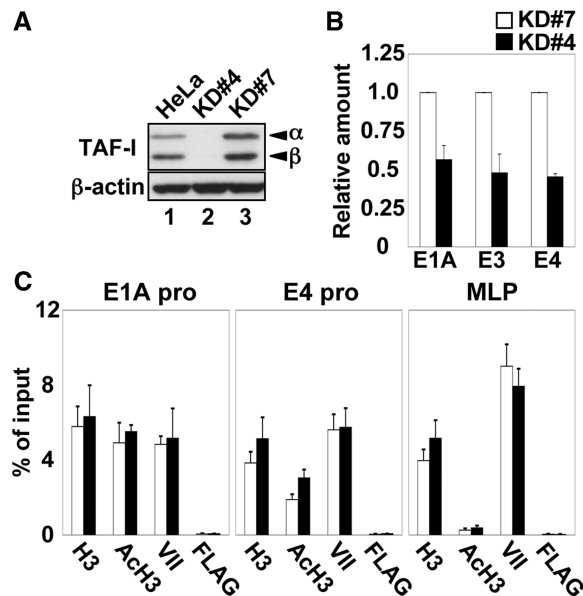
Figure 3. Effect of knockdown of TAF-I expression on viral chromatin. (**A**) Expression level of TAF-I in KD cell lines. Western blot analyses were performed with lysates from HeLa cells (lane 1), KD cell lines clone 4 (lane 2) or clone 7 (lane 3). (**B**) RT–qPCR assays. KD cell lines clone 4 and 7 were infected with HAdV5 at an MOI of 100 and total RNAs were purified at 4 hpi. cDNAs were synthesized with reverse transcription and subjected to qPCR using primer sets for E1A, E3, E4 and β-actin mRNAs. Viral mRNA levels were normalized by β-actin mRNA level and graphed. (**C**) ChIP assays. KD cell lines clone 4 and 7 were infected with HAdV5 at an MOI of 100. At 4 hpi, cells were collected and subjected to ChIP assays as described in the legend for Figure 1B.

agarose electrophoresis of DNA purified from samples (Supplementary Figure S3). Transfected cells were subjected to ChIP assays (Figure 4A). ChIP assays revealed that the viral DNA was associated with protein VII, histone H3 and AcH3 in Ad-core-transfected cells, as was the case in infected cells (Figure 4A, lanes 16–24). On the other hand, in TP-DNA-transfected cells, only a trace level of association of AcH3 was detected at 24 hpt, while histone H3 was bound at the comparable level to that of Ad-core-transfected cells at all time points tested here (Figure 4A, lanes 1–6). In Ad-core-transfected cells, the amount of input DNA at 24 hpt was more than that of 16 hpt (Figure 4A, lanes 29 and 30). This increment of input DNA was due to viral DNA replication because the addition of HU suppressed it (data not shown). We also performed RT–PCR assays at 12 and 24 hpt (Figure 4B) and found that the expression level of viral mRNAs in Ad-core-transfected cells was much higher than that in TP-DNA-transfected cells, while that of β-actin mRNA remains unchanged in both cells (Figure 4B, lanes 7, 8, 10 and 11). It is noted that in Ad-core-transfected cells, mRNA of MLP was detected only at 24 hpt (Figure 4B, lane 11) and this expression was dependent on viral DNA replication (data not shown), indicating that cells transfected with Ad-core could reproduce the expression pattern of viral genes in infected cells. These results strongly suggest that protein VII can enhance viral gene expression in infected cells. This could be due to high

efficiency of nuclear transport and/or high stability of the viral DNA bound with core proteins. To address this possibility, we analyzed the amount of the viral DNA in cytoplasmic and nuclear fractions of transfected cells (Figure 4C). HeLa cells were transfected with Ad-core or TP-DNA and cultured in the presence of HU to prevent viral DNA replication. At 12 and 24 hpt, transfected cells were harvested and separated into cytoplasmic and nuclear fractions. The separation was confirmed by western blotting with anti-Hsp90 (for cytoplasmic marker) and antihistone H3 (for nuclear marker) antibodies (Figure 4C, upper panels). Viral DNA was purified from each fraction and analyzed by PCR with specific primers for viral DNA (Figure 4C, lower panel). We found that the amount of the viral DNA in nuclei of Ad-core-transfected cells was comparable to that in nuclei of TP-DNA-transfected cells (Figure 4C, lower panel, lanes 1–3, 7–9, 13–15 and 19–21), indicating that protein VII is not likely to enhance the nuclear transport and the stability of the viral DNA.

Protein VII enhances transcription initiation

It was reported that protein VII could interact with E1A protein, a transactivator for viral early gene transcription (13). Based on this, a model was proposed that protein VII recruits E1A protein to viral chromatin, leading to the upregulation of viral early gene transcription. Therefore, the transcription enhancement effect of core proteins seen in Figure 4 might be brought from the interaction between protein VII and E1A protein. Alternatively, if protein VII could enhance transcription with its own property, the transcription enhancement effect could be observed independent of the presence of Ad proteins and/or adenoviral DNA sequences. Thus, to examine the direct effect of protein VII on transcription in the absence of any Ad gene products and DNA sequences, we reconstituted protein VII–DNA complexes using recombinant protein VII (Figure 5A, left panel) and a non-adenoviral plasmid DNA (pSV-Luc, Figure 5B), which contains the luciferase gene as a reporter. The reconstituted complexes were first analyzed by an agarose gel electrophoresis (Figure 5A, right panel). By the addition of increasing amounts of protein VII, plasmid DNA did not enter the gel, indicating that protein VII forms nucleoprotein complexes (or aggregates) with pSV-Luc (Figure 5A, right panel, compare lanes 2 with lanes 3–8). To examine whether protein VII have a positive role in transcription, the reconstituted complexes were transfected in HeLa cells, and the luciferase activity was measured at 24 hpt (Figure 5C). As shown in Figure 5C clearly, the addition of protein VII enhanced the luciferase activity. When 100 ng of protein VII was added to 100 ng of pSV-Luc, the luciferase activity was increased to the maximal level (5- to 6-fold) compared with the case of pSV-Luc alone. This result indicates that protein VII is able to enhance transcription in the absence of any Ad products. We also found that protein VII could enhance transcription from a reporter plasmid regulated by Ad enhancer/promoter (Supplementary Figure S4A),
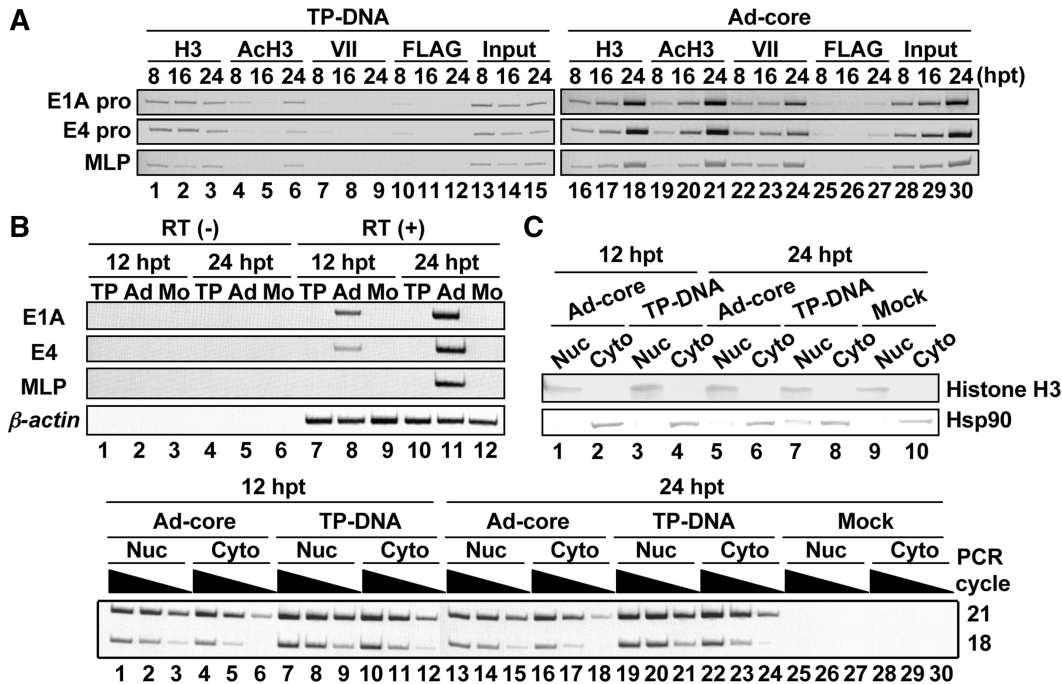
Figure 4. Ad-core/TP-DNA transfection assays. (**A**) ChIP assays of viral chromatin in transfected cells. HeLa cells were transfected with TP-DNA (left three panels) or Ad-core (right three panels). At 8, 16 and 24 hpt, cells were collected, and cell lysates were subjected to ChIP assays using indicated antibodies. Semi-quantitative PCR was performed using primer sets for E1A pro, E4 pro or MLP and immunoprecipitated DNA as templates. PCR products were separated on a 7% polyacrylamide gel and visualized by staining with EtBr. Input DNAs (lanes 13–15 and 28–30) were purified from 2% of cell lysates used for ChIP. (**B**) Semi-quantitative RT–PCR assays. HeLa cells were transfected with Ad-core (Ad; lanes 2, 5, 8 and 11), TP-DNA (TP; lanes 1, 4, 7 and 10), or mock-DNA (Mo; lanes 3, 6, 9 and 12), and total RNAs were purified at 12 (lanes 1–3 and 7–9) and 24 hpt (lanes 4–6 and 10–12). cDNAs were synthesized without (lanes 1–6) or with (lanes 7–12) reverse transcription. Semi-quantitative PCR was performed using primer sets for E1A, E4, MLP and β-actin mRNAs. PCR products were analyzed as described in the legend for (A). (**C**) The amount of nuclear and cytoplasmic viral DNA in transfected cells. Ad-core and TP-DNA were used for transfection (100 ng DNA) into HeLa cells. Transfected cells were cultured in the presence of HU to prevent viral DNA replication. At 12 (lower panel, lanes 1–12) and 24 hpt (lanes 13–30), nuclear and cytoplasmic fractions were prepared from Ad-core- (lower panel, lanes 1–6 and 13–18), TP-DNA- (lanes 7–12 and 19–24), and mock-transfected cells (lanes 25–30) as described in 'Materials and Methods' section. To confirm the separation of each fraction, nuclear (upper panels, lanes 1, 3, 5, 7 and 9) and cytoplasmic fractions (lanes 2, 4, 6, 8 and 10) were subjected to western blot analyses using antihistone H3 and anti-Hsp90 antibodies for nuclear and cytoplasmic marker proteins, respectively. The viral DNA of nuclear (lower panel, lanes 1–3, 7–9, 13–15, 19–21 and 25–27) and cytoplasmic fractions (lanes 4–6, 10–12, 16–18, 22–24 and 28–30) was amplified by semi-quantitative PCR with a primer set for the MLP region. PCR products were analyzed as described in the legend for (A). For each fraction, 4-fold serial dilution (1, 1/4 and 1/16 volume) was used as templates for PCR.

indicating that this effect of protein VII is not sequence-specific.

Next, to examine the effect of protein VII on the chromatin structure of transfected DNA, we performed ChIP assays with several antibodies (Figure 5D). We chose two regions for this ChIP assay; one is the promoter region (SV40 pro) upstream of the luciferase ORF, and the other is the ORF region of β-lactamase gene (Amp ORF), which is thought not to be transcribed in transfected cells. We found that protein VII is still bound to the plasmid DNA in transfected cells when mixed prior to transfection (Figure 5D, VII). We also found that TAF-I is bound to the plasmid DNA only when transfected together with protein VII (Figure 5D, TAF-I). In contrast, histone H3 was detected independently of the presence of protein VII (Figure 5D, H3). We also performed ChIP assays with anti-Sp1 antibody because the SV40 promoter contains several Sp1-binding sites (34). The binding level of Sp1 at the SV40 promoter was increased >2-fold in the presence of protein VII, suggesting that the enhancement of transcription by protein VII is at least in part due to the

increase of Sp1 recruitment, that is, one of important transcription initiation steps (Figure 5D, Sp1). Taken together, these results suggest that protein VII increases the initiation level of transcription, resulting in transcription enhancement. To examine the effect of protein VII on the transfection efficiency and/or DNA stability, the reconstituted complexes were transfected in HeLa cells, and at 24 hpt, transfected cells were separated into cytoplasmic and nuclear fractions as performed in Figure 4C. The amounts of pSV-Luc in nuclear fraction were quantified using qPCR with primers for the SV40 promoter (Figure 5E and Supplementary Figure S4B). The amount of pSV-Luc in the nucleus was the almost same regardless of the presence of protein VII, indicating that protein VII does not enhance the transfection efficiency and/or DNA stability under this condition.

Involvement of TAF-I in enhancement effect by protein VII

Finally, to examine whether TAF-I is needed in protein VII-mediated enhancement of transcription, KD of TAF-I
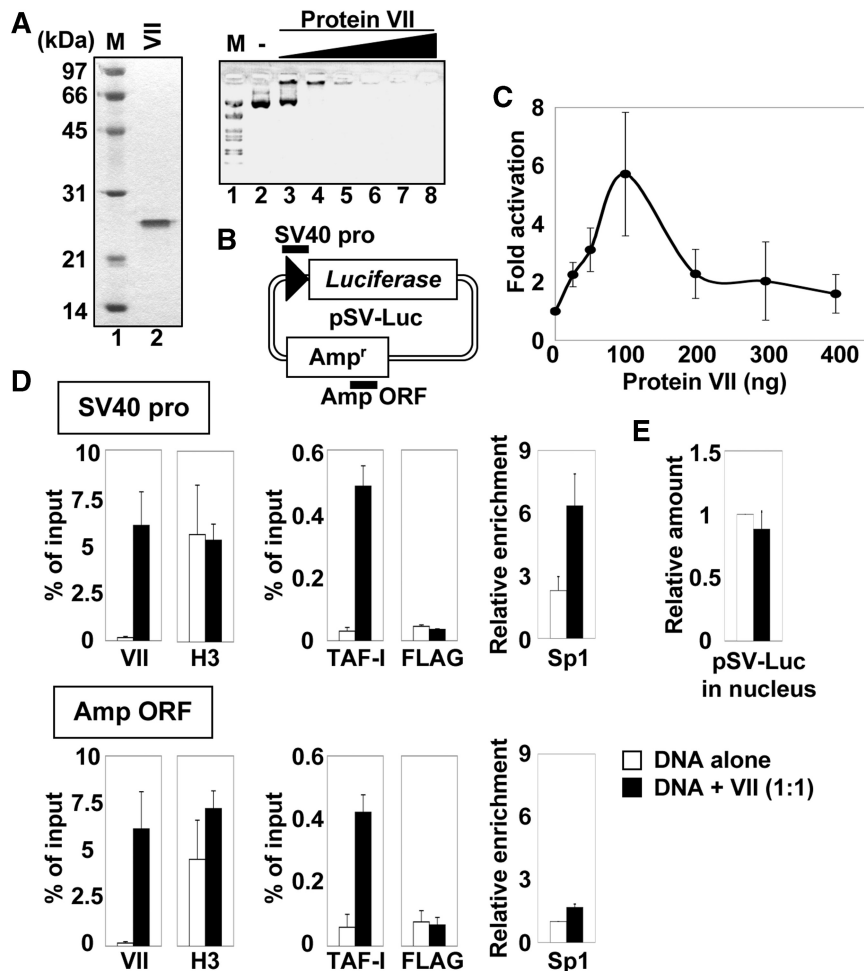
Figure 5. Reconstitution of protein VII–DNA complexes. (**A**) Electrophoresis mobility shift assays. Left panel shows Coomassie Brilliant Blue staining of recombinant protein VII (100 ng, lane 2) separated by a 12.5% SDS–PAGE. Lane 1 shows molecular size markers (M). pSV-Luc (100 ng) was incubated at 37°C for 15 min in the absence (right panel, lane 2) or presence of protein VII (25, 50, 100, 200, 300, 400 ng for lanes 3, 4, 5, 6, 7 and 8, respectively). The samples were separated on a 1% agarose gel in $0.5 \times$ TBE buffer, and DNAs were visualized by staining with EtBr. Lane 1 shows DNA size markers (M). (**B**) The illustration of the pSV-Luc structure. Filled triangle and open boxes represent the promoter region and the ORF region, respectively. Two black bars are target regions for ChIP assays. (**C**) Luciferase assays. HeLa cells were transfected with the reconstituted complexes shown in (A). At 24 hpt, cell lysates were prepared, and the luciferase activity was measured. (**D**) ChIP assays. pSV-Luc (100 ng) was incubated without (DNA alone) or with protein VII (100 ng) and used for transfection into HeLa cells as described in (A). At 24 hpt, cells were harvested, and cell lysates were subjected to ChIP assays. qPCR was performed using primer sets for SV40 promoter (SV40 pro) and ORF region of β-lactamase gene (Amp ORF) and immunoprecipitated DNA as templates. Note that the binding level of Sp1 is calculated as fold enrichment relative to negative control (anti-FLAG antibody) and then normalized by that at Amp ORF in naked DNA-transfected cells. (**E**) The amount of pSV-Luc in the nucleus of transfected cells. Cells transfected as described in (D) was harvested at 24 hpt, and nuclear and cytoplasmic fractions were prepared as performed in Figure 4C. DNAs recovered from nuclear fraction were subjected to qPCR using primer sets for SV40 pro and β-actin gene (for cellular genome number). The amounts of pSV-Luc in the nucleus were normalized by cellular genome number and graphed.

expression was performed using siRNA specific for TAF-I, siTAF-I. The expression level of TAF-I in siTAF-I-treated cells was decreased to ∼10% of that in control siRNA (siControl)-treated cells (Figure 6A). siTAF-I- or siControl-treated cells were transfected with pSV-Luc alone or with protein VII, and then the effect of TAF-I KD on the chromatin structure of transfected DNA and the recruitment of Sp1 to the promoter were examined (Figure 6B). TAF-I KD resulted in slight change of the binding level of histone H3 (Figure 6B, H3). In siControl-treated cells, protein VII could enhance the binding of Sp1 at the promoter region, as described earlier (Figure 6B, Sp1). In contrast,

when siTAF-I-treated cells were transfected with protein VII–DNA complex, protein VII could not enhance Sp1 binding, while the binding level of protein VII was almost the same in both cells (Figure 6B, VII and Sp1). When cells were transfected with pSV-Luc alone, the recruitment of Sp1 to the promoter was not affected by TAF-I KD, indicating that TAF-I is not directly involved in Sp1 recruitment (Figure 6B, Sp1), but TAF-I is required for the recruitment of the transcription factor when protein VII is present. Therefore, it is suggested that the enhancement effect by protein VII is due to ternary complex formation with TAF-I.
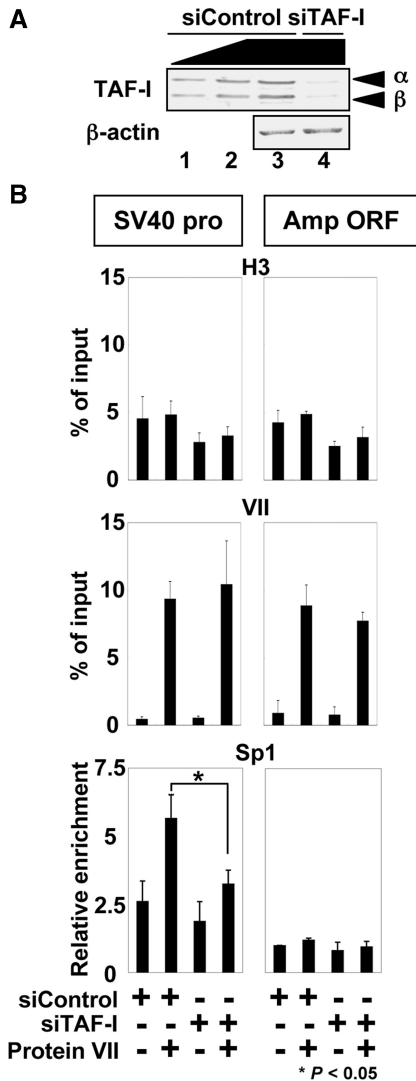
Figure 6. Effect of knockdown of TAF-I expression on Sp1 recruitment. (**A**) Expression level of TAF-I. Western blot analyses were performed with lysates from HeLa cells treated with control siRNA (siControl, lanes 1–3) or siRNA for TAF-I (siTAF-I, lane 4). For siControl-treated cells, 25% (lane 1) and 50% (lane 2) volume of lysate were also loaded. (**B**) Transfection of siRNA-treated cells with reconstituted protein VII–DNA complexes. siControl- or siTAF-I-treated cells were transfected with reconstituted protein VII–DNA complexes, and cell lysates were subjected to ChIP assays as described in Figure 5D. *P*-values are calculated using Student's *t*-test.

DISCUSSION

Several previous reports suggested the possible structure of viral chromatin in infected cells, but the exact structure remains unclear. ChIP and re-ChIP assays in this study clearly showed that cellular histones as well as protein VII are associated with the almost entire region of viral chromatin during early phases of infection and this 'chimeric' chromatin functions as a template for viral early transcription (Figure 1 and Supplementary Figure S1). It is noted that the binding levels of core histones were lower than those on cellular chromatin (Supplementary Figure S1C). The lower nucleosome density in early phases of infection

was also reported in the cases of herpes simplex virus type 1 and human cytomegalovirus (35–37). Thus, this might be a common feature for some DNA viruses during lytic infection cycles. We also found that on viral chromatin, acetylation of histone H3 occurs in a good correlation with viral transcription (Figure 2B). Recently, these dynamic changes of histone modifications linked with transcription were also observed in other DNA viruses, such as SV40 and herpes simplex virus type 1 (38,39). Therefore, the transcription regulation coupled with histone modification could also be a general mechanism for DNA viruses. Post-translational modification of histones contributes to some local changes of the chromatin structure and/or functions as a specific target for chromatin-binding factors (30). Among a variety of histone modifications, acetylation at K9 and/or K14 of histone H3 is recognized as an 'active' chromatin mark for transcription. On the adenoviral chromatin, acetylation of histone H3 occurs in two fashions, i.e. in a transcription-independent manner observed at active promoter regions and dependent manner seen at other regions (Figure 2 and Supplementary Figure S1D). Because the transcription inhibition by α-amanitin did not affect the acetylation level of histone H3 at active promoter regions (Figure 2B), this acetylation could occur actively at these regions, although the exact role of this acetylation in viral transcription is unclear at present. On the other hand, the acetylation at the regions within the transcription units (including inactive promoters) is largely dependent on transcription (Figure 2B), suggesting that the acetylation occurs being coupled with elongation of RNA polymerase II, as the case reported in SV40 (38). We also observed other histone modification correlated with viral transcription in infected cells (unpublished data). These results suggest the possibility that the chromatin structure and/or histone modification have a role in viral transcription, as is the case for cellular chromatin. It is also worthwhile to note that the addition of α-amanitin causes a drastic reduction of the binding level of protein VII (Figure 2A). This result may suggest that transcription contributes to the maintenance of the viral chromatin structure. It was reported that nuclear dots formed by protein VII are abolished during viral transcription and the addition of α-amanitin inhibits it (33). This report proposed that transcription releases protein VII from viral chromatin. This report, however, did not address the localization of the viral DNA in the course of infection. It is possible that the intracellular localization of the viral DNA is changed together with protein VII as viral transcription proceeds, and thus nuclear dots formed by protein VII may disappear. If so, the addition of α-amanitin might inhibit the localization changes of protein VII and the viral DNA rather than the release of protein VII, resulting in the remaining nuclear dots. We have shown here that protein VII could be a component of the template for viral early gene transcription (Figure 1C) and has a positive role in transcription (Figures 4 and 5). We also observed using ChIP assays that in the presence of HU, protein VII remains associated with viral chromatin even at 16 hpi (data not shown). Therefore, we conclude that protein

VII remains associated with the viral DNA during early phases of infection, although we could not exclude the possibility that protein VII is released from some specific region or a minor population of viral chromatin. We also could not rule out the possibility that some population of protein VII is replaced by histones, since the binding level of histone H3 is gradually increased and that of protein VII is reciprocally decreased at some region even in the absence of α-amanitin (e.g. Figure 1B, MLP).

TAF-I, a remodeling factor for protein VII–DNA complexes, has a histone chaperone activity and reported to regulate cellular gene expression through its activity (21). However, KD of TAF-I expression resulted in no effect on the binding level of histone H3 on viral chromatin (Figure 3C). Our preliminary data suggest that other histone chaperones seem to be involved in histone deposition onto viral chromatin (unpublished data). Thus, it is suggested that the role of TAF-I in viral transcription is unlikely to depend on its histone chaperone activity, although we could not exclude the possibility that TAF-I regulates the structure and/or the binding mode of nucleosome on viral chromatin. In addition, the acetylation level of H3 on viral chromatin was not affected by TAF-I KD, although TAF-I is also reported as a component of INHAT (22). pp32, also a component of INHAT (22), is reported to be associated with viral chromatin (31) and it is reported that HDAC1 (histone deacetylase 1) forms a complex with TAF-I and pp32 (40). However, KD of pp32 expression has no effect on the binding level of AcH3 on viral chromatin like TAF-I KD (data not shown). We observed that the addition of trichostatin A, a HDAC inhibitor, affected neither the viral mRNA levels nor the binding level of AcH3 on viral chromatin (data not shown). Therefore, it is likely that TAF-I and pp32 (and HDAC1) are not involved in viral transcription as components of INHAT.

We also showed a functional role of protein VII in cells (Figures 4 and 5). Previously, it was reported that protein VII shows a negative effect on transcription when expressed in *Xenopus* oocytes (13). In their condition, however, the expression level of protein VII relative to the DNA amount was not well considered. In our condition, protein VII was mixed together with DNA and then introduced into cells. The enhancement effect reached the maximal level when protein VII and DNA were mixed at the equal weight ratio (1:1) (Figure 5C). This ratio (∼30.8 bp/one protein VII molecule) is close to that of the virion (∼36 000 bp/833 copies of protein VII (41) = 43.2 bp/one protein VII molecule). In the condition employed here, the import level of the viral DNA into the nucleus was not enhanced by protein VII (Figures 4C and 5E). Hindley *et al.* (42) reported using semi-*in vitro* assays that protein VII is imported by transportin into the nucleus through NPC and a transportin inhibitor inhibits the nuclear import of the viral DNA, and proposed that the import of the viral DNA is coupled with the transportin-mediated import of protein VII. We may consider an alternative nuclear import pathway for transfected Ad-core. In the case of infection, virions are first attached to NPC through the interaction with CAN/Nup214, and then the nuclear import of Ad-core occurs concomitantly with the disassembly of the virion (7). It is quite likely that a viral component(s) other than protein VII is involved in this coupling process. Thus, the nuclear import of transfected Ad-core or reconstituted protein VII–DNA complexes could be different from that of Ad-core released from virions at NPC.

It was reported that transiently transfected plasmid DNA does not show the clear nucleosome ladders when digested with MNase, although core histones bind to the transfected DNA (43). It is possible that histones are deposited on transfected DNA randomly, resulting in some negative effect on the template activity. Therefore, naked DNA (TP-DNA or pSV-Luc alone) could be subjected to the random deposition of histones, and thus show lower level of transcription in cells (Figures 4B and 5C). On the other hand, protein VII might regulate the chromatin structure in *cis* to escape from or overcome the negative effect by histone deposition, although the detailed mechanism remains unclear at present. One possible mechanism is that protein VII protects DNA from random deposition of histones because biochemical analyses indicated that protein VII on the DNA inhibits the nucleosome formation locally (data not shown). Protein VII alone, however, could not enhance transcription and TAF-I is needed in this process since TAF-I KD reduced the enhancement effect of protein VII (Figure 6B). We previously reported using biochemical analyses that TAF-I remodels protein VII–DNA complexes to make them more accessible for transcription factors, and enhances transcription from Ad-core, at least by facilitating the formation of the transcription initiation complex (11,18,20). In good agreement with this, protein VII, together with TAF-I, enhances transcription in cells at least in part by increasing the recruitment of transcription factors, one of the important steps of the transcription initiation (Figure 5D). Taken together, we would propose a possible mechanism that protein VII could protect *cis* elements essential for transcription (including the binding sites of transcription factors) from random deposition of histones, but keep the accessibility of *trans*-acting factors to these regions through the remodeling by TAF-I (Figure 7). We speculate that this chromatin structure organized by protein VII leads to and/or coordinates with proper deposition of histones and/or histone modifications on viral chromatin, resulting in efficient viral transcription in early phases of infection. Thus, protein VII could establish the chromatin structure competent for transcription. Consistent with this idea, we observed using *in vivo* footprinting assays that the chromatin structure surrounding the promoter region in the presence of protein VII differs from that in the absence of protein VII (data not shown). It was also reported that linker histone H1, but not core histones, is poorly associated with transiently transfected DNA (43). Histone H1 was reported to have an important role in the disassembly of the Ad virion and the nuclear import of Ad genome through NPC, possibly by interacting with hexon, one of capsid proteins (7). Therefore, it is important to ask whether histone H1 binds to viral chromatin and plays a role in viral transcription.

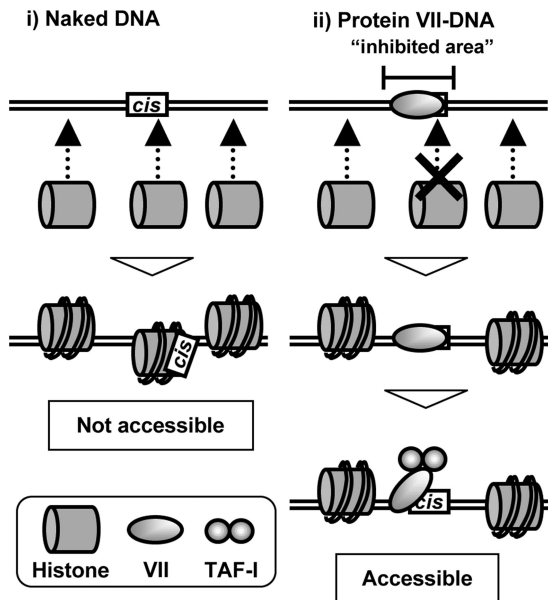In this report, we have shown a functional role of viral chromatin in the regulation of viral gene expression in

Figure 7. A hypothetical model for the enhancement of transcription by protein VII. In the nucleus, naked DNA is subjected to random deposition of histones (Left). If *cis* elements essential for transcription are covered with histones, *trans*-acting factors could not access easily. On the other hand, if protein VII is bound in the vicinity of the *cis* elements, nucleosome assembly at the surrounding area might be inhibited (right). TAF-I could remodel protein VII–DNA complexes, concomitantly allowing the access of *trans*-acting factors.

early phases of infection. The chromatin structure plays an important role in DNA replication in eukaryote cells (44), so that protein VII and/or histones might also have a role in viral DNA replication. Thomas and Mathews showed with superinfection experiments that the activation of late genes requires viral DNA replication in *cis* (45). It is reasonable to assume that during viral DNA replication, viral chromatin undergoes some structural changes to be a competent template for the late gene transcription. We observed that histone H3 also binds to viral chromatin in late phases of infection (unpublished data). Therefore, it is possible that histones are incorporated into viral chromatin during or after viral DNA replication and have an important role in activation and regulation of the late genes. The deposition mechanism of a transcription factor(s) and histones during DNA replication is to be an important key. Studies to clarify the structural change of viral chromatin during viral DNA replication and its role for the regulation of late gene expression are needed.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

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