FEBS 27901 FEBS Letters 555 (2003) 521–527

Ternary complex formation between DNA-adenovirus core protein VII and TAF-Iβ/SET, an acidic molecular chaperone

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Received 2 September 2003; revised 10 November 2003; accepted 12 November 2003

First published online 21 November 2003

Edited by Hans-Dieter Klenk

Abstract The adenovirus (Ad) genome complexed with viral core proteins designated Ad core is the template for transcription of early genes and the first round of replication in Ad-infected cells. A cellular protein designated template-activating factor-I (TAF-I) is found to be involved in remodeling of the Ad core in vitro. Here we found that TAF-I interacts with the Ad DNA through core protein VII in infected cells in early phases of infection. In vitro binding assays using recombinant proteins showed that TAF-I forms ternary complexes with DNA–protein VII complexes.

Key words: Adenovirus; Chromatin immunoprecipitation; Histone chaperone; Replication; Transcription

1. Introduction

The adenovirus (Ad) genome DNA of about 36 000 bp is condensed in the virion with viral basic proteins, core proteins V and VII, and polypeptide X, forming a chromatin-like structure. Protein VII with a molecular mass of 19 kDa is a major DNA binding protein of the Ad genome–protein complexes and has limited amino acid sequence homology with histone H3 and a sperm basic protein [1]. Protein V forms an outer shell around the Ad DNA–protein VII complex and associates more loosely with Ad DNA than protein VII [2,3]. Polypeptide X, a small highly basic peptide of 19 amino acids, is suggested to play a role in the Ad DNA condensation [4,5]. Protein VII and polypeptide X are synthesized as precursors, whereas protein V is as itself. Ad virions are uncoated stepwise in the cytoplasm. The Ad genome–protein complex is bound to nuclear pore complexes [6], and then imported into the nucleus through nuclear pore complexes [7]. Protein V is dissociated from the Ad genome during or right after its nuclear import, while protein VII remains associated with the Ad DNA throughout early phases of infection [8,9]. This nucleoprotein complex composed of the Ad genome DNA and protein VII, here designated Ad core, is a bona fide template for transcription of immediate-early and early viral genes and the first round of DNA replication.

We have identified template-activating factor-I (TAF-I)/SET [10–12], TAF-II/NAP-1 [13,14] and TAF-III/nucleophosmin/B23 [14] from uninfected HeLa cell extracts as stimulatory factors in cell-free Ad DNA replication and transcription systems using the Ad core as template. It is shown that TAF-I binds to the Ad core in vitro and enhances the nuclease sensitivity of the Ad core [15]. Therefore, it is suggested that TAF-I remodels the Ad core by forming a ternary complex composed of DNA, core proteins and TAF-I, thereby making the replication and transcription machineries accessible to DNA. However, the exact mechanism of remodeling of the Ad core by these factors has not been well elucidated. Studying detailed mechanisms would shed light on the molecular mechanism of remodeling of the cellular chromatin by these proteins, since TAF-I, -II and -III are found to function as histone chaperones [14–16].

In this report, we first showed that TAF-I interacts with the Ad DNA through protein VII in early phases of infection in infected cells. Furthermore, we analyzed the binding mode of TAF-I to protein VII and protein VII–DNA complexes using recombinant proteins. These analyses have revealed that TAF-I is capable of forming stoichiometric ternary complexes with the Ad DNA–VII complex.

2. Materials and methods

2.1. Protein preparation

The gene corresponding to the precursor of protein VII (pre-VII) was amplified by polymerase chain reaction (PCR) from the human adenovirus type 5 (HAdV-5) genomic DNA using a set of primers, 5′-GCCTCGAGATGTCCATCCTTATATCGCCC-3′ and 5′-GCCTCGAGCTAGTTGCGCGGGGGGCGGG-3′. Nucleotide sequences were confirmed by sequencing the DNA. PCR products were digested with *Xho*I, and cloned into the *Xho*I site of plasmid pET14b (Novagen) for expression in *Escherichia coli* of a pre-VII tagged with amino-terminal hexahistidines (His-pre-VII). His-pre-VII was expressed in the *E. coli* strain BL21(DE3) in LB medium containing 0.1 mg/ml ampicillin by the addition of isopropyl-β-D-thiogalactopyranoside at a final concentration of 0.2 mg/ml. The cells were disrupted by sonication, and an insoluble fraction containing His-pre-VII was recovered by centrifugation. His-pre-VII was solubilized with 6 M urea and purified through a Ni-NTA (Novagen) column in the presence of 6 M urea according to the manufacturer's instruction. Purified His-pre-VII was dialyzed against water, and then subjected to lyophilization. His-pre-VII was dissolved in buffer A (20 mM Tris–HCl pH7.9, 0.5 M NaCl and 6 M urea) and further purified through HiPrep Sephacryl S-200 column chromatography (Amersham Biosciences). Purified His-

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doi:10.1016/S0014-5793(03)01336-X

pre-VII was dialyzed against water and stored at −30°C until use. Protein VII was prepared by digestion of His-pre-VII with Ad protease in the condition as previously described [17–19]. His-pre-VII was digested at 37°C for 6 h in a mixture containing 0.5 mg/ml His-pre-VII, 0.5 mg/ml salmon sperm DNA, 50 μM polypeptide VIc, a protease activator peptide NH_2-GVWSLKRRCF-COOH, and 10 μg/ml hexahistidine-tagged Ad protease (His-Ad protease), 20 mM Tris–HCl pH 7.9. Then, the concentrations of NaCl and urea were adjusted to 0.5 M and 6 M, respectively. The mixture was loaded on a UnoS column (Bio-Rad), and protein VII was eluted with 20 ml of a linear gradient of 0.5–1.5 M NaCl in buffer A. Purified protein VII was dialyzed against water and stored at −30°C until use. Polypeptide VIc [17] was synthesized by the Fmoc method and purified by high performance liquid chromatography. Recombinant His-Ad protease was prepared as follows: the open reading frame (ORF) of the Ad protease was cloned from HAdV-2 by PCR and inserted into pQE9 vector (Qiagen). *E. coli* M15 strain was transformed with the plasmid DNA. His-Ad protease was expressed and purified as described for His-TAF-I (see below). Recombinant human TAF-I with a hexahistidine tag (His-TAF-I) at its amino-terminus was prepared as described previously [11,20]. Recombinant (r) TAF-I without histidine tag (rTAF-I) was prepared as follows: TAF-I ORF DNA fragment was prepared by digestion of pET14b-TAF-I plasmid DNA with *Bam*HI and *Nde*I, and inserted into pET3a treated with the same restriction enzymes. rTAF-I was expressed in *E. coli* strain BL21(DE3). Cells were lysed by sonication in buffer B containing 20 mM Tris–HCl pH 7.9 and 0.2 M NaCl. rTAF-I recovered in a soluble fraction was loaded on a DE52 (Whatman) column and eluted with a linear gradient of 0.2–0.4 M NaCl in buffer B. rTAF-I was purified through UnoQ column (Bio-Rad) chromatography. rTAF-I was eluted with a linear gradient of 0.25–0.55 M NaCl in buffer B. rTAF-I was further purified by a HiPrep Sephacryl S-200 column chromatography and stored at −80°C until use.

2.2. Antibodies

Rat polyclonal antiserum against pre-VII was generated by immunization of a 5-week-old male rat (SD, Tokyo Jikkenn Doubutu) with 100 μg His-pre-VII in Freund's complete adjuvant (Sigma). The animal was boosted twice with 30 μg and 50 μg pre-VII in Freund's incomplete adjuvant, respectively, at 2-week intervals. Mouse monoclonal anti-TAF-Iβ antibody (KM1721) [21] was used for Western blotting and immunoprecipitation.

2.3. Chromatin immunoprecipitation (ChIP) assay

ChIP assays were carried out according to the manual of the ChIP assay kit (Upstate Biotechnology) with minor modifications. HeLa cells (1×10^6 cells) were infected with HAdV-5 at a multiplicity of 100 plaque-forming units (PFU)/cell. Hydroxyurea (HU) was added at a final concentration of 3 mM at 2 h post infection (h.p.i.) when the Ad DNA replication was to be blocked. At indicated time points after infection, cells were cross-linked with formaldehyde at a final concentration of 1% at room temperature for 10 min, followed by addition of glycine at a concentration of 0.125 M. Cells were collected in a 1.5-ml tube and lysed by sonication in 0.4 ml of sodium dodecyl sulfate (SDS) lysis buffer (1% SDS, 10 mM EDTA, 50 mM Tris–HCl pH 7.9 and 1 mM phenylmethylsulfonyl fluoride (PMSF)). After centrifugation, a supernatant fraction was diluted 10-fold with ChIP dilution buffer (16.7 mM Tris–HCl, pH 7.9, 167 mM NaCl, 1.2 mM EDTA, 1.1% Triton X-100, 0.01% SDS and 1 mM PMSF). The lysate was pre-cleared by rotating with protein A-Sepharose beads pre-masked with salmon sperm DNA (0.2 mg/ml) and bovine serum albumin (0.5 mg/ml) for 30 min. One-eighth volume of the lysate was incubated with appropriate antibodies where indicated. After incubation with pre-masked protein A-Sepharose beads for 30 min, immune complexes were recovered. The beads were washed successively with high-salt wash buffer (20 mM Tris–HCl pH 7.9, 2 mM EDTA, 500 mM NaCl, 1% Triton X-100 and 0.1% SDS), and LiCl wash buffer (10 mM Tris–HCl pH 7.9, 1 mM EDTA, 0.25 M LiCl, 1% NP-40 and 1% deoxycholate). The beads were washed with TE (10 mM Tris–HCl pH 7.9, 0.5 mM EDTA) and transferred to a new 1.5-ml tube. Proteins were eluted with 0.2 ml of freshly prepared elution buffer (0.1 M $NaHCO_3$ and 1% SDS). Cross-linking was reversed by adding NaCl at a final concentration of 0.2 M and incubation at 65°C overnight. After addition of Tris–HCl pH 6.5 (final 40 mM) and EDTA (final 10 mM), the eluate was treated with proteinase K. DNA was extracted with phenol/chloroform, precipitated with ethanol, and dissolved in 50 μl of TE. At the same time DNA was recovered from a portion of pre-cleared lysates and designated input DNA. A target sequence was detected by PCR with specific primers where indicated. The PCR products were separated by a 2% agarose gel and stained with SYBR Gold (Molecular Probes).

2.4. Electrophoretic mobility shift assay

DNA fragments of 34 bp were prepared by digestion of pUC119 with *Msp*I followed by purification of the DNA fragment excised from polyacrylamide gel. Reconstitution of DNA–protein VII complexes was performed in a buffer (total volume of 10 μl) containing 40 mM Tris–HCl pH 7.9, 150 mM NaCl, 12.5% glycerol, 50 ng DNA and 80 ng protein VII by incubation at 37°C for 15 min. For ternary complex formation, His-TAF-I was added to the DNA–protein VII mixture and incubated at 37°C for 15 min. The mixture was subjected to a 7.5% polyacrylamide gel electrophoresis (PAGE) in 0.5×TBE (Tris-borate/EDTA). After the electrophoresis, DNA was stained with SYBR Gold (Molecular Probes) and visualized by FluoroImager 595 (Molecular Dynamics). For transfer of proteins in the polyacrylamide gel to a nitrocellulose membrane, a gel was immersed in the SDS–PAGE electrophoresis buffer for 30 min and rinsed briefly with a transfer buffer (25 mM Tris–HCl, 192 mM glycine, pH 8.3). Proteins were then transferred to a nitrocellulose membrane using a semi-dry protein blotting apparatus (Bio-Rad). His-TAF-I was visualized with enhanced chemiluminescent (ECL) reagent after serial incubation of anti-TAF-I antibody, biotin-conjugated anti-mouse IgG and avidin-conjugated horseradish peroxidase.

3. Results

3.1. In vivo interaction of TAF-I with adenovirus core complexes

TAF-I was identified as a factor that stimulates replication of the Ad core DNA in vitro [10]. Glycerol gradient analyses using purified TAF-I and Ad cores showed that TAF-I is bound to the Ad core [15]. Since TAF-I is incapable of binding to DNA, TAF-I would bind to the Ad core through core proteins (see below). Here, to see whether TAF-I is associated with the Ad core in Ad-infected cells, we tried to detect the Ad DNA in complexes isolated by immunoprecipitation with anti-TAF-I and anti-pre-VII antibodies (Fig. 1). To this end, polyclonal anti-pre-VII antibody was generated, and the specificity of this antibody was examined by Western blotting using recombinant His-pre-VII, protein VII, and extracts prepared from uninfected and infected cells (Fig. 1A). This antibody recognized both recombinant His-pre-VII and protein VII (lanes 1 and 2). Protein VII and pre-VII in extracts of infected cells were specifically recognized by this antibody (lanes 3 and 4). HeLa cells were infected with HAdV-5 and subjected to cross-linking with formaldehyde at 3, 6 and 12 h.p.i. (Fig. 1B) The cells were disrupted by extensive sonication, and cell lysates were subjected to immunoprecipitation. The DNA associated with the immunoprecipitate was isolated, and analyzed by PCR with a specific primer set for the major late gene (ML) ORF region of the Ad genome DNA (for details see Fig. 4). Protein VII was associated with the Ad DNA in early phases of infection (Fig. 1B, upper panel, lanes 13 and 14). Furthermore, association of the Ad DNA with TAF-I was also detected in early phases of infection (lanes 7 and 8). Thus, it is concluded that the Ad DNA is associated with complexes containing TAF-I in Ad-infected cells in early phases of infection. The amount of input Ad DNA at 12 h.p.i. was more than that at 6 h.p.i. (lanes 2 and 3), suggesting that DNA replication initiates between 6 and 12 h.p.i. The amount of the Ad DNA associated with TAF-I decreased slightly at 12 h.p.i. (lane 9). The Ad DNA

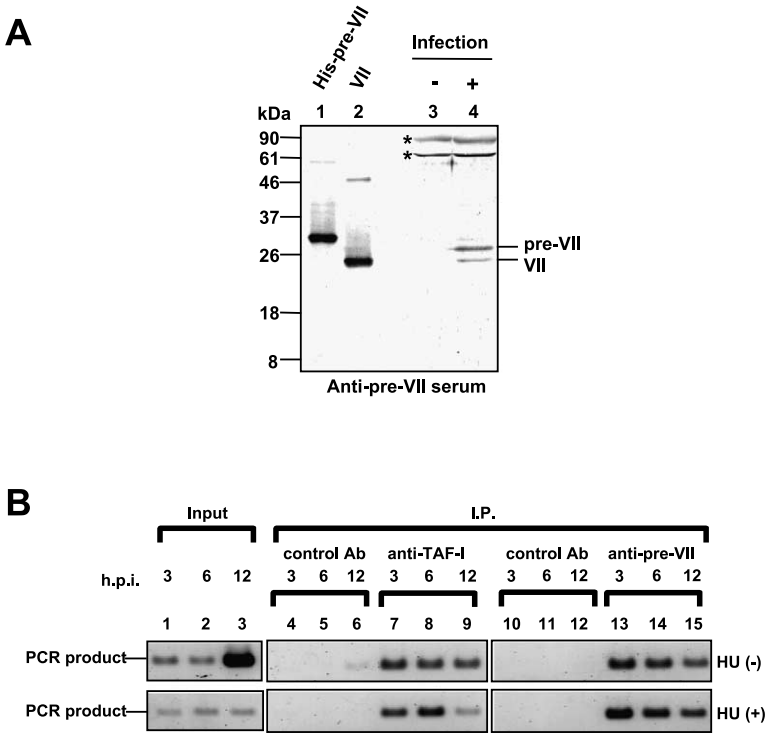

Fig. 1. Interaction of TAF-I with Ad DNA in infected cells. A: Polyclonal antibody directed against bacterially produced His-pre-VII. HeLa cells were mock-infected (lane 3) or infected with HAdV-5 at a multiplicity of infection of 100 PFU (lane 4). Cells were harvested at 24 h.p.i., and cell extracts were prepared by sonication. These cell extracts, recombinant His-pre-VII (lane 1) and protein VII (lane 2), were subjected to Western blotting analysis with anti-pre-VII antibody. Pre-VII and protein VII were transferred to a polyvinylidene difluoride membrane in a transfer buffer containing 0.05% SDS, while TAF-I was transferred in the absence of SDS. The proteins were visualized by incubation with biotin-conjugated anti-rat IgG and avidin-conjugated alkaline phosphatase, and color was developed by addition of cleavable chromophore. Asterisks indicate background bands which appeared without first antibody (data not shown). B: ChIP assay. HeLa cells (1×10^6 cells) were infected with CsCl-purified HAdV-5 as described previously [10] at a multiplicity of 100 PFU. HU was added at 2 h.p.i. (bottom panel). At 3 (lanes 1, 4, 7, 10 and 13), 6 (lanes 2, 5, 8, 11 and 14) and 12 h.p.i. (lanes 3, 6, 9, 12 and 15), the cells were cross-linked with formaldehyde and then collected. Cell lysates prepared as described in Section 2 were subjected to immunoprecipitation with antibodies against TAF-I (lanes 7–9) and protein VII (lanes 13–15). The same amount of mouse anti-flag IgG antibody (Sigma) and the same volume of rat preimmune serum was used as a control antibody for anti-TAF-I antibody (IgG) and anti-pre-VII antibody, respectively. Input lanes contain 0.4% of the input lysates used for experiments shown in lanes 4–15. The DNA associated with immunoprecipitates was purified, and amplified semi-quantitatively using PCR with a set of No. 5 primers indicated in Fig. 4A.

associated with protein VII also decreased at 12 h.p.i. (lane 15). These observations suggest that TAF-I and protein VII are dissociated from the Ad DNA during the progression of infection. Essentially similar results were obtained with other primer sets (data not shown). Note that protein VII is derived from the incoming Ad core, since protein VII is synthesized as a pre-VII in late phases of infection after the onset of DNA replication [22]. To confirm that TAF-I is associated with the Ad DNA before the onset of replication, immunoprecipitation assays were performed using cell lysates prepared from cells maintained in the presence of HU, a replication inhibitor (Fig. 1B, bottom panel). The amount of input DNA remains constant up to 12 h.p.i. (lanes 1–3), indicating that replication is inhibited. The amount of Ad DNA immunoprecipitated with anti-TAF-I antibody at 3 and 6 h.p.i. in the presence of HU is virtually the same as that in the absence of HU, indicating that the Ad DNA interacts with TAF-I prior to the onset of Ad DNA replication and the de novo synthesis of late proteins including pre-VII. The amount of TAF-I-associated Ad DNA and protein VII-associated Ad DNA decreased at 12 h.p.i. in the presence of HU, suggesting that TAF-I and protein VII are dissociated from the Ad DNA without replication.

3.2. *Ternary complex formation of TAF-I with DNA–protein VII complexes*

It is presumed that TAF-I mediates remodeling of the Ad core through forming a ternary complex composed of Ad DNA–protein VII–TAF-I [15]. Here, we examined if such a ternary complex can be formed in vitro using DNA, purified recombinant protein VII and TAF-I proteins. We prepared protein VII by processing purified His-pre-VII with Ad protease, since the expression level of a mature form of protein VII in *E. coli* is quite low while that of His-pre-VII is high. In addition, it is possible that the processing reaction may be involved in formation of functional protein VII. Formation of ternary complexes was monitored by an electrophoretic mobility shift assay. Nucleoprotein complexes were first reconstituted by mixing a 34-bp-long DNA and protein VII (Fig. 2A, lanes 2). Under the experimental conditions, DNA complexed with protein VII did not enter the gel, possibly due to the assumption that highly basic protein VII irrespective of the presence of DNA moves to the anode. After formation of nucleoprotein complex, His-TAF-I was added to the mixture and further incubated. The complexes retained in origin could be aggregates. Addition of His-TAF-I to DNA–protein VII complexes resulted in resumption of free DNA, suggesting

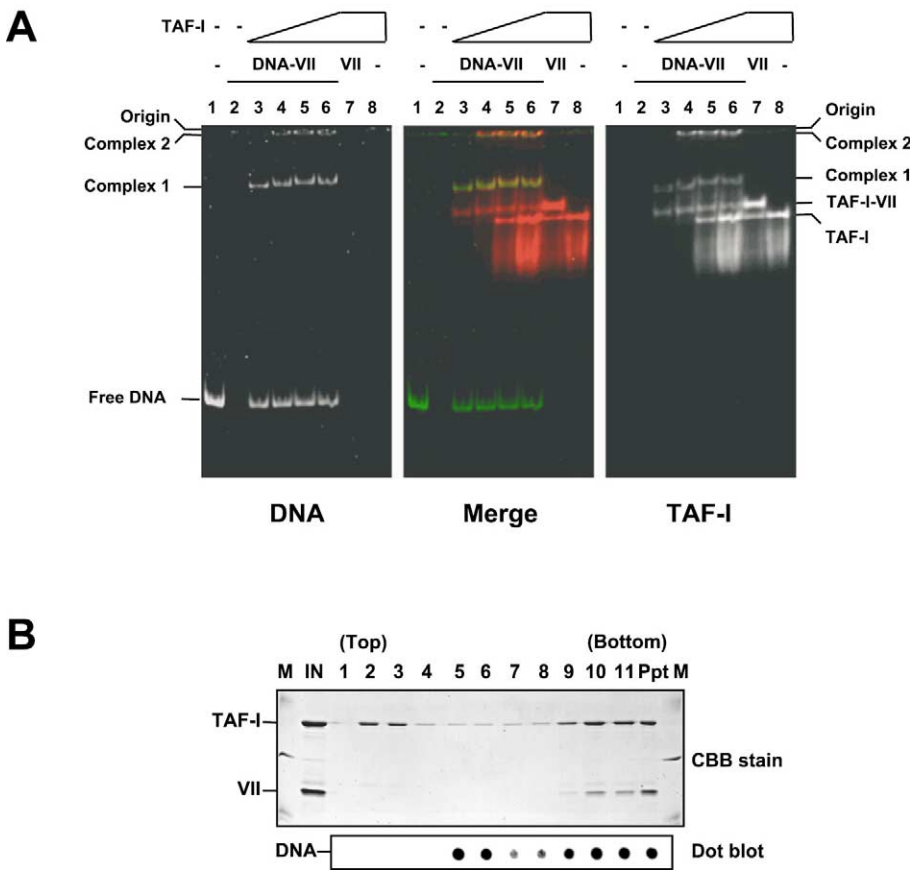

Fig. 2. Ternary complex formation of TAF-I with DNA–protein VII complexes. A: Electrophoretic mobility shift assay. Nucleoprotein complexes of DNA–protein VII (lanes 2–6) were reconstituted at 37°C for 15 min by mixing 34-bp-long DNA (50 ng = 2.2 pmol) with protein VII (80 ng = 4 pmol). The mixtures were further incubated in the absence (lane 2) and presence of His-TAF-I (4, 8, 16, 32 pmol for lanes 3, 4, 5 and 6, respectively). The samples were separated on a 7.5% polyacrylamide gel in 0.5 × TBE buffer. DNA alone, a mixture of His-TAF-I and protein VII (lane 7), and His-TAF-I alone (lane 8) were also analyzed. DNA was visualized by staining with SYBR Gold (left panel), while His-TAF-I was detected by Western blotting using anti-TAF-I antibody (right panel). Images of left and right panels were merged (green, DNA; red, TAF-I) with Adobe Photoshop 5.0 (middle panel). B: Glycerol density assay using reconstituted DNA–protein VII complexes and rTAF-I. Nucleoprotein complexes of DNA–protein VII were reconstituted by mixing linearized plasmid DNA (3 μg) of 3.4 kb with protein VII (3 μg = 150 pmol) at 37°C for 15 min. DNA–protein VII complexes were further incubated with rTAF-I (6 μg = 180 pmol) and subjected to centrifugation at 15°C for 4 h in a SW55 rotor (Beckman) at 45 000 rpm through a 10–40% glycerol gradient in 10 mM Tris–HCl pH 7.9, 0.5 mM EDTA and 150 mM NaCl. A portion (2%) of input proteins and proteins in fractions collected from the top were separated on a 12.5% SDS–PAGE and visualized by staining with Coomassie brilliant blue. DNA in each fraction was dot-blotted and hybridized with [α-^{32}P]dCTP-radiolabeled probe prepared with Prime-It II Random Primer Labeling Kit (Stratagene). Almost all DNA–protein VII complexes without rTAF-I sedimented and attached to the bottom wall designated 'Ppt' fraction (data not shown).

that His-TAF-I remodels and disrupts DNA–protein VII complexes including aggregates. His-TAF-I also facilitated formation of distinct bands designated 'complex 1' and 'complex 2' (lanes 3–6). Western blotting of the gel with the anti-TAF-I antibody showed that His-TAF-I is present in four bands, complex 1 and complex 2 (lanes 3–6), His-TAF-I–protein VII (lane 7), and His-TAF-I (lane 8), suggesting that DNA–protein VII–His-TAF-I ternary complexes were formed as complex 1 and complex 2 (see the merged panel).

The formation of the ternary complex of core proteins, a long DNA (3.4 kb), and rTAF-I was further examined by glycerol gradient assays (Fig. 2B). The nucleoprotein complexes were subjected to a 10–40% glycerol gradient in the presence of 150 mM NaCl. The amount of DNA in each fraction collected from the top was quantified using dot blot hybridization, and proteins in each fraction were separated on a 12.5% SDS–PAGE and stained with Coomassie brilliant blue. Free DNA in an independent centrifugation was recovered in fractions 5–7, while both rTAF-I and protein VII free of DNA were recovered in top fractions (data not shown). When rTAF-I was added to DNA–protein VII complexes, a portion of rTAF-I was recovered in fractions 9–11, where both DNA and protein VII are present. These observations together with the results shown in Fig. 2A strongly suggest the formation of the DNA–protein VII–TAF-I ternary complex.

3.3. Stoichiometry of the TAF-I–protein VII binary complexes

In order to know the structural basis for the mechanism of Ad core remodeling by TAF-I, the stoichiometry of the binary complex composed of TAF-I and protein VII was examined (Fig. 3). Mixtures of rTAF-I and protein VII were resolved by a 7.5% native PAGE, and proteins were visualized by staining with Coomassie brilliant blue (Fig. 3A). TAF-I is a dimeric acidic protein [20] and migrated toward the anode (lane 1), while protein VII is a highly basic protein and did not enter the gel (lane 6). When rTAF-I and protein VII were mixed at a molar ratio of 2:1 (one TAF-I dimer and one protein VII), a band migrating more slowly than the band corresponding to

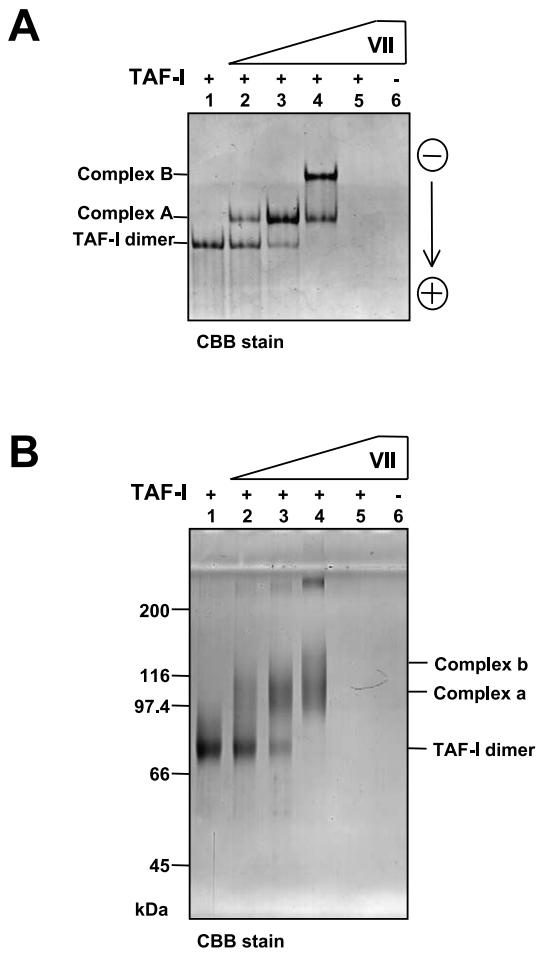

Fig. 3. Stoichiometry of TAF-I–protein VII binary complex. A: Native PAGE analysis of complexes of rTAF and protein VII. rTAF-I (2.5 μg) was incubated with protein VII (1/4-, 1/2-, 1-, 2-fold molar ratio to rTAF-I for lanes 2, 3, 4 and 5, respectively) in a buffer containing 20 mM HEPES–NaOH pH7.9, 150 mM NaCl, and 5% glycerol. The mixtures, rTAF-I (lane 1), and protein VII (lane 6) were subjected to separation on a 7.5% polyacrylamide gel in 0.5×TBE buffer, and proteins were visualized by staining with Coomassie brilliant blue. B: Cross-linking assay. The same aliquots as in A were cross-linked with 0.05% GA at 37°C for 10 min, and then SDS sample buffer (250 mM Tris–HCl pH 6.8, 40% glycerol and 8% SDS) was added to stop the cross-linking reaction. The proteins were separated by a 7.5% SDS–PAGE and visualized by staining with Coomassie brilliant blue. Bands with a molecular mass more than 200 kDa could be highly aggregated due to excess cross-linking.

the TAF-I dimer designated 'complex A' was detected (lanes 2 and 3). When the molar ratio was adjusted to 1:1 (one TAF-I dimer and two protein VII), the other slowly migrating band designated 'complex B' was detected (lane 4). Addition of protein VII at a molar ratio more than 1:1 resulted in change of the color of the solution to cloudy white, suggesting that aggregation was formed (data not shown). The aggregates may move to the cathode, so that no protein bands were observed (lane 5). The stoichiometry was examined by measuring the amount of each protein in complexes A and B. To this end, the gel slices corresponding to complexes A and B were excised, and proteins eluted from the gel slices were separated by SDS–PAGE and visualized by staining with Coomassie brilliant blue. These experiments showed that complex A consists of 1.2 mol of TAF-I dimer and 1 mol of protein VII, while complex B consists of 1.3 mol of TAF-I dimer and 2 mol of protein VII (data not shown). The stoichiometry was further confirmed by cross-linking assays (Fig. 3B). Aliquots of samples of Fig. 3A were cross-linked with a chemical cross-linker, glutaraldehyde (GA). Proteins were separated by SDS–PAGE, and stained with Coomassie brilliant blue. The apparent molecular mass of rTAF-I and protein VII estimated by SDS–PAGE was 39 kDa and 26 kDa, respectively ([10] and data not shown). The band of 78 kDa is a dimeric form of rTAF-I as previously described (lane 1) [20]. Under conditions for formation of complex A (Fig. 3A, lane 3), and for formation of both complex A and complex B (Fig. 3A, lane 4), complex a and complex b with molecular masses of 106 kDa and 130 kDa were observed (Fig. 3B, lanes 2 and 3). Based on the molecular mass, complex a and complex b could be a complex of one dimeric form of rTAF-I and one protein VII or a complex of one dimeric form of rTAF-I and two protein VII, each corresponding to complex A and complex B, respectively. Thus, it is concluded that 1 mol of TAF-I dimer form complexes with either 1 or 2 mol of protein VII.

3.4. Localization of TAF-I on the Ad genome

We found the interaction of TAF-I with protein VII in infected cells (Fig. 1) and confirmed the ternary complex formation in vitro. Then, we tried to examine the sequence specificity for localization of TAF-I and protein VII on the Ad DNA using various primer sets (Fig. 4A) for ChIP-PCR (Fig. 4B). Three independent experimental results are summarized in Fig. 4C. The histogram of the distribution of TAF-I and protein VII on the Ad genome DNA showed that TAF-I is not uniformly distributed within the Ad DNA (Fig. 4C). In particular, the E1a promoter region was relatively free of TAF-I (see Section 4). TAF-I was localized slightly less in regions of E1b ORF, E3 ORF and E4 ORF than the other regions. The distribution pattern of protein VII was correlated to some degree with that of TAF-I. Since this result shows that TAF-I is localized where protein VII is localized, it is likely that there is no difference in binding affinity of TAF-I to protein VII depending on the region in the Ad DNA.

4. Discussion

In this report, we have shown the interaction of TAF-I with the Ad DNA through protein VII in early phases of infection. From the observation that the Ad DNA interacts with protein VII throughout early phases of infection [8] together with our observation in this report, it is likely that transcription- and replication-competent template is composed of DNA–protein VII–TAF-I complexes.

ChIP assays using anti-TAF-I antibody showed that as the infection process proceeds, the amount of Ad DNA associated with TAF-I decreases independent of Ad DNA replication, while the amount of input DNA remained unchanged prior to the onset of replication (Fig. 1). It is therefore suggested that TAF-I and protein VII are dissociated from the Ad DNA. One of the possible driving forces for dissociation of TAF-I and protein VII could be the transcription process. Our assumption is as follows. The structure of the Ad core is partially remodeled by binding of TAF-I [15]; as transcription factors/machineries could access and bind to DNA, TAF-I and protein VII may be dissociated; the region free of TAF-I and protein VII could be more accessible for transcription

factors/machineries, thereby being maintained in a transcription-active state. This notion may be the case for the promoter region of E1a, one of the immediate-early genes, where the dissociation of TAF-I and protein VII was significant (Fig. 4). The binding of TAF-I may not be enough for other promoter regions to start transcription, such as the ML promoter region. TAF-I stimulates transcription from the E1a promoter more effectively than that from the ML promoter in a cell-free transcription system with nuclear extracts and the Ad core as template [23]. Nuclease sensitivity assays showed that TAF-I remodels the E1a promoter region of the Ad core but less the ML promoter region [15]. Therefore, not only TAF-I but also other factors and/or processes such as replication reaction and factors induced by progression of infection could be required. Earlier studies on the Ad core structure during the infection [8,24–26] suggest that the Ad core is rapidly organized into a structure resembling that of transcription-active cellular chromatin. If so, a question remains whether the remodeled state is kept only by transcription factors/machineries or with histones. The histone binding to the Ad DNA during early phase of infection is still controversial [8,26].

Electrophoretic mobility shift assays revealed that TAF-I facilitates the release of the free form of DNA and also binds to the DNA–protein VII complex. Even in the presence of an excess amount of TAF-I, DNA–protein VII complexes were not completely disrupted. Since TAF-I mediates both disruption and assembly of DNA–basic protein complexes [15], we assume the co-existence of free DNA, protein VII–DNA, TAF-I–protein VII, and the ternary complexes around the equilibrium condition.

It is worthwhile to consider the mechanism of the formation of the ternary complex. The structure of the DNA–protein VII complex in virions has been studied biochemically and physicochemically [2,25,27–29], but the precise structure of the DNA–protein VII complex has not been revealed. The amino-terminal half and the carboxy-terminal half of protein VII are rich in basic amino acid residues, and both regions are suggested to be involved in DNA binding [2,28]. It is suggested that protein VII binds to the phosphate backbone of DNA [2]. TAF-I has the characteristic acidic tail and forms a dimer through its amino-terminal region [20]. Two kinds of the TAF-I–protein VII binary complex can be formed, one consisting of one TAF-I dimer and one protein VII (complex A in Fig. 3), and the other of one TAF-I dimer and two protein VII (complex B in Fig. 3). It is possible to assume that in complex A each acidic tail in a TAF-I dimer interacts with each one of two basic regions of protein VII, while in

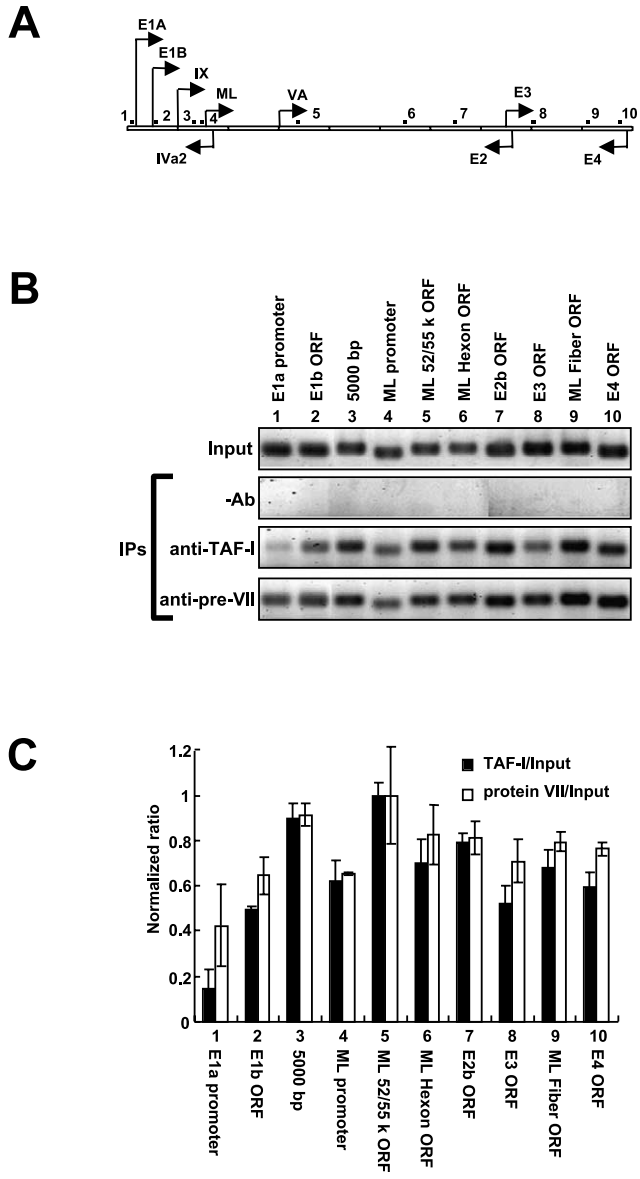

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Fig. 4. Distribution of TAF-I on the Ad genome in early phases of infection. A: The diagram of the location of PCR products on HAdV-5 DNA detected in B. The arrows show the transcription initiation sites of HAdV-5. Short bars numbered with 1–10 represent the location of primer sets used in B, corresponding to lanes 1–10, respectively. B: Distribution of TAF-I and protein VII on the Ad DNA. Cell lysates were prepared from cross-linked Ad-infected cells at 6 h.p.i. as described in the legend for Fig. 1 and Section 2, and subjected to ChIP analysis with antibodies against TAF-I and pre-VII. The DNA associated with the immunoprecipitates was semi-quantitatively analyzed by PCR using primer sets shown in A. Nucleotide sequences of primer sets are as follows: 5′-GGGTCAAAGTTGGCGTTTTA-3′ and 5′-CAAAATGGCTAGGAGGTGGA-3′ for the E1a promoter region, 5′-GCTTGGGAGTGTTTGGAAGA-3′ and 5′-CGGTGTGGAAAAATCCAAAG-3′ for the E1b coding region, 5′-GCATGTTTTCCCTGACCAAA-3′ and 5′-CTGGACGAGCACCGACTACT-3′ for the 5000-bp region, 5′-GCGGTCCTCCTCGTATAGAA-3′ and 5′-CCCACCCCCTTTTATAGCC-3′ for the ML promoter region, 5′-GCTGGAGCAAAACCCAAATA-3′ and 5′-TATCTTGCGGGCGTAAAACT-3′ for the ML ORF 52/55-kb region, 5′-CGCAGTGGTCTTACATGCAC-3′ and 5′-CACACGGTTATCACCCACAG-3′ for the hexon coding region, 5′-AGAAGAACATGCCGCAAGAC-3′ and 5′-TCGAAGGCGAGCTTAAGTGT-3′ for the E2b ORF DBP region, 5′-GCAGCCAGGTGACACTACAG-3′ and 5′-AAAGCTGCGTCTGCTTTTGT-3′ for the E3 ORF region, 5′-TAAAGTACGGGGCTCCTTTG-3′ and 5′-GAGGGCCCTGTCCTAGTCTT-3′ for the ML ORF fiber region, and 5′-TGGCGTGGTCAAACTCTACA-3′ and 5′-GATTTTTACAATGGCCGGACT-3′ for the E4 ORF region. C: Summary of distribution of TAF-I and protein VII on the Ad genome. Intensities of the bands in B were measured using NIH Image from three independent experiments. Intensities of 'input', for example, were normalized relative to the maximum intensity among 'input' values obtained with various primer sets in each experiment. The band intensities in ChIP assays with anti-TAF-I antibody and anti-pre-VII antibody were normalized in a similar way, and further normalized by each 'input' value determined for each primer set. Normalized values are indicated as bars with S.D.

complex B one of the basic regions of protein VII is free from one TAF-I molecule. Thus, in the ternary complex one of the basic regions of VII could bind to DNA, the other basic region being kept bound to TAF-I.

TAF-I binds not only to protein VII but also to pre-VII in vivo as well as in vitro (unpublished results). This suggests that TAF-I may play a role(s) in the events of late phases, such as virion assembly. If pre-VII forms ternary complexes like protein VII, there could be a mechanism by which ternary complexes are to be changed to binary complexes, pre-VII–Ad DNA. Further studies are required in this direction.

Acknowledgements: We thank Drs. M. Miyaji-Yamaguchi and H. Mihara for their help in preparing recombinant TAF-I proteins and synthetic peptides. This work was supported in part by a Grant-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology of Japan and by a grant from the Bioarchitect Research Program from RIKEN (K.N.).

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