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Physical and functional interaction between a nucleolar protein nucleophosmin/B23 and adenovirus basic core proteins

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Abstract **We identified nucleophosmin/B23 as a component of template-activating factor-III that stimulates the DNA replication from the adenovirus DNA complexed with viral basic core proteins. Here, we have studied the functional interaction of B23 with viral core proteins. We found that B23 interacts with viral basic core proteins, core protein V and precursor of core protein VII (pre-VII), in infected cells. Biochemical analyses demonstrated that B23 suppresses formation of aggregates between DNA and core proteins and transfers pre-VII to DNA. These results indicate that B23 functions as a chaperone in the viral chromatin assembly process in infected cells.**

Keywords: Adenovirus; B23; Chromatin; Core protein; Nucleolus

1. Introduction

The adenovirus (Ad) genome is a linear double-stranded DNA of approximately 36000 base pairs (bp) in length, and packed into the non-enveloped icosahedral particle. The viral DNA is covalently linked with virally encoded terminal protein and condensed in the virion with viral core protein VII, core protein V, and polypeptide X, thus forming a chromatin-like structure, hereafter designated as Ad core [1–3]. Core protein VII, a 19 kDa protein with limited amino acid sequence similarity to histone H3 and a basic sperm-specific protein, is a major component of Ad core and tightly associated with the Ad genome [4]. Core protein V associates loosely with Ad DNA and forms an outer shell around Ad core to link it with the capsid by binding with a dimer of polypeptide VI [5–7]. The infecting virus disassembles gradually in the cytoplasm after entry and penetration into host cells [8–11]. Core protein V is removed from Ad core immediately after entry into the nucleus, whereas core protein VII remains associated with the genome [12,13]. Therefore, transcription of the early genes and the first round of viral DNA replication start using Ad DNA complexed with core protein VII as a template in infected cells. During late stages of infection, the precursor of core protein VII (pre-VII) and core protein V package the newly replicated DNA into Ad core. The detail molecular mechanism of disassembly and assembly of Ad core is not clear at present.

We identified nucleophosmin/B23 as a component of Template Activating Factor (TAF)-III that stimulates the DNA replication from Ad core [14]. Two splicing variants of B23, B23.1 and B23.2, which differ only in their C-terminal regions, are expressed in a variety of growing cells. Both B23.1 and B23.2 contain highly acidic domains, and the C-terminal region unique for B23.1 is essential for its RNA binding activity. The nucleolar localization of B23 is disrupted upon Ad infection, and core protein V was shown to be responsible for re-localization of B23 from the nucleolus to the whole cell [15]. Thus, it is possible that B23 is involved in the Ad life cycle. However, the functional interaction between B23 and factors associated with Ad infection has not been described yet.

To explore the function of B23 in the Ad life cycle, we have studied the interaction between B23 and Ad core proteins. We found that B23 interacts with both viral core protein V and pre-VII during late stages of infection. Biochemical analyses demonstrated that B23 can induce dissociation of core proteins from DNA–core protein aggregates. In addition, B23 was found to mediate formation of nucleoprotein complexes containing pre-VII. Taken together, it is indicated that B23 functions as a chaperone for viral chromatin assembly.

2. Materials and methods

2.1. Plasmid construction and protein purification

cDNA corresponding to the protein V was amplified by polymerase chain reaction (PCR) from the human adenovirus type 5 (HAdV-5) genomic DNA using a primer set, 5′-AAGCTCGCATATGTCCAAGCGCAAAATCAAA-3′ and 5′-AAGCTAAGGATCCTTAAACGATGCTGGGGTGGTA-3′. The amplified PCR product was cloned into NdeI- and BamHI-digested pET14b (Novagen). His-protein V expressed in the *E. coli* strain BL21 codon plus RIL (STRATAGENE) was purified using His-bind resins (Novagen) according to the manufacturer's instruction. Core protein VII and pre-VII, and GST-tagged proteins were expressed and purified as previously described [16,17].

2.2. Immunoprecipitation assay

HeLa cells were infected with HAdV-5 at multiplicity of infection (MOI) of 10. At 24 h post infection (h.p.i.), cells (1×10^7) were collected and lysed on ice for 10 min in 1 ml of IP buffer (50 mM Tris–HCl pH 7.9, 1 mM PMSF, 0.1% Triton X-100, and 1 mg/ml bovine serum albumin (BSA)) containing 150 mM NaCl followed by extensive sonication. Cell extracts recovered as a supernatant fraction by centrifugation were mixed with anti-protein V (a generous gift from Dr. W.C. Russel), anti-core protein VII [16], or anti-B23 (Zymed) antibodies, and incubated at 4 °C for 3 h. Then, Protein A Sepharose CL4B

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beads (10 μl of resin; Amersham Pharmacia) were added and further incubated for 1 h with gentle agitation. Immunoprecipitated proteins were analyzed by SDS–PAGE followed by Western blotting.

2.3. Electrophoretic mobility shift assay

The 147 bp-long DNA fragment containing the *5S rRNA* gene was amplified by PCR. DNA was first mixed with core proteins, and then increasing amounts of B23.1 were added, or core proteins pre-incubated with increasing amounts of B23 were mixed with DNA. Both reactions were carried out at 37 °C for 15 min in a buffer containing 40 mM Tris–HCl pH 7.9, 100 mM NaCl, 0.1 mg/ml of BSA, and 10% glycerol and protein–DNA complexes were analyzed by 6% PAGE in 0.5XTBE.

3. Results and discussion

3.1. Interaction of B23 with core proteins V and VII in Ad-infected cells

To explore the function of B23 in the Ad life cycle, we first addressed whether B23 interacts with viral core proteins by immunoprecipitation experiments. HeLa cells were infected with HAdV-5, and cell lysates were prepared from cells at 24 h.p.i. Immunoprecipitation assays were carried out with antibodies against B23, core protein V, and core protein VII. Western blot analyses revealed that both core protein V and pre-VII were co-immunoprecipitated with B23 (Fig. 1A, lane 6). We also confirmed this interaction by immunoprecipitation assays using anti-core protein VII and V antibodies (Fig. 1B, lanes 1–6 and lanes 7–12, respectively) followed by Western blotting with anti-B23 antibody. To further examine whether the interaction between B23 and basic core proteins was the consequence of artificial association between acidic and basic proteins, immunoprecipitation assays with antibodies against TAF-Iβ and B23 were carried out. TAF-I is a highly acidic protein that was identified as a stimulatory factor for the Ad DNA replication [18]. As shown in Fig. 1A and B, both core protein V and pre-VII were co-immunoprecipitated with B23, whereas only pre-VII was co-immunoprecipitated with TAF-I (Fig. 1C). The theoretical isoelectric point (p*I*) of TAF-I (4.12) is lower than that of B23.1 (4.64). In addition, we previously found that small acidic protein pp32 with theo-

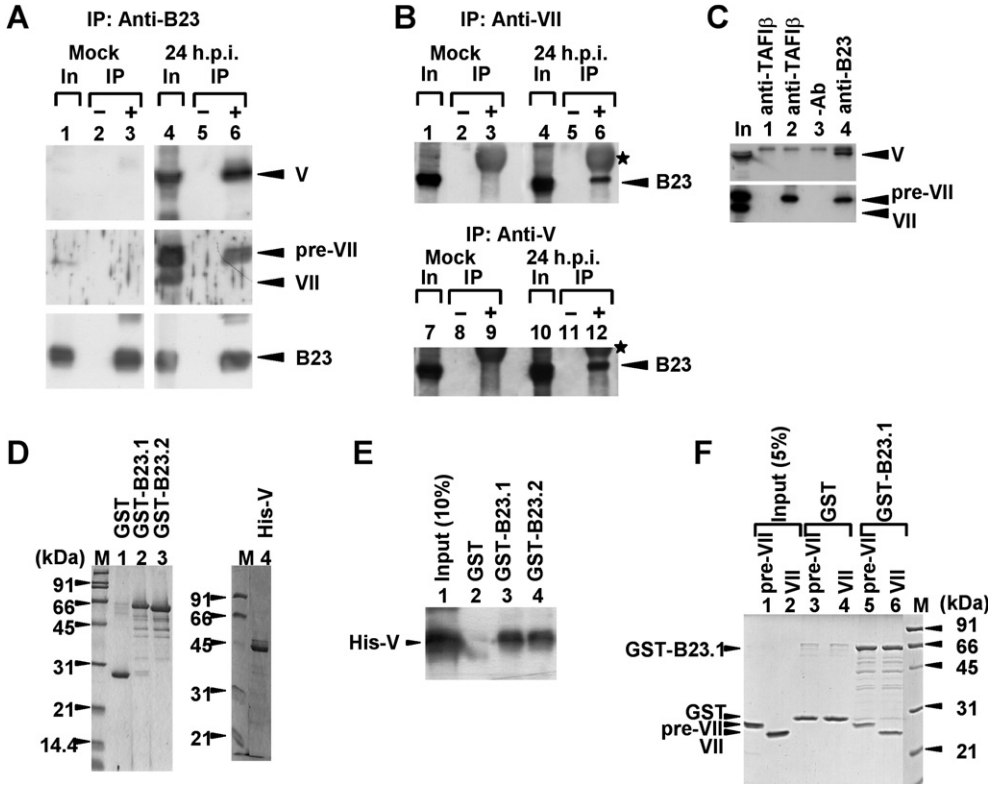

Fig. 1. Interaction between B23 and core proteins in infected cells. (A, B) Immunoprecipitation from Ad-infected cell extracts. Cell lysates prepared from mock- or Ad-infected HeLa cells were subjected to immunoprecipitation without or with anti-B23 antibody (A) or anti-core protein V and anti-core protein VII antibodies (B). B23 and core proteins VII and V were detected by Western blotting. Positions of the proteins were indicated at the right side of the panel. Asterisks indicate the immunoglobin heavy chain. (C) Immunoprecipitation with anti-TAF-I and anti-B23 antibodies. Cell lysates prepared from mock (lane 1) and Ad-infected cells (lanes 2–4) were subjected to immunoprecipitation without (lane 3) or with anti-TAF-Iβ [19] (lanes 1 and 2) or anti-B23 (lane 4) antibody. Proteins were separated on 10% SDS–PAGE followed by Western blotting with anti-core protein V or VII antibodies (top and bottom panels, respectively). (D) Purified recombinant proteins. Purified GST, GST–B23.1, GST–B23.2, and His-core protein V (lanes 1–4, respectively) were separated on a 10% SDS–PAGE and visualized by CBB staining. Lane M indicates molecular weight markers. (E) GST-pull down assays with core protein V. GST, GST–B23.1, and GST–B23.2 (lanes 2–4, respectively) (1 μg each) were incubated with His-core protein V (1 μg) in IP buffer containing 200 mM NaCl and incubated at 4 °C for 1 h. Then 20 μl of glutathione Sepharose CL4B beads (Amersham Pharmacia) were added and further incubated for 1 h. Proteins bound to glutathione-Sepharose beads were separated on a 12.5% SDS–PAGE and analyzed by Western blotting with anti-core protein V antibody. Purified core protein V (0.1 μg, lane 1) was also shown. (F) GST-pull down assays with core protein VII and pre-VII. Purified pre-VII (lanes 3 and 5) and core protein VII (lanes 4 and 6) (1 μg each) were mixed with either GST or GST–B23.1 (1 μg each) as in Fig. 1E. Proteins bound to Glutathione-Sepharose beads were separated on a 12.5% SDS–PAGE and visualized by CBB staining. Lane M indicates molecular weight markers. Positions of GST, GST–B23.1, pre-VII and core protein VII were indicated at the left side of the panel.