Histone- and chromatin-binding activity of template activating factor-I

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Abstract Template activating factor-I (TAF-I) is a histone-binding chromatin remodeling factor. We recently found that TAF-I is capable of mediating decondensation of *Xenopus* sperm chromatin by releasing sperm-specific basic proteins. Here we present evidence that TAF-I preferentially binds to histone H3 among four core histones. Immunofluorescent staining revealed that TAF-I binds to the decondensed sperm chromatin, of which protein components predominantly consist of histones H3 and H4.

Key words: Chromatin remodeling; Histone; Template activating factor-I

1. Introduction

Remodeling of chromatin structure contributes to various aspects of gene activity, such as transcription, replication, repair and recombination. Factors involved in chromatin remodeling include histone modifying enzymes, multi-subunit ATP-dependent chromatin remodeling factors and histone chaperones (for recent reviews, see [1,2]). Histone chaperone was defined as the factor which deposits histones to nucleosomes, the structural units of chromatin [3].

We have been using the adenovirus genome complexed with viral basic proteins, designated adenovirus core, for a model system of cellular chromatin and identified template activating factor-I (TAF-I) as a host factor required for DNA replication and transcription of the adenovirus core [4,5]. Our previous works have shown that TAF-I has histone-binding and nucleosome assembly and remodeling activities, and suggested that TAF-I is a novel histone chaperone [6–8]. Purification and cDNA cloning revealed that TAF-I is a very acidic factor consisting of a homo- or heterodimer of the 41 kDa TAF-Iα and the 39 kDa TAF-Iβ proteins [4,9]. Recently we also found that recombinant human and *Xenopus* TAF-Iβ decondenses *Xenopus* sperm chromatin [10]. TAF-Iβ releases sperm-specific basic proteins from the sperm chromatin through direct interaction. We report here that TAF-Iβ remains bound to the decondensed sperm chromatin after releasing the sperm-specific basic proteins. Since TAF-Iβ preferentially binds to core histones H3/H4 in solution, we suggest that TAF-I associates with the chromatin through its interaction with histones H3/H4.

2. Materials and methods

2.1. Preparation of proteins

Recombinant human TAF-I (hTAF-I) with a six-histidine tag at its amino-terminus was prepared as described previously [9,10]. Glutathione *S*-transferase (GST) and GST-fusion hTAF-Iβ proteins were prepared as described [10]. Core histones, histone H2A/H2B and H3/H4 were purified from HeLa cells as described by Simon and Felsenfeld [11].

2.2. GST pull-down assay

Eighty micrograms of GST or GST-hTAF-Iβ protein was mixed with 12 μg of core histones or 6 μg of histones H2A/H2B or H3/H4 in 1 ml of buffer A (20 mM Tris–HCl pH 7.5, 1 mM EDTA, 10% glycerol, 1 mM dithiothreitol (DTT) and 0.25 mM phenylmethylsulfonyl fluoride) containing 100 mM NaCl at 4°C for 60 min. The mixture was then incubated with 20 μl of glutathione-Sepharose 4B by gentle agitation at 4°C for 60 min. Protein complexes bound to the Sepharose beads were precipitated by centrifugation and washed extensively with buffer A containing 150 mM NaCl, then with buffer A containing 200 mM NaCl. Proteins that were eluted with buffer A containing 1 M NaCl were concentrated by precipitation with trichloroacetic acid and analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

Plasmid DNAs pCH1.3, H3/pRSET and H4/pRSET, which contain the *Xenopus* histone H1, H3 and H4 coding sequences, respectively, under the T7 promoter were used to prepare ^{3}H-labeled histones [12,13]. Cell-free transcription-coupled translation in rabbit reticulocyte lysate was performed using 2 μg of each plasmid in a 100 μl reaction with the TNT T7 Quick Coupled Transcription/Translation System (Promega) in the presence of ^{3}H-labeled lysine and arginine. After 2 h incubation at 30°C, 15 μg of GST or GST–hTAF-Iβ protein was added to 45 μl of the lysate and the mixture was adjusted to a total volume of 170 μl with buffer A containing 100 mM NaCl and incubated at 4°C for another hour. The mixture was then incubated with 15 μl of glutathione-Sepharose 4B by gentle agitation. Protein complexes bound to the Sepharose beads were precipitated by centrifugation and washed extensively with buffer A containing 100 mM NaCl, then with buffer A containing 250 mM NaCl. Proteins were eluted with 10 mM reduced glutathione and analyzed by SDS-PAGE.

2.3. Decondensation of Xenopus sperm chromatin

Demembraned *Xenopus* sperm chromatin was prepared as described [14]. The demembraned sperm chromatin was incubated at a final concentration of 5×10^{3} sperm/μl with TAF-I at room temperature for 60 min in 10 μl of reaction mixture containing 8 mM HEPES pH 7.5, 100 mM KCl, 2 mM $MgCl_2$, 200 mM sucrose and 0.8 mM DTT. After the incubation, a 2 μl aliquot of the reaction mixture was added to 5 μl of 7.4% formaldehyde on a slide glass. The slide was dried and incubated with 1% bovine serum albumin (BSA) in phosphate-buffered saline (PBS) for 30 min. After washing with two changes of PBS containing 0.3% Triton X-100, the slide was incubated with first antibodies in PBS containing 1% BSA for 45 min. Protein G-purified mouse monoclonal antibodies which recognize the TAF-Iβ specific region or TAF-Iα/β common region were used [7]. The slide was

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Abbreviations: TAF-I, template activating factor-I; GST, glutathione *S*-transferase; DTT, dithiothreitol; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; BSA, bovine serum albumin; PBS, phosphate-buffered saline

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washed as above and incubated with secondary antibody, goat anti-mouse IgG antibody labeled with FITC. After washing the sample was stained with Hoechst 33258 and visualized under a fluorescent microscope. To analyze the chromatin-bound proteins, after 60 min incubation with TAF-I the chromatin (1.5×10^6 sperm) was precipitated by centrifugation at 15 000 rpm for 10 min. The chromatin was suspended in buffer A containing 50 mM NaCl, and then HCl was added at a final concentration of 0.5 N. The mixture was incubated on ice for 10 min and insoluble proteins were removed by centrifugation for 10 min. The HCl-soluble proteins were concentrated by precipitation with trichloroacetic acid and analyzed by SDS-PAGE.

3. Results and discussion

In the previous study it has been shown that TAF-Iβ binds to core histones through an ionic interaction [7,8]. To determine which histone(s) interacts with TAF-I in solution, we performed a GST pull-down assay. Core histones or fractionated histones H2A/H2B or H3/H4 which were purified from human HeLa cells were incubated with GST-fusion human TAF-Iβ. Protein complexes were precipitated by binding to the glutathione-Sepharose beads and washed with a buffer containing 200 mM NaCl. Histones eluted at 1 M NaCl from GST or GST–TAF-Iβ were analyzed by SDS-PAGE (Fig. 1). The results showed that when mixed together four core histones interacted with TAF-Iβ (lane 5), consistent with the previous study of the pull-down assay with histone beads [7]. Histones H3/H4 did interact with TAF-Iβ, although their amounts recovered in the 1 M NaCl eluate were much less than those when a mixture of four core histones was incubated with TAF-Iβ (compare lanes 5 and 6). In contrast, histones H2A/H2B were barely detected in the eluate when those fractionated histones were incubated with TAF-I (lane 7), suggesting very little interaction of fractionated H2A/H2B with TAF-Iβ in solution. However, these results do not rule out the possibility that TAF-I could bind to histones H2A/H2B. In solution histones H3/H4 exist as tetramers $(H3/H4)_2$ and H2A/H2B exist as dimers [15]. Our results suggest that TAF-I may interact not only with H3/H4 but also with H2A/H2B if H2A/H2B dimers are present together with H3/H4 tetramers (compare lanes 5 and 7). An interesting possibility is that TAF-I might facilitate the interaction between H4 and H2B, given that TAF-I functions as a nucleosome assembly factor [6]. In any case, these results suggest that TAF-Iβ interacts with core histones with a preference for histones H3/H4. We next examined whether histones H3 and H4 independently interact with TAF-I (Fig. 2). Labeled histone H1, H3 and H4 proteins were prepared in a cell-free system with rabbit reticulocyte lysate. GST pull-down assay using the labeled histones revealed the interaction of histone H3 with TAF-Iβ. A trace amount of histone H4 was found to interact with TAF-I, while interaction of linker histone H1 with TAF-I was not detected.

Recently we found that TAF-I mediates the decondensation of *Xenopus* sperm chromatin [10]. During *Xenopus* spermatogenesis, most histones H2A/H2B are replaced with sperm-specific basic proteins, SP1–6 [16]. *Xenopus* sperm chromatin thus contains SPs and all four core histones, but the amounts of histones H2A and H2B are considerably less than those of H3 and H4 (Fig. 3, lane 1, SP1 is not detectable in this gel). Upon

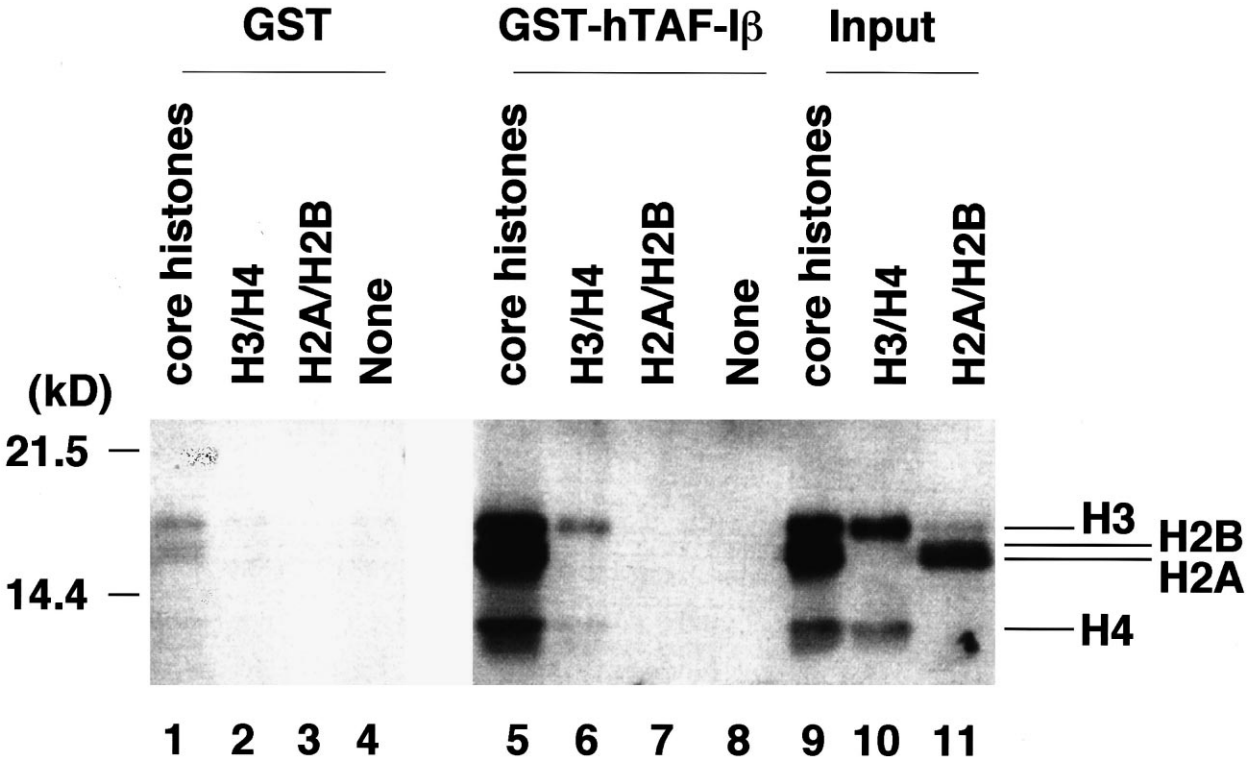

Fig. 1. GST pull-down assay with purified core histones. GST (lanes 1–4) and GST–hTAF-Iβ (lanes 5–8) proteins were incubated with HeLa core histones (lanes 1 and 5), fractionated histones H3/H4 (lanes 2 and 6) and histones H2A/H2B (lanes 3 and 7) and without any proteins (lanes 4 and 8). The GST pull-down assay was performed as described in Section 2 and proteins eluted with a buffer containing 1 M NaCl were analyzed in SDS-PAGE, followed by silver staining. Input histones were electrophoresed in parallel (lanes 9–11). The positions of core histones and marker proteins are indicated.

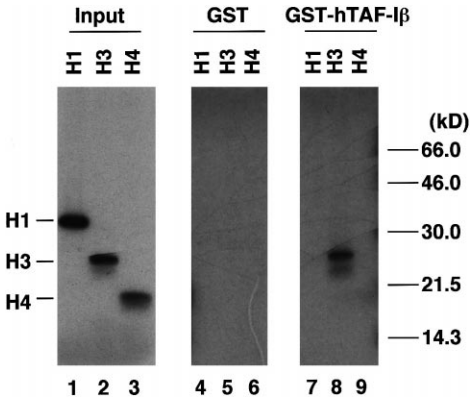

Fig. 2. TAF-I preferentially binds to histone H3. ^{3}H-labeled histones H1, H3 and H4 were prepared in a cell-free translation with rabbit reticulocyte lysate. The reticulocyte lysate programmed to synthesize histones H1 (lanes 4 and 7), H3 (lanes 5 and 8) and H4 (lanes 6 and 9) was incubated with GST (lanes 4–6) or GST–hTAF-Iβ (lanes 7–9) and the protein complexes were precipitated as described in Section 2. Proteins eluted with reduced glutathione were analyzed in SDS-PAGE and detected by fluorography. Lanes 1–3 are aliquots of the reticulocyte lysates used in the GST pull-down assay. The positions of histones and marker proteins are indicated.

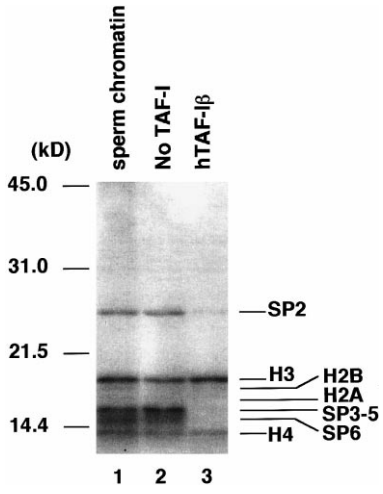

Fig. 3. TAF-I releases sperm-specific basic proteins from *Xenopus* sperm chromatin. *Xenopus* sperm chromatin was incubated without (lane 2) or with (lane 3) hTAF-Iβ under conditions for chromatin decondensation. The chromosomal basic proteins were analyzed in SDS-PAGE and stained with Coomassie brilliant blue. Basic proteins prepared from the sperm chromatin (without incubation) were analyzed in parallel (lane 1). The positions of core histones, sperm-specific basic proteins (SP2–6) and marker proteins are indicated.

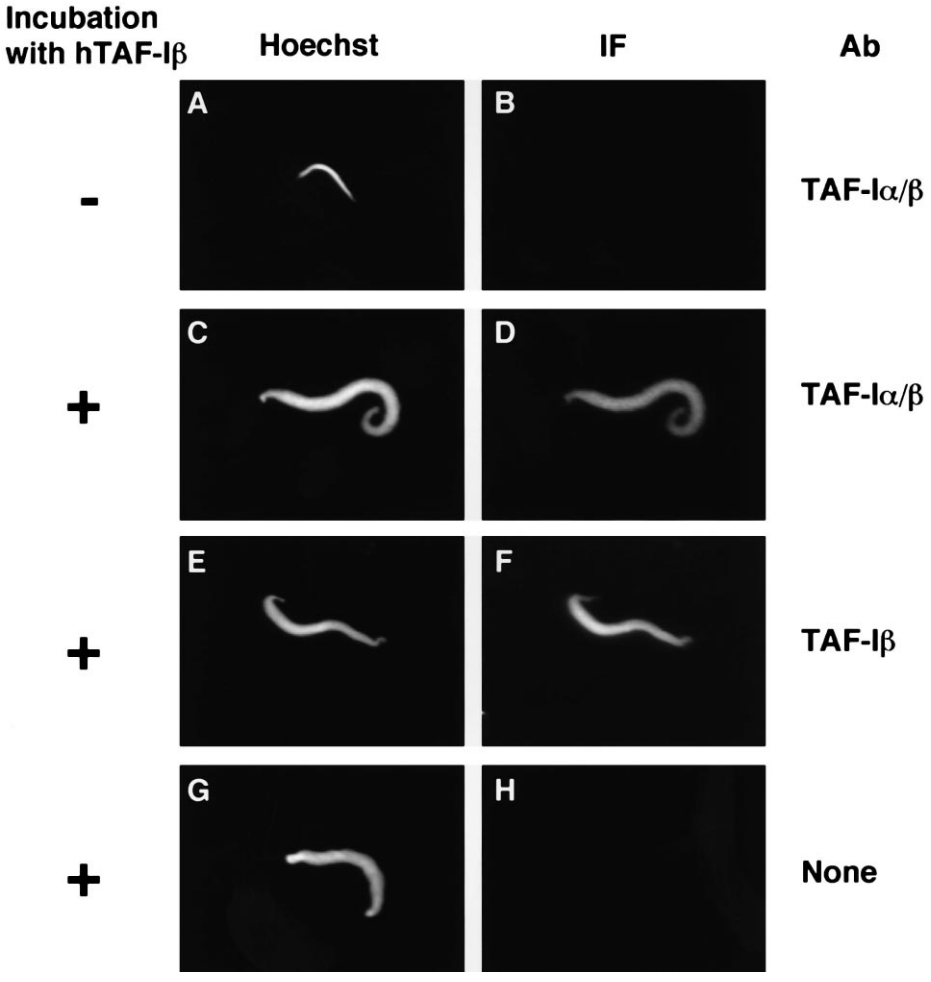

Fig. 4. TAF-I binds to the decondensed sperm chromatin. *Xenopus* sperm chromatin was incubated without (A and B) or with (C–H) hTAF-Iβ under conditions for chromatin decondensation. The DNA of the sperm chromatin was stained with Hoechst 33258 (A, C, E and G). Indirect immunofluorescence was performed with monoclonal antibodies against the TAF-Iα/β common region (B and D) and the TAF-Iβ specific region (F) and with no first antibody (H).

incubation with TAF-Iβ, the sperm chromatin was decondensed concomitant with the release of most of the SPs from it (Fig. 3, lane 3, for decondensation see Fig. 4). As a result, the decondensed chromatin contains predominantly histones H3 and H4 as its major chromosomal basic proteins. We have found using GST–hTAF-Iβ in the decondensation assay that TAF-Iβ interacts with the released SPs in solution [10]. Since TAF-Iβ interacts with histones H3/H4, we tried to examine whether TAF-Iβ also binds to the chromatin. To do this, indirect immunofluorescence experiments with anti-TAF-I antibodies were performed (Fig. 4). The sperm chromatin decondensed with TAF-Iβ was reactive with monoclonal antibodies against the TAF-Iβ specific region or the TAF-Iα/β common region, indicating that a part of TAF-Iβ remains bound to the chromatin after the decondensation reaction.

Since TAF-I is acidic, it is unlikely that TAF-I directly binds to the chromosomal DNA. Indeed, in our preliminary experiments we failed to detect any DNA-binding activity of TAF-I ([8] and data not shown). Rather it is reasonable to assume that immunofluorescent staining with anti-TAF-I antibodies likely reflects the interaction between TAF-I and histones H3 and/or H4 in the decondensed sperm chromatin. In this regard it is of interest to note that both TAF-Iα and TAF-Iβ have a long acidic region at their C-termini and that the deletion mutant of TAF-I lacking this acidic region is not capable of interacting with core histones or mediating the sperm chromatin decondensation [8,10]. It still remains to be elucidated whether TAF-I remodels the sperm chromatin by interacting with histones H3 and H4 in addition to releasing SPs from the chromatin.

There are several factors in *Drosophila* embryo extracts which mediate decondensation of *Xenopus* sperm chromatin [17–19]. One of these factors, p22/CRP1, has been shown to bind to the decondensed sperm chromatin after the decondensation reaction with *Drosophila* extracts [20]. In *Xenopus* egg extracts nucleoplasmin is possibly the most abundant histone chaperone which decondenses the sperm chromatin [21,22]. We have not observed binding of TAF-I to the sperm chromatin decondensed with *Xenopus* egg extracts, possibly due to the lower abundance of endogenous TAF-I in the egg extract in comparison to that of nucleoplasmin. However, immunopurified *Xenopus* TAF-I from the egg extract was active in decondensation of sperm chromatin [10]. The results presented here together with those described previously show that after the decondensation reaction TAF-I exists as two forms: one is soluble and associated with the released SPs, while the other is bound to the chromatin. It is to be examined if TAF-I remains bound to the chromatin or is released from it eventually after the formation of full nucleosomes with four somatic core histones.

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