Template Activating Factor-I Remodels the Chromatin Structure and Stimulates Transcription from the Chromatin Template*

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To study the mechanisms of replication and transcription on chromatin, we have been using the adenovirus DNA complexed with viral basic core proteins, called Ad core. We have identified template activating factor (TAF)-I from uninfected HeLa cells as the factor that stimulates replication and transcription from the Ad core. The nuclelease sensitivity assays have revealed that TAF-I remodels the Ad core, thereby making transcription and replication apparatus accessible to the template DNA. To examine whether TAF-I remodels the chromatin consisting of histones, the chromatin structure was reconstituted on the DNA fragment with core histones by the salt dialysis method. The transcription from the reconstituted chromatin was completely repressed, while TAF-I remodeled the chromatin and stimulated the transcription. TAF-I was found to interact with histones. Furthermore, it was shown that TAF-I is capable not only of disrupting the chromatin structure but also of preventing the formation of DNA-histone aggregation and transferring histones to naked DNA. The possible function of TAF-I in conjunction with a histone chaperone activity is discussed.

The eukaryotic nucleosome, a unit of chromatin, consists of 146 base pairs (bp) of DNA and a histone octamer containing two copies each of histone H2A, H2B, H3, and H4. It has been thought that some modifications of the chromatin structure would be needed before the initiation of replication or transcription (reviewed in Ref. 1). Some factors are shown to gain access to the chromatin DNA directly in vitro (2), while some others do so with the aid of proteins, such as yeast or human SWI/SNF (reviewed in Refs. 3 and 4), Drosophila NURF (5), and related factors (reviewed in Refs. 6 and 7), which facilitate the change of interaction between DNA and histone octamer. Furthermore, the gene activity is also regulated by enzymatic modification of histone octamer. Each histone possesses sites in its N-terminal region that can be hyperacetylated, and their acetylation and/or deacetylation are closely related to the gene activity (1, 8–12).

In order to study the molecular mechanism for activation of transcription and replication from chromatin templates, we have been using the adenovirus DNA complexed with the viral basic core proteins (Ad core) as a model system. The Ad genome is a double-stranded DNA of about 36,000 bp and forms the chromatin-like structure in the virion and in the infected cells. About 200 bp of DNA per viral nucleosome is coiled around six copies of the viral core protein VII, and each unit of viral nucleosome is bridged by the core protein V (13). Immediately after infection, early genes are transcribed, and some of their products together with the host factors NFI, II, and III put forward the genome DNA replication (14, 15). Newly synthesized DNA does not remain naked but transiently forms a complex with the cellular histones (16). Late genes are transcribed from the newly replicated DNA, and core proteins and other viral capsid proteins are synthesized. Since histones are not present in the Ad virion, cellular histones on the newly replicated viral DNA are to be removed and replaced with newly synthesized viral core proteins before being packaged into the progeny virus capsid. This type of replacement seems similar to that of histones with protamine during spermatogenesis. Although basic mechanisms for replication and transcription of the Ad genome DNA have been evaluated with in vitro systems using naked DNA templates, in vitro replication and transcription from the Ad core do not take place with the factors needed for these reactions on the naked DNA template (17–19). Since the viral DNA in infected cells is also complexed with either basic viral core proteins or histones to form the chromatin structure, the access of trans-acting factors involved in replication and transcription to their cognate sites is restricted. Therefore, it is reasonable to presume that the remodeling of the viral chromatin takes place before the initiation of replication and/or transcription.

Recently, we have identified from uninfected HeLa cells template activating factor (TAF)-I, which stimulates the replication from the Ad core (18). TAF-I also stimulates the transcription from the E1A promoter on the Ad core but not effectively from the major late promoter (MLP) (19). There are two subtypes of TAF-I, designated as TAF-Iα and TAF-Iβ, both of which have a common amino acid sequence except that N-terminal 30-amino acid sequences are specific for each subtype. TAF-I has a long acidic tail in its C-terminal region that is required for the activation of the Ad core replication and transcription (20, 21). The stimulatory activity of TAF-Iβ is higher than that of TAF-Iα. TAF-Iβ is the same as the product of the set gene, which is fused to the can gene by the translocation in an acute undifferentiated leukemia (21, 22). TAF-I shows low but distinct amino acid sequence homology to nucleosome assembly protein (NAP)-1, which was originally identified as the factor involved in chromatin assembly (23). It is indicated that
NAP-I can replace for TAF-I in the stimulation of replication and transcription from the Ad core, and that TAF-I has NAP-I activity (20, 21). Therefore, both proteins are structural and functional homologue of each other.

Here we investigate the mechanisms for the stimulation of transcription by TAF-I from both E1A and ML promoters on the Ad core and the reconstituted chromatin consisting of histones. TAF-I stimulates the transcription not only from the E1A promoter on the Ad core but also from the chromatin template reconstituted on the DNA containing the MLP. The nucleic sensitivity assays have revealed that TAF-I stimulates the transcription from these templates by altering core protein-DNA or histone-DNA interaction. Furthermore, the Far Western analyses reveal that TAF-I binds to each core histone and its binding affiniti to histone H3/H4 complex is higher than that to H2A/H2B complex. TAF-I binds to core histones through its acidic region and prevents the formation of aggregation between DNA and core histones. Our results lead to the possibility that one of the putative physiological functions of TAF-I may be to suppress the random aggregation of DNA-basic proteins such as DNA-histones. Since other proteins are also identified as histone chaperone, the redundancy of these histone chaperones raises the question how their roles are assigned and cooperated in a cell.

EXPERIMENTAL PROCEDURES

Southern Blot Analysis—Ad core DNA and recombinant (r) TAF-I were prepared as described (20, 21). Ad core (50 ng), plasmid pLAI (5 ng) (19), or pSmaF (5 ng) (19) was incubated at 30 °C for 30 min in 25 mM Hepes-NaOH, pH 7.9, 12.5 mM MgCl2, 60 mM KCl, 1 mM diethiothreitol with or without rTAF-I. Then 5 units of restriction endonuclease, PvuII (Toyobo) or NcoI (Toyobo), was added and incubated at 37 °C for 5 min. The reaction was stopped by adding the stop buffer (10 mM Tris-HCl, pH 9.5, 0.5% SDS, 10 mM EDTA). DNA was purified, separated by electrophoresis on 1.0% agarose gel in TAE buffer (40 mM Tris-acetate and 1 mM EDTA), and transferred to Hybond N (Amersham Pharmacia Biotech). The membrane was subjected to the hybridization with the radiolabeled DNA probe complementary to the test genes and visualized by autoradiography.

Chromatin Reconstitution by the Salt Dialysis Method—Chromatin structure was reconstituted on the plasmid DNA or end-labeled DNA fragments by the salt dialysis method (24). The 453-bp-long DNA fragment containing the region between nucleotide positions 5789 and 6242 (where the left terminus of the Ad DNA is position 1) was prepared from plasmid pSmaF by digestion with HindIII and XhoI. Core histones, histone H2A/H2B, and histone H3/H4 were separately prepared as described by Simon and Felsenfeld (25) using hydroxylapatite column chromatography. Ten micromoles of the 453-bp-long DNA or pSmaF was mixed with core histones (8 µg) in 2 mM NaOH, pH 7.9, 12.5 mM MgCl2, 60 mM KCl, 1 mM dithiothreitol with or without rTAF-I. 1 pmol each of restriction nuclease, PvuII (Toyobo) or NcoI (Toyobo), was added and incubated at 37 °C for 5 min. The reaction was stopped by adding the stop buffer (10 mM Tris-HCl, pH 7.9, 0.5% SDS, 10 mM EDTA). DNA was purified, separated by electrophoresis on 1.0% agarose gel in TAE buffer (40 mM Tris-acetate and 1 mM EDTA), and transferred to Hybond N (Amersham Pharmacia Biotech). The membrane was subjected to the hybridization with the radiolabeled DNA probe complementary to the test genes and visualized by autoradiography.

Chromatin Reconstitution by the Salt Dialysis Method—Core histones, histone H2A/H2B, and histone H3/H4 were separately prepared as described (20, 21). Ad core (50 ng), plasmid pLAI (5 ng) (19), or pSmaF (5 ng) (19) was incubated at 30 °C for 30 min in 25 mM Hepes-NaOH, pH 7.9, 12.5 mM MgCl2, 60 mM KCl, 1 mM diethiothreitol with or without rTAF-I. Then 5 units of restriction endonuclease, PvuII (Toyobo) or NcoI (Toyobo), was added and incubated at 37 °C for 5 min. The reaction was stopped by adding the stop buffer (10 mM Tris-HCl, pH 7.9, 0.5% SDS, 10 mM EDTA). DNA was purified, separated by electrophoresis on 1.0% agarose gel in TAE buffer (40 mM Tris-acetate and 1 mM EDTA), and transferred to Hybond N (Amersham Pharmacia Biotech). The membrane was subjected to the hybridization with the radiolabeled DNA probe complementary to the test genes and visualized by autoradiography.

Disruption of the Ad Core Structure by TAF-I—Our previous study showed that TAF-I stimulates transcription from the E1A promoter on the Ad core but not from the MLP (19). Here we have examined the effect of TAF-I on the structural change of the Ad core around these promoter regions. The Ad core was subjected to digestion with restriction enzymes after incubation with or without TAF-I. Nucleic sensitivity was monitored by the Southern blot analysis using the radiolabeled DNA probe complementary to either the E1A promoter region spanning nucleotide positions 455–628 from the left end of the adenovirus type 5 or the MLP region spanning nucleotide positions 5779–6242 (Fig. 1A). On the E1A promoter region, TAF-I augments the accessibility of nucleases to DNA in the Ad core and the amounts of DNA fragments generated by PvuII or NciI are increased in a dose-dependent manner (Fig. 1B, upper panel). This suggests that TAF-I induces the conformational change of the Ad core structure, so that nucleases easily gain access to DNA. On the other hand, TAF-I does not have such intensive effect on the MLP region as seen in the E1A promoter region (Fig. 1B, bottom panel). Although small amounts of PvuII or NciI fragments are generated by the digestion of TAF-I (25–277), a large portion of the MLP region in the Ad core remains uncut. The fact that TAF-I induces this conformational change of the Ad core in the E1A promoter region but not in the MLP region is in good agreement with the difference of these promoter activities on the Ad core stimulated by TAF-I, although the detailed mechanism for this selectivity of TAF-I is not known at present.

Next we examined the effect of nucleoside triphosphates
Fig. 1. Restriction endonuclease digestion of the Ad core. A, the diagram of the restriction sites of the Ad DNA E1A and ML promoter regions. The arrows show the transcription initiation sites of the E1A and the ML genes. Numbers relative to the left end (nucleotide position 1) of the Ad genome indicate the sites of PvuII (nucleotide positions 455 and 628 for the E1A promoter region and 4980, 6082, and 6558 for the MLP region) and NciI (422, 478, 550, and 820 for the E1A promoter region and 5359, 5585, 5988, and 6557 for the MLP region). DNA fragments containing the regions spanning nucleotide positions between 455 and 628 for the E1A promoter region, and between 5789 and 6242 for the MLP region, were used as a probe. B, effect of TAF-I on the nuclease accessibility. pLa1 and pSmAF (5 ng each for lanes 1 and 6) or the Ad core (50 ng for lanes 2–5 and 7–10) were incubated at 30 °C for 30 min without lanes 1, 2, 6, and 7) or with 50 ng (lanes 3 and 8), 100 ng (lanes 4 and 9), and 150 ng (lanes 5 and 10) of rTAF-I. Five units of restriction enzyme, PvuII (lanes 5–7) or NciI (lanes 6–10), was added and incubated at 37 °C for 5 min. The digestion patterns were monitored by the Southern blot analysis using radiolabeled DNA probe specific for either the E1A promoter region (upper panel) or the MLP region (bottom panel). C, nucleotide-independent disruption of the Ad core structure. The Ad core (50 ng) was incubated at 30 °C for 30 min without lane 1) or with 150 ng of rTAF-I (lanes 2–5) in the presence (3 mM ATP for lane 3, 3 mM GTP for lane 4, and 3 mM dATP for lane 5) or absence (lanes 1 and 2) of NTP PvuII digestion and the Southern blot analysis were performed as described in B. (NTPs) on the TAF-I activity. As shown in Fig. 1C, the TAF-I activity is totally independent of the presence of ATP, GTP, or dATP. This is consistent with the observation that TAF-I does not have ATPase activity (data not shown). This observation suggests that the mechanism for chromatin remodeling by TAF-I is different from that by ATP-dependent chromatin remodeling complexes. TAF-Iβ had the stronger chromatin remodeling activity than TAF-Iα (data not shown), which is in agreement with the fact that the stimulatory activity for the Ad core replication and transcription by TAF-Iβ is more than that by TAF-Iα (18, 20, 21).

Since the long acidic tail present in the C-terminal region of TAF-I is required for the stimulation of the Ad core DNA replication and transcription from the E1A promoter (20, 21), it is possible that TAF-Iα associates with basic core proteins through the acidic region and that this interaction is involved in the conformational change of the Ad core. In addition, it is not known whether TAF-Iα removes the core proteins from the Ad core complex. To examine this, the Ad core incubated with or without TAF-Iα was subjected to the glycerol gradient assay. When the Ad core is incubated with TAF-Iβ, TAF-Iα is co-sedimented with the Ad core (Fig. 2). This suggests that TAF-Iα binds to the Ad core through core proteins, although the protein seen between TAF-Iα and β (lanes 8–10 in both panels A and B) associated with the Ad core is a possible target of TAF-Iα. However, this would not be the case, since the stoichiometric amounts of TAF-Iα to core proteins are required for the maximal TAF-Iα activity (18, 19, 21) and the ratio of this protein to TAF-Iα is much smaller than that of core proteins to TAF-Iα. It is worth noting that the Ad core treated with TAF-Iβ distributes in broader ranges compared with the non-treated Ad core. These results suggest that TAF-Iα interacts with the Ad core forming an Ad DNA-core protein-TAF-Iα tertiary complex, and that the less dense Ad cores are produced due to the conformational change. It is not known whether TAF-Iα interacts with core protein V, VII, or both.

TAF-I Stimulates the Transcription from Chromatin Templates—Since TAF-I remodels the Ad core and stimulates the transcription from the Ad core, it is important to test whether this is also the case for the cellular type chromatin. Then, to examine the effect of TAF-I on the chromatin template consisting of core histones, we have reconstructed the chromatin structure. In order to avoid the possible contamination of non-histone proteins into the reconstituted chromatin when chromatin assembly extracts of Xenopus or Drosophila are used for the chromatin reconstitution, the salt dialysis method was employed. For reconstitution of the chromatin structure, the 453-bp-long DNA fragment containing the Ad MLP spanning nucleotide positions from –260 to +190 relative to the transcription start site (+1) and core histones purified from HeLa cells were used.

The mechanism of the initiation of transcription from the MLP has been well studied by use of naked DNA templates. The 453-bp-long DNA fragment contains not only TATA box and MLTF/USF binding sites but also downstream element factor binding site. TATA box and MLTF/USF binding sites are the minimal requirement for the in vitro transcription reaction (26), and the binding of downstream element factor to the specific DNA sequence between nucleotide positions +86 and +95 relative to the transcription start site further stimulates the transcription from the MLP (27). The histone-DNA complexes generated by the salt dialysis method were fractionated on a sucrose gradient, and the nucleoprotein complex in each fraction was directly analyzed by electrophoresis on an agarose gel (Fig. 3A). Histone octamer seems randomly positioned on the DNA fragment, and mono-, di-, or trinucleosomes cannot be...
nucleosomes reconstituted on the radiolabeled DNA fragment recovered in fractions 13–17 would contain mono- and dinucleosomes. In fact, when nucleoprotein complex reconstituted on the 453-bp DNA fragment in each fraction was subjected to the MNase digestion assay, only a 150- and 150- and 300-bp fragments, respectively, were recovered from fractions 13 and 17 (data not shown). From these results, we concluded that fractions 13 and 17 mainly contain mono- and dinucleosomes, respectively. These fractions were pooled and used in subsequent experiments.

Next, the transcription reaction was performed (Fig. 4A) using the reconstituted chromatin as a template and nuclear extracts prepared from HeLa cells as enzyme/factors source. Transcripts were detected by the primer extension method as described previously (19). When template DNA is assembled into nucleosome, transcription is repressed (compare lanes 1, 2, and 7). Transcription repression on the dinucleosome is more extensive than that on the mononucleosome (lanes 2 and 7).

Because the promoter region and the transcription start site may remain open when mononucleosome is formed around 3′-end of the DNA fragment, transcription would proceed. This would be the reason why the transcription from the dinucleosome template is completely repressed (lane 7) while that from the mononucleosome is not (lane 2). Of importance is that this transcription repression is relieved by TAF-I (lanes 3–6 and 8–11). As the increase of this transcription level by TAF-I is not observed when naked DNA is used as a template (Ref. 19 and data not shown), this transcription derepression should be induced by the conformational change of the chromatin structure by TAF-I. To confirm this, the nuclease sensitivity assays were performed. When nucleosome is treated by increasing amounts of TAF-I (Fig. 4B), the nucleosome sensitivity is increased and 293- and 160-bp DNA fragments are generated. These results indicate that TAF-I somehow remodels not only the Ad core but also the chromatin structure and thereby stimulates the transcription from DNA-basic protein complexes in vitro. While the level of the nucleosome sensitivity reflecting the level of the remodeling of the structure of the reconstituted chromatin is increased in a TAF-I dose-dependent manner, transcription is not strictly stimulated as the function of increasing amounts of TAF-I. It is known that only a fraction of DNA can be utilized as template for the in vitro transcription reaction even when naked DNA is used. Therefore, it is likely that the amount of remodeled DNA by TAF-I is not optimum for the in vitro transcription reaction. SWI/SNF and related complexes have been shown to stimulate the chromatin remodeling depending on the DNA binding factors that would lead to the promoter specificity of these chromatin remodeling factors (1, 3–7). The activity of TAF-I seems independent of promoter specific factors, since these factors are not required for TAF-I-mediated transcription activation (19).
to test the affinity of TAF-I to each histone complex. We have, therefore, tested the binding of TAF-I to histone H2A/H2B and H3/H4 complexes, which are present as hetero-dimer and -tetramer, respectively, in a cell. Five picomoles each of core histones, H2A/H2B, H3/H4, and rTAF-I-β as a positive control or BSA as a negative control were spotted on a PVDF membrane and the membrane was subjected to the Far Western analysis (Fig. 5C). The 32P-labeled TAF-I protein does not bind to BSA but to the TAF-I-β at the low but distinct level (lanes 5 and 4), since TAF-Iα and β forms both hetero- and homo-oligomer both in vivo and in vitro (18).2 TAF-I is capable of interacting with core histones (lane 1) being in good agreement with the results from the pull-down assay (29), the results shown in Fig. 5B, and the glycerol gradient assay (data not shown). As shown in Fig. 5C, the binding of TAF-I to H3/H4 complex (lane 3) is greater than that to H2A/H2B complex (lane 2). The binding activity of TAF-I to H3/H4 complex is about 68% compared with core histones, while that to H2A/H2B complex is only 13%. This result suggests that TAF-I preferentially interacts with H3/H4 rather than H2A/H2B. Then, the domain of TAF-I involved in association with histones was determined. Wild type and mutant rTAF-I proteins shown in Fig. 5D were subjected to the Far Western analysis using core histones labeled by PKA as a probe. As shown in Fig. 5E, core histones bind to the wild type-TAF-I-β (lane 1). On the other hand, rTAF-I-β(1–225), which completely lacks the acidic region, loses the histone binding activity (lane 2). rTAF-Iβ(26–277), which lacks the N-terminal region specific for TAF-I-β, binds to histones with the same extent as the wild type TAF-I-β (lane 3), and the histone binding activity of rTAF-Iβ(133–277) is about half of that of the wild type (lane 4). These observations suggest that the acidic region of TAF-I is crucial for histone binding and the N-terminal region between the amino acid positions 26 and 132 would be more or less involved in this activity.

Next, we analyzed the chromatin assembly activity of TAF-I using the gel mobility shift assay (Fig. 6). The end-labeled, 453-bp-long DNA fragment was mixed with core histones that had been pre-incubated in the presence or absence of TAF-I. TAF-I does not bind to naked DNA (lanes 1 and 2). When DNA is directly mixed with histones, large histone-DNA aggregates that fail to enter the gel and random complexes are observed (lane 3). In contrast, when core histones are pre-incubated with TAF-I, distinct DNA-protein complexes are formed (lanes 4–7) in a dose-dependent manner of TAF-I. A fraction of the histone-DNA complex formed in the presence of TAF-I migrates to the same position as that of the nucleosome reconstituted on the same DNA fragment by the salt dialysis method (lane 8). The amount of this complex is increased when increasing amounts (50, 100, 200, and 500 ng for lanes 4, 5, 6, and 7, respectively) of TAF-I are mixed. When the structure of this DNA-protein complex was examined by the MNase digestion assay, the discrete 150-bp-long DNA fragment, a unit of nucleosomal DNA, was not clearly detected (data not shown). It is therefore presumed that the DNA-histone complex mediated by TAF-I would be loosely assembled and not form the complete nucleosome structure. However, it is noted that TAF-I prevents the large DNA-histone aggregation (compare lanes 3–7).

**DISCUSSION**

We have described the effects of TAF-I on the reconstituted chromatin. TAF-I was originally identified from HeLa cells as the factor that stimulates replication and transcription from the adenovirus DNA-core protein complex in a chromatin-like structure (18, 19). Here we have shown that TAF-I also stimulates transcription from the reconstituted chromatin consist-

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2 M. Okuwaki and K. Nagata, unpublished data.
The histone binding activity of TAF-I. A, histones purified from HeLa cells. Purified core histones (lane 1), histone H2A/H2B (lane 2), and H3/H4 (lane 3) were prepared as described under "Experimental Procedures," separated by electrophoresis on 15% SDS-PAGE, and stained with Coomassie Brilliant Blue. B and C, Far Western analysis using 32P-labeled TAF-Iβ as a probe. Each histone protein separated as shown in A was transferred to a PVDF membrane and subjected to Far Western analysis. The position of each histone is indicated (B). Five picomoles each of core histones (lane 1), H2A/H2B (lane 2), H3/H4 (lane 3), rTAF-Iβ (lane 4), and BSA (lane 5) was spotted on to a PVDF membrane and incubated with 32P-labeled rTAF-Iβ (C). D, schematic diagram of mutant TAF-I proteins. rTAF-Iβ(1–277), rTAF-Iβ(1–225), rTAF-Iβ(26–277), and rTAF-Iβ(133–277) used for the Far Western analysis are represented schematically. Shaded and filled boxes indicate TAF-Iβ-specific and acidic regions, respectively. The numbers in the parentheses are the amino acid number from the N terminus of wild type TAF-Iβ.

The histone binding activity of TAF-I remodels the Ad core structure on the E1A promoter region, while it does not have such an effect on the MLP region on the Ad core (Fig. 1). In contrast, TAF-I is capable of stimulating the transcription and remodeling the structure of the reconstituted chromatin formed on the DNA fragment containing the MLP (Fig. 4). These results suggest that TAF-I would not have any specificity of DNA sequence, although the MLP on the Ad core is not effectively remodelled by TAF-I. The contradiction of these results would be explained by the difference of the Ad core structure between the E1A promoter and MLP regions. The MLP region may be packed more compactly than E1A promoter region and the MLP in the reconstituted nucleosome. In fact, when the Ad core is used as a template for the transcription, the stimulation of the transcription from the MLP is dependent on the genome DNA replication (19). It is presumed that the MLP region on the Ad core would be more much insensitive to TAF-I than the E1A promoter region on the Ad core and the MLP in the reconstituted chromatin. Therefore, the structural change of the Ad core within the MLP region coupled with the genome DNA replication would be needed for the transcription from the MLP. In infected cells, the transcription from the MLP is activated dramatically after the onset of the genome DNA replication compared with its transcription activity in early phases of infection. The molecular basis of this transcription switching between early and late phases via replication has not been well clarified. The transcription activation from the MLP by the switch could not be explained simply by the increase of the genome copy number. The Ad genome is complexed with viral basic core proteins in the virion and in the cell during early stages of infection, and newly synthesized DNA would be complexed with histones of host cells and assembled into the chromatin structure following the genome replication (16). When replication occurs, the Ad core structure is drastically disrupted and trans-acting factors could easily gain access to the parental genome template and/or the newly synthesized DNA. It is suggested that the chromatin structure would be reconstituted on the newly synthesized DNA complexed with transcription factors. Workman et al. (30) demonstrated that the transcription from chromatin reconstituted on the DNA in the presence of MLTF/USF is active, while the transcription from the chromatin template reconstituted without MLTF/USF is repressed. The mechanism of the transcription activation from the MLP by trans-acting chromatin remodeling factors is the other possibility as we have demonstrated in this study. More precise studies are needed because the transcription level relieved by TAF-I is only 10–20% of that
incubated without (lanes 3) or with 100 ng (lanes 4, 5, 6, and 7, respectively) of rTAF-I. After incubation at 30°C for 30 min, the labeled 453-bp-long DNA fragment (10 ng) was added and further incubated for 1 h and separated by electrophoresis on 0.8% agarose gel. DNA in the absence of core histones incubated without (lane 1) or with 100 ng (lane 2) of rTAF-I, and reconstituted nucleosome ( dinucleosome fraction for lane 8) are also shown.

Fig. 6. The gel mobility shift assay. Core histones (20 ng) were incubated without (lane 3) or with rTAF-Iβ (50, 100, 200, and 500 ng for lanes 4, 5, 6, and 7, respectively) in the transcription reaction mixture. After incubation at 30°C for 30 min, the labeled 453-bp-long DNA fragment (10 ng) was added and further incubated for 1 h and separated by electrophoresis on 0.8% agarose gel. DNA in the absence of core histones incubated without (lane 1) or with 100 ng (lane 2) of rTAF-Iβ, and reconstituted nucleosome ( dinucleosome fraction for lane 8) are also shown.

on the naked DNA. These observations raise the possibility that TAF-I may cooperate with other factors in remodeling the chromatin structure in vivo.

SWI/SNF, NURF, and other factors that require ATP hydrolysis are suggested to function together with sequence-specific DNA-binding proteins. Recently, Mizuguchi et al. (31) demonstrated that NURF complexes remodel the chromatin structure and stimulate the transcription dependent on GAL4 fused to SWI/SNF or NURF-like complexes. Since the DNA fragment used in this study contains the binding sites described above, our system would be useful to further assign roles of these factors. Furthermore, Orphanides et al. (32) reported that NTP-hydrolysis independent accessory factor, termed FACT, is needed for the elongation step of the transcription from the chromatin templates, although the detailed mechanism of the reaction mediated by FACT is unknown at present. The functional nature of FACT would be different from that of the factors that function as histone chaperone including TAF-I, since FACT cannot re-model the chromatin structure.

TAF-I also suppresses the random aggregation of histones, possibly through complex formation with core histones as shown in Figs. 5 and 6. NAP-I and nucleoplasm and N1/N2 and CAF-I bind preferentially to histones H2A/H2B and histones H3/H4, respectively (reviewed in Ref. 33). This study showed that TAF-I preferentially interacts with histones H3/H4 rather than H2A/H2B, although more precise experiments under physiological conditions are needed. Since there are a variety of acidic proteins that have the histone binding activity (35), the function of each protein and its behavior within a cell should be carefully investigated. In addition, the histone-DNA complex formed by TAF-I would not be a complete chromatin structure. The glycerol gradient assay indicated that the Ad core treated with TAF-I forms the tertiary complex (Fig. 2). Since the mobility of the major nucleoprotein complex in the presence of TAF-I as shown in lane 7 of Fig. 6 was similar to that of the reconstituted chromatin, TAF-I would not be present in the complex. However, it is possible that TAF-I is present in complexes tailing toward the gel origin. It has been reported that NAP-I assembles the chromatin structure cooperatively with ATP-dependent complex (34). It is possible that the nucleoprotein complex formed by TAF-I (Fig. 6) is loosely assembled and another factor would be needed to form the complete chromatin structure. It has been shown that polyions such as polyglutamic acid and bulk RNA are also able to prevent the random aggregation between DNA and histones, and mediate the histone transfer process (reviewed in Refs. 33 and 35). The internal deletion mutant of TAF-Iβ, which has the same long acidic tail as wild type TAF-Iβ, loses the stimulatory activity for the replication from the Ad core (20), suggesting that not only acidity but also the proper conformation of the acidic region in TAF-I is required for its activity.

The function of TAF-I in vivo is unknown. It is reported that components of SWI/SNF complex are enriched in the active chromatin and nuclear matrix fragments (36). From the fact that TAF-I has been originally purified from cytoplasm fractions, TAF-I seems to leaked out easily from the nucleus, although TAF-I has a nuclear localization signal and it is retained in the nucleus in part through its acidic region (29). It is an open question how the TAF-I activities for disruption of the chromatin structure and prevention of random aggregation between DNA and histones are controlled in a cell. Since the level of TAF-I proteins are not significantly fluctuated through the cell cycle (29), qualitative change rather than quantitative change would be needed. It is tentatively speculated that some modifications may operate in the regulation of the TAF-I activity. Recently, it has been reported that Xenopus TAF-Iβ homologue specifically binds to a B-type cyclin (37). Human TAF-I is found to inhibit the activity of protein phosphatase 2A (38) by the multi-protein complex formation (39). Based on these facts, the TAF-I activity is possibly regulated during the cell cycle by phosphorylation and/or TAF-I may regulate phosphorylation/dephosphorylation. On this line, TAF-I has been shown to be phosphorylated in vivo in its N-terminal region (40), although a specific kinase(s) that phosphorylates TAF-I is unknown. From these observations, TAF-I would be a multi-functional protein.

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