Histone acetylation-independent transcription stimulation by a histone chaperone

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ABSTRACT

Histone chaperones are thought to be important for maintaining the physiological activity of histones; however, their exact roles are not fully understood. The physiological function of template activating factor (TAF)-I, one of the histone chaperones, also remains unclear; however, its biochemical properties have been well studied. By performing microarray analyses, we found that TAF-I stimulates the transcription of a sub-set of genes. The transcription of endogenous genes that was up-regulated by TAF-I was found to be additively stimulated by histone acetylation. On performing an experiment with a cell line containing a model gene integrated into the chromosome, TAF-I was found to stimulate the model gene transcription in a histone chaperone activity-dependent manner additively with histone acetylation. TAF-I bound to the core histones and remodeled the chromatin structure independent of the N-terminal histone tail and its acetylation level in vitro. These results suggest that TAF-I remodels the chromatin structure through its interaction with the core domain of the histones, including the histone fold, and this mechanism is independent of the histone acetylation status.

INTRODUCTION

Chromatin remodeling is a crucial step prior to transcription initiation. Various chromatin regulatory factors have been identified. ATP-dependent chromatin remodeling (ADCR) complexes and histone modification enzymes, such as histone acetyltransferases (HATs) and deacetylases (HDACs) are associated with the dynamic regulation of the chromatin structure and gene activity (1). The cooperative function of these two groups is well documented (2). For instance, SWI/SNF, an ATP-hydrolyzing factor of the ADCR complexes, is recruited to the gene promoter region through the recognition of acetylated histones, and it sequentially promotes alteration of the nucleosome structure (3). In addition to these two groups, the histone chaperone family is also involved in chromatin regulation and histone metabolism (4). In vitro studies have demonstrated that histone chaperones bind to core histones and facilitate assembly and disassembly of the nucleosome structure in an ATP-independent manner. Nucleosome assembly protein-1 (NAP-1), one of the best-characterized histone chaperones, facilitates assembly and disassembly of nucleosomes containing both canonical and variant histones in vitro (5,6). It is reported that NAP-1 facilitates disassembly of nucleosome in concert with ADCR and histone acetylation in vitro (7,8). However, there exists little information on the physiological role of histone chaperones and their cooperative function with ADCR complexes and/or histone modifications.

By using the adenovirus (Ad) chromatin-like genome (Ad core) as a template, we found that template activating factor (TAF)-I, a histone chaperone, acts as a stimulatory factor for in vitro adenovirus DNA replication (9). Two isoforms exist, namely, TAF-Iα and TAF-Iβ, and these form homo- or hetero-dimers through their dimerization domain (10). TAF-Iβ is encoded by the putative oncogene SET (11). TAF-I is implicated in the regulation of several cellular processes including mRNA stability, cell-cycle regulation, signal transduction and apoptosis (12–16). We demonstrated that TAF-I has histone chaperone activity (17,18). Dimerization and the acidic amino acid cluster are essential for the histone chaperone activity and the stimulation of Ad core DNA replication in vitro (10,17,18). TAF-I stimulates the cell-free transcription from the cellular-type chromatin template in vitro (17). Thus, it is quite possible that TAF-I plays a basic role in the assembly and disassembly of the cellular chromatin structure and the regulation of gene activity. It is indicated that TAF-I is a component of an inhibitor of the histone acetyltransferase complex (INHAT) (19). In addition, TAF-I interferes with the DNA binding of transcription factors, such as Sp1 and KLF5, and thereby represses

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transcription (20, 21). In contrast to these repressive roles of TAF-I, it has been demonstrated that TAF-Iβ is involved in the stimulation of Hoxa9 and KAI1 gene transcription (22, 23). It is also reported that TAF-I augments the transcriptional activity of CBP, a HAT/co-activator protein (24). When considered together, these results suggest that TAF-I may regulate transcription positively or negatively in a gene-specific manner, possibly in concert with histone acetylation. However, the molecular mechanism of the gene-specific function of TAF-I and the functional interaction between TAF-I and histone modifications are not well understood.

Here, we have addressed the mechanism of transcription stimulation by TAF-I in concert with histone acetylation. cDNA microarray analyses demonstrated that TAF-I is involved in the positive or negative transcription regulation of a sub-set of genes. The transcription regulation of genes regulated by TAF-I is independent of the level of histone acetylation. Furthermore, TAF-I stimulated transcription from a model gene is integrated in the chromosome in a histone chaperone activity-dependent manner, but independently of the histone acetylation level. Consistent with this finding, we found that the histone N-terminal tail is not required for the histone chaperone activity of TAF-I.

MATERIALS AND METHODS

Cell cultures

Monolayer cultures of HeLa cells and WT (clone 7) and TAF-I KD (clone 4) HeLa cell lines (25) were maintained at 37°C in the minimal essential medium (MEM; Nissui) containing 10% FBS. CHO-AA8-Luc Tet-Off control cell line (CHO-luc) (CLONTECH) was maintained at 37°C in the minimum essential alpha medium (α-MEM; GIBCO) containing 10% FBS. In CHO-luc cells, the luciferase gene transcription is regulated by a tetracycline-responsive VP16 fusion activator (TetR-VP16) (26). In order to investigate the effect of TAF-Iβ under the condition where the luciferase expression does not reach the saturation level, we set the tetracycline concentration (1 μg/ml) so as to keep the luciferase expression at the basal level.

Plasmid DNAs and recombinant proteins

Plasmid DNAs, pCAGGS, an eukaryotic expression vector (27), pCHA-TAF-Iβ and pCHA-TAF-IβΔC3, were prepared as described previously (22). To generate pCHA-TAF-IβPM, for expression of TAF-Iβ containing point mutants in its dimerization domain, a DNA fragment was prepared by digestion of pET-14bTAF-IβPM plasmid DNA (10) with NdeI and BamH1. The cohesive ends of this fragment were treated with Klenow fragment to be blunt and ligated into MluI-digested pCHA, the ends of which had also been filled with Klenow fragment. Hexa-histidine- (His-) tagged TAF-Iβ, TAF-IβΔC3 and TAF-IβPM were prepared as described previously (10).

Antibodies

Antibodies used in this study are as follows: For TAF-Iα and β, mouse anti-TAF-I monoclonal antibody (KM1726) and for TAF-Iβ, mouse monoclonal antibody (KM1720) (28); for experimental control of ChIP, mouse anti-Flag (M2) monoclonal antibody; for Hsp90, rabbit polyclonal antibody [kindly provided by Drs Miyata Y. and Nishida E. (Kyoto University)] (29); for TBP, rabbit polyclonal antibody (Santa Cruz); for HA-tag, rat monoclonal (3F10) antibody (Roche), for histone H3, rabbit polyclonal antibody (abcam); for acetylated histone H3 (K9, K14), rabbit polyclonal antibody (Upatate); for histone H4, rabbit polyclonal antibody (abcam); for acetylated histone H4 (K5), rabbit polyclonal antibody (abcam).

Microarray analyses

Total RNA was isolated from WT and TAF-I KD HeLa cell lines using the guanidine method (30). The concentration of RNA in each sample was determined using a spectrophotometer. The RNA was processed and hybridized to the Code Link™ human whole genome array, according to the manufacturer’s protocols (GE Healthcare). Chips were scanned and analyzed using Microarray software (arrayWoRx®).

RT–PCR

WT and TAF-I KD HeLa cells were treated without or with a HDAC inhibitor, trichostatin A (TSA) at the concentration of 0.1 μM for indicated periods. cDNA was synthesized from the total RNA (2 μg) using Superscript III reverse transcriptase (RT; Invitrogen) and oligo-dT primer. PCR was performed using synthesized cDNAs and a set of specific primers complementary to human AKAP12, KRT17, TFAP2C, SERPINI1, cTnC, β-actin, and RPL13A (31) mRNAs by pre-determined PCR cycles under which PCR products are logarithmically amplified. Primer sequences were as follows: AKAP12 forward direction, 5′-GGAGACCATCAACCATCACAG-3′; AKAP12 reverse direction, 5′-CAGTTGATGGATGACAGTG-3′; KRT17 forward direction, 5′-GAGGATTGTTCTTTACTCAAG-3′; KRT17 reverse direction, 5′-TCATACAGCAGATTGTTGA-3′; TFAP2C forward direction, 5′-GGCCACCAACTGTTGAAAGA-3′; TFAP2C reverse direction, 5′-GCGTTCTGTATGCTCTG-3′; TFAP2C reverse direction, 5′-GACATGTTCTATATGGTCT-3′; SERPINI1 forward direction, 5′-GACATGTTGTGCTGCACT-3′; SERPINI1 reverse direction, 5′-TTTGGACTTTACTATCATCTT-3′; cTnC forward direction, 5′-GTAGAGCAGCTGACAGAAG-3′; cTnC reverse direction, 5′-TTGTCACACACTGCGAAGAAG-3′; β-actin forward direction, 5′-ATGGGTCAGAAGGATTCT-3′; β-actin reverse direction, 5′-GGTCATCTTCTCCT-3′; RPL13A forward direction, 5′-CATCGTGCG-AATAATTGTTGTA-3′; RPL13A reverse direction, 5′-GACAGCTTGACAGAGG-3′, and RPL13A reverse direction, 5′-GCACGGCCCTGAGGAGCC-3′. The PCR products were separated on a 6% PAGE, visualized by staining with EtBr, and quantified with NIH Image.

Luciferase assay

CHO-luc cells transfected with plasmid DNAs where indicated were washed with phosphate-buffered saline (PBS) and lysed in a cell lysis buffer [25 mM Tris–HCl (pH 7.9), 10% Glycerol and 0.1% Triton X-100] by five freezing-thawing cycles. The cell lysates and a luciferase substrate (Promega) were mixed, and the luciferase activity was measured by Lumat LB9506 (BERTHOLD). Transfection
was performed using TransIT LT1 transfection reagent (Mirus).

Chromatin immunoprecipitation (ChIP)

HeLa cells were fixed with 1% formaldehyde at room temperature for 10 min, and ChIP was carried out essentially according to the protocol from Upstate Biotechnology using antibodies where described. The recovered DNA was amplified by PCR with the primer sets. Primer sequences used were as follows: β-actin promoter forward direction, 5'-ATGCTGCAGGCGGAGC-3'; β-actin promoter reverse direction, 5'-TGGAGTCGACGCTC-3'; cTnC promoter forward direction, 5'-TGTAGCTTGCATCGAGCGC-3'; and, cTnC promoter reverse direction, 5'-TGGAGTCCAC-3'. Transfected CHO-luc cells were fixed with 1% formaldehyde at room temperature for 10 min, and ChIP was carried out. A primer set, 5'-CTGGTACCCGGGT-3' and 5'-GGCCTCACCAGGT-3', was used to amplify human cytomegalovirus (HCMV) minimal promoter region which directs transcription of the integrated luciferase gene. The PCR products were separated on a 6% PAGE, visualized by staining with EtBr, and quantified with NIH Image.

Chromatin reconstitution and micrococcal nuclease (MNase) digestion assay

Chromatin was reconstituted on a plasmid DNA, pUC119-ML2, with core histones prepared from mock-treated cells, hyperacetylated core histones prepared from cells treated with sodium n-butyrate (NaBu), a HDAC inhibitor (32), and tail-less core histones by the salt dialysis method as described previously (17). To generate pUC119-ML2, a DNA fragment containing the region between nucleotide position 4979 and 6242 (where the left terminus of adenovirus type 2 DNA is defined as position 1) was excised from pSmaF (33) by digestion with PstI and HindIII and cloned into PstI and HindIII-digested pUC119. Reconstituted chromatin (50 ng DNA) was subjected to partial digestion with MNase in a digestion buffer [10 mM Tris–HCl (pH 7.9), 70 mM NaCl, 3 mM CaCl2 and 1.5 mM MgCl2] at 37°C. Digested DNA was purified, separated by 1.4% agarose gel electrophoresis, and visualized by SYBR Gold (Molecular probe) staining.

RESULTS

Involvement of TAF-I in the transcription regulation in vivo

It has been reported that TAF-I is involved in the expression of various genes. We attempted to confirm this using the microarray technique and TAF-I knock-down (KD) cells. Previously, we had established stable HeLa cell lines in which the expression of TAF-I was knocked down by siRNA (25). Here, we used one of the TAF-I KD cell lines, in which the expression levels of the proteins TAF-Ia and TAF-Ib were reduced to ~10% relative to that of the wild-type cell (WT) (Figure 1A). Comparison of the gene expression profile of TAF-I KD with that of WT cells by microarray analysis indicated that several genes are down-regulated or up-regulated due to the reduction of the expression level of TAF-I, suggesting that TAF-I stimulates and represses subsets of genes. Among the 29172 array probes that we tested, we detected 106 probes that showed more than a 2-fold down-regulation in the signal and 48 probes that showed more than a 2-fold up-regulation in the signal. We selected genes that were well annotated, and summarized the results (Supplementary Table 1). We confirmed the mRNA expression level of some of the genes by using semi-quantitative RT–PCR (Figure 1B, and data not shown). Figure 1B shows the RT–PCR result which indicates that AKAP12 and KRT17 are the down-regulated genes and TFAP2C and SERPINH1 are the up-regulated genes. This suggests that the alteration pattern of these genes matches well with the results of the microarray analysis. To study the positive function of TAF-I, we decided to focus on certain down-regulated genes in the TAF-I KD cells. Among the genes examined so far, the slow/cardiac troponin C (cTnC) gene (34) was observed to be one of the typical genes whose expression level was drastically reduced in the TAF-I KD cells (Figure 1C). To examine whether TAF-Ib is associated with the cTnC gene promoter, we performed ChIP assays by using an antibody against TAF-Ib (Figure 1D). TAF-Ib interacted with the cTnC promoter but not the β-actin promoter region, suggesting that TAF-I regulates the cTnC gene expression at the level of transcription by binding to the promoter region.

TAF-I stimulates the model gene transcription in a histone chaperone activity-dependent manner

By microarray analyses, we found that TAF-I stimulates the transcription of a sub-set of genes, such as cTnC. Further, to quantitatively analyze the effect of TAF-I on the transcription from the chromatin template, we exploited the properties of the cell line CHO-luciferase (CHO-luc); in CHO-luc the model reporter gene is integrated in the chromosome such that the luciferase gene transcription is regulated in the context of the chromatin structure. By using this system, we found that the luciferase expression is stimulated by TAF-Ib in a dose-dependent manner (Figure 2A and B). We did not detect the TAF-I-dependent transcription stimulation when the luciferase reporter plasmid was transiently co-transfected with a TAF-Ib expression vector (data not shown), indicating that TAF-I-dependent stimulation is specific for the gene integrated in the chromosome.

To examine whether this stimulatory effect of TAF-I is dependent on its histone chaperone activity, we prepared histone chaperone activity-deficient TAF-Ib mutants (Figure 2A–C). Since the acidic amino acid cluster and dimerization of TAF-I are essential for the remodeling of the Ad core and histone chaperone activity, we used two mutants -one lacking the C-terminal acidic amino acid cluster region, termed TAF-IbΔC3, and the other containing point mutations in the coiled-coil dimerization domain, termed TAF-IbPME (10,11). The acidic amino acid cluster is essential for the INHAT activity, while dimerization of TAF-Ib is not completely required (19). In contrast, neither the acidic amino acid cluster nor the dimer formation is essential for the II-P2A activity of TAF-Ib (16). These suggest that the dimerization domain is crucial for the maximal chaperone
activity but not essential for the INHAT and the I2PP2A activities. In contrast to TAF-IbWT (wild-type), neither TAF-IbD3C nor TAF-IbPME stimulated luciferase expression, suggesting that the stimulation of the luciferase gene transcription is dependent on the histone chaperone activity of TAF-Ib. Next, we examined the chromatin remodeling activity of TAF-Ib, and compared this activity of TAF-IbWT with that of each TAF-Ib mutant by using the nuclease sensitivity assay with recombinant His-tagged TAF-Ib derivatives (Figure 2D and E) (10). The chromatin structure was reconstituted using purified cellular core histones on a plasmid DNA in vitro (17). After incubation with each TAF-Ib protein, the reconstituted chromatin was subjected to nuclease sensitivity assays by using micrococcal nuclease (MNase). While the reconstituted chromatin was highly insensitive to digestion by MNase, TAF-IbWT enhanced the nuclease sensitivity of the reconstituted chromatin (Figure 2E, compare lanes 2–4 and 5–7); this was in good agreement with the previous report, which stated that TAF-IbWT efficiently remodels the chromatin structure (17). In contrast, the nuclease sensitivity of chromatin was slightly induced by TAF-IbPME (Figure 2E, lanes 5–7 and 11–13). The nuclease sensitivity was not significantly induced by TAF-IbD3C (Figure 2E, lanes 8–10), while TAF-IbD3C showed slightly higher activity than TAF-IbPME. These results suggest that the chromatin remodeling activity of TAF-Ib is dependent on both dimer formation and the acidic cluster region. It is likely that the hydrophilic surface of the dimerized coiled-coil region has a slight chromatin remodeling activity as shown by the experiments using the Ad core (35). When these results are considered together, it may be suggested that TAF-Ib
remodels the chromatin structure of the model gene and stimulates its transcription in CHO-luc cells. Next, to determine whether over-expression of TAF-Iβ affects histone acetylation around the model gene promoter region, we examined the histone acetylation level in the model gene promoter region by ChIP assays. In CHO-luc cells that over-express each TAF-Iβ derivative, the amounts and the acetylation levels of histone H3 and H4 were not significantly altered (Figure 3A). These results indicate that TAF-Iβ does not affect the histone acetylation of the model gene promoter. Next, to determine whether TAF-Iβ is associated with the model gene promoter region, we carried out ChIP assays using a TAF-Iβ antibody (Figure 3B). The expression level of each HA-tagged TAF-Iβ derivatives. Cell extracts (total amount of protein; 1.5 μg) prepared from CHO-luc cells transfected with each amount of DNA for expression of TAF-Iβ mutants [see (A), under the graph] were separated by a 10% SDS–PAGE, and each HA-tagged TAF-Iβ protein was detected by immunoblotting with anti-HA antibodies. Results of three independent experiments are shown. (C) Representation of relative transcription stimulatory activities of TAF-Iβ derivatives. The band intensity of each TAF-Iβ derivatives (B) were quantitatively measured with NIH image. The luciferase activity (A) was normalized by each protein expression level, and the relative activity is summarized, where the average of experiments using the TAF-Iβ WT is set as 1. Results are represented as mean values ± SDs from three independent experiments. (D) Purified recombinant proteins. Recombinant His-tagged TAF-Iβ (WT), TAF-IβΔC3 (ΔC3), TAF-IβPME (PME) (400 ng each) generated in the bacterial expression system were purified according to the manufacture’s instruction, separated by a 15% SDS–PAGE, and visualized by staining with Coomassie brilliant blue. Lane M shows molecular weight markers. (E) MNase sensitivity assay. Reconstituted chromatin (50 ng DNA) was incubated at 30°C for 30 min without (lanes 1–4) or with His-tagged TAF-Iβ (1.2 μg, lanes 5–7), His-tagged TAF-IβΔC3 (960 ng, lanes 8–10) and His-tagged TAF-IβPME (1.2 μg, lanes 11–13). The mixture was then subjected to partial digestion without (lane 1) or with MNase (0.0003 U for lanes 2, 5, 8 and 11, 0.0015 U for lanes 3, 6, 9 and 12, 0.0075 U for lanes 4, 7, 10 and 13) at 37°C for 6 min. DNAs were purified, separated by electrophoresis on a 1.4% agarose gel and visualized by SYBR Gold staining. Lane M shows DNA size markers. The nucleosome-derived DNA ladders are shown by bullets.
compared to WT (Figure 3B, lanes 10 and 12, and D). TAF-I
bD
C3 was also associated with the model gene promoter with lower efficiency than TAF-I
b
WT (Figure 3B, lanes 10 and 11, and D). These results suggest that dimer formation but not the acidic amino acid cluster of TAF-I
b
is essential for its association with chromatin. It is interesting to note that the acidic cluster is not required for the chromatin binding of TAF-I. Nevertheless, it is concluded that the histone chaperone/chromatin remodeling activity of TAF-I is important for stimulation of the transcription of the model gene. TAF-I and histone acetylation additively stimulates the transcription

Next, we examined the effect of histone acetylation on the model gene transcription stimulated by TAF-I
b
(Figure 4). CHO-luc cells expressing TAF-I
b
WT were incubated without or with increasing concentrations of TSA, a class I HDAC inhibitor (36). The luciferase expression was stimulated by the over-expressed TAF-I
b
(1st and 2nd bars). The luciferase expression was slightly stimulated by the addition of 3 nM of TSA (1st and 3rd bars), significantly stimulated in combination with over-expressed TAF-I
b
(1st–4th bars), and greatly stimulated by the addition of 10 nM of TSA (1st and 5th bars). Under this condition however the over-expressed TAF-I
b
was not stimulatory (5th and 6th bars). The expression levels of both endogenous and exogenous TAF-I
b
were not affected by TSA treatment (Figure 4, upper panel). These results indicate that the model gene transcription is stimulated by TAF-I
b
additively or slightly more than additively with histone acetylation when the basal transcription level of the model gene remains at a low level. The stimulatory effect of TAF-I
b
decreased when the model gene transcription was sufficiently stimulated by a high level of histone acetylation or the transcription activator TetR-VP16 (data not shown). Based on these results, it is presumed that TAF-I is associated with genes expressed at an intrinsically low level. This possibility is supported by the observation of the endogenous cTnC gene (Figure 1D), since the transcription of the cTnC gene is maintained at a low level in HeLa cells as compared with that in the cardiac ventricle and slow skeletal muscle cells (34). We compared the acetylation level of histone H3 and the amount of TBP (TATA-binding protein) at the cTnC promoter with those at
the β-actin promoter using ChIP assays. The results of ChIP assays clearly demonstrated that both the acetylation level of histone H3 and the amount of TBP at the cTnC promoter were lower than those of the β-actin promoter (Figure 1D). These results suggest that the transcription of the cTnC gene occurs at a basal level, and TAF-I may be involved in the basal transcription step.

To confirm this finding using the model gene, we examined the effect of histone acetylation induced by TSA on the transcription level of the endogenous cTnC gene in TAF-I KD cells. If TAF-I functions as INHAT on the cTnC gene, TSA may restore the cTnC transcription level in the KD cells more efficiently than that in the WT cells. In contrast, if TAF-I positively affects the HAT function, TSA may restore the cTnC transcription level in the WT cells more efficiently than that in the KD cells. Total RNA was prepared from the cells incubated with TSA and subjected to semi-quantitative RT–PCR (Figure 5A), and the results are summarized in Figure 5B. In both WT and TAF-I KD cells, the cTnC transcription was up-regulated during the incubation periods in the presence of TSA, whereas the ribosomal protein L13A (RPL13A) gene transcription remained unaltered (31). The transcription level of the cTnC in the WT cells was higher than that in the TAF-I KD cells at all time points, suggesting that TAF-I functions positively in the cTnC transcription. Furthermore, the stimulation profiles of both WT and TAF-I KD cells were parallel, suggesting that the cTnC gene transcription is stimulated by TAF-I additively with histone acetylation.

Association of TAF-Iβ with chromatin independent of the histone acetylation level in vivo

It has been reported that TAF-Iβ represses the EBI1 gene transcription in a manner similar to INHAT (37). In this gene regulation, the association of TAF-Iβ with the promoter region is repressed by histone acetylation. By using the model cell line, we have demonstrated that the transcription of the model gene is stimulated by TAF-Iβ additively with histone acetylation. Since TAF-Iβ stimulated the model gene transcription independent of the level of histone acetylation, there is a high-possibility that the association of TAF-Iβ with the model gene is not affected by histone acetylation. To confirm this, we examined the amount of TAF-Iβ associated with the model gene promoter under various levels of histone acetylation. By using antibodies against TAF-Iβ and acetylated histone H3, we performed ChIP assays with CHO-luc cells that over-express TAF-Iβ and were treated with TSA (Figure 6). The ChIP assays showed that the amounts of DNA co-immunoprecipitated with an antibody against the acetylated histone H3 increased gradually with the incubation periods in the presence of TSA. This indicates that the histone associated with the model gene promoter are acetylated as a function of the incubation time with TSA. In contrast, the amount of DNA co-immunoprecipitated with an antibody against TAF-Iβ remained unaltered (lower panel). These results suggest that TAF-Iβ is associated with the model gene promoter independent of the histone acetylation level.

Histone tail and the acetylation-independent histone chaperone activity of TAF-I

Next, we examined whether acetylation of the histone N-terminal tail affects the histone binding activity of TAF-Iβ
incubation with increasing amounts of TAF-Iβ (Figure 7E, lanes 5–7). The nuclease sensitivity of the chromatin reconstituted with Ac-core histones was slightly more sensitive than that of the other reconstituted chromatin structures (Figure 7E, lanes 5, 9 and 13). This is consistent with the previous finding that hyperacetylated chromatin forms an extended conformation as compared to the hypoacetylated chromatin (40). The nuclease sensitivity of the chromatin reconstituted with N-, Ac- and TL-core histones showed equal increase in a TAF-Iβ dose-dependent manner (Figure 7E, lanes 5–7, 9–11 and 13–15). These results indicate that TAF-Iβ remodels the chromatin structure irrespective of the histone tail and its acetylation.

**DISCUSSION**

Based on the result of the microarray analyses, we have demonstrated that TAF-I is involved in transcription stimulation and repression of gene sub-sets (Supplementary Table 1 and Figure 1B). Currently, we have not reached a consensus on the mechanism of gene down-regulation in TAF-I KD cells. However, it is noted that among the genes up-regulated by TAF-I KD, TPD52L1, TFAP2C, DNAJC12 and TFF1 genes are reported to be estrogen-responsive (1,41–43). The transcription of these genes was increased by estrogen in the estrogen receptor (ER)-positive breast cancer cells, such as MCF-7 cells. Hence, we propose that these estrogen-responsive genes are repressed by TAF-I possibly through its INHAT activity in the ER-negative cells such as HeLa cells.

TAF-Iβ stimulated transcription additively with histone acetylation induced by the low but not the high-concentration of HDAC inhibitors (Figure 4). Since the order of acetylation of the various lysine residues in histones is not random (44), it is possible that the initially acetylated sites make the nucleosomal DNA more accessible to the remodeling factors by facilitating a conformational transition of the nucleosome. Consistent with this finding, it has been reported that the low concentration of HDAC inhibitor causes local structural changes of the nucleosomes and enhances the basal transcription from the MMTV promoter independent of a hormone ligand, while the high-concentrations of inhibitors inhibits the transcription (45). We demonstrated that TAF-Iβ targets the C-terminal histone fold domain rather than the N-terminal histone tail of the histones in nucleosomes (Figure 7B and E). It is possible that TAF-Iβ contacts the DNA-wrapped core region of this transcription-competent chromatin and loosens the histone fold–DNA interaction independent of histone acetylation, thereby promoting the formation of the basal transcription machinery or attenuation of resumption of the competent chromatin to the repressed chromatin structure. Once genes are sufficiently activated by forming the loosened nucleosome structure, TAF-I may not be required. We found that the MNase sensitivity is greatly increased at the model gene promoter region in the CHO-lac cells after incubation with 1 mM of TSA for 24 h (data not shown). It is possible that histones are highly acetylated and most of the histones might be removed from the promoter region, so that a large amount of histone chaperone is not required. Although we have focused on the transcription stimulatory
function of TAF-I in this study, it is possible that our finding on the TAF-I-mediated mechanism may be applicable to transcription inhibition as well. Remodeling of the chromatin structure by TAF-I might promote the binding of a repressor(s) to the DNA, thereby repressing the transcription independent of its INHAT function.

Why TAF-I does not play a role similar to that of INHAT at the cTnC gene and the model gene promoters used in this study remains to be determined. A current model of the INHAT function indicates that TAF-I binds stably to nucleosomes and represses the access of HATs by masking the N-terminal histone tail (19). However, we could not detect stable TAF-I-nucleosome complexes in vitro and in vivo (data not shown). We found that only chromatin from cells cross-linked with formaldehyde, but not from naive cells, co-immunoprecipitated with TAF-I (Figures 1D and 3B, and data not shown). These results indicate the possibility that association of TAF-I with chromatin is weak and/or
transient in vivo because TAF-I is a member of chaperones. Although TAF-I and chromatin cannot form a stable complex, a transient contact of TAF-I with the nucleosomes may be sufficient to remodel the chromatin structure (Figures 2E and 7E). However, this could be less effective in remodeling and is not a gene-specific process. In fact, the molecular number of the TAF-Iβ dimer required for remodeling was 5 to 15 times more than that of histones in our in vitro chromatin remodeling assays (Figures 2E and 7E). It is possible that TAF-I alone cannot function efficiently as a transcriptional stimulatory factor or INHAT in vivo. We hypothesize that other cellular factors/cofactors would support the gene-specific function of TAF-I for recruiting TAF-I on a specific gene locus and/or modulating the function of TAF-I. Although TAF-IβAC3 could be associated with the model gene promoter region, it was found to be incapable of stimulating transcription in vivo (Figures 2C and 3D) and remodeling the chromatin structure in vitro (Figure 2E). These suggest that recruiting TAF-I on the specific chromatin region could be dependent on proper dimer formation and/or the resultant exposure of the hydrophilic surface by dimer formation (10) rather than the interaction between histones and the acidic cluster of TAF-I. Further, it is interesting to hypothesize that the C-terminal acidic cluster is a functional domain of TAF-Iβ for the chromatin remodeling, and the N-terminal region containing the coiled-coil dimerization region with the hydrophilic surface is a binding domain for the gene-specific factor to recruit TAF-I on the chromatin. For example, THAP7, a recently identified TAF-I interacting protein, recruits TAF-Iβ to a specific promoter and down-regulates the transcriptional activity of the retinoic acid receptor and ER (46). It is worthwhile to note that 120–225 amino acid residues of TAF-Iβ is essential for the binding to THAP7, and the N-terminal region containing the coiled-coil region greatly enhances this interaction, while the C-terminal acidic cluster is dispensable (46). Fe65 behaves like an adaptor protein that assembles TAF-I and Tip60 on the KAI1 gene chromatin, and consequently stimulates its transcription (23). We demonstrated that TAF-I may specifically targets MLL on certain gene loci (22). In this sense, systematic identification of genes regulated by TAF-I and genomic regions associated with TAF-I could be useful to obtain a cue. We propose that transcriptome and interactome analyses can greatly aid in the investigation and classification of the gene regulatory roles for not only TAF-I and but also other histone chaperones.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR online.

ACKNOWLEDGEMENTS

The authors thank Drs H. Haruki and S. Saito for construction of TAF-I KD HeLa cells and the useful discussion and suggestion about the reporter gene assay and preparation of pCHA-TAF-IβPME plasmid, respectively. This work was supported by grants-in-aid from the Ministry of Education, Culture, Sports, Science and Technology of Japan (K.N. and M.O.), and a grant for the project of Tsukuba Advanced Research Alliance (K.N.). Funding to pay the Open Access publication charges for this article was provided by a grant for the project of Tsukuba Advanced Research Alliance.

Conflict of interest statement. None declared.

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