Functional Interaction of the DNA-binding Transcription Factor Sp1 through Its DNA-binding Domain with the Histone Chaperone TAF-I*

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Transcription involves molecular interactions between general and regulatory transcription factors with further regulation by protein-protein interactions (e.g. transcriptional cofactors). Here we describe functional interaction between DNA-binding transcription factor and histone chaperone. Affinity purification of factors interacting with the DNA-binding domain of the transcription factor Sp1 showed Sp1 to interact with the histone chaperone TAF-I, both α and β isoforms. This interaction was specific as Sp1 did not interact with another histone chaperone C1A nor did other tested DNA-binding regulatory factors (MyoD, NFκB, p53) interact with TAF-I. Interaction of Sp1 and TAF-I occurs both in vitro and in vivo. Interaction with TAF-I results in inhibition of DNA-binding, and also likely as a result of such, inhibition of promoter activation by Sp1. Collectively, we describe interaction between DNA-binding transcription factor and histone chaperone which results in negative regulation of the promoter. This novel regulatory interaction advances our understanding of the mechanisms of eukaryotic transcription through DNA-binding regulatory transcription factors by protein-protein interactions, and also shows the DNA-binding domain to mediate important regulatory interactions.

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1 The abbreviations used are: DBD, DNA-binding domain; GST; glutathione S-transferase; CBB, Coomassie Brilliant Blue; HA, hemagglutinin; TOF-MS, time-of-flight mass spectrometry; MALDI-TOF, matrix-assisted laser desorption/ionization time-of-flight.
DBD, we have in the present study affinity-purified interacting factors and analyzed their functional effects on Sp1. Here we show interaction of Sp1 through its DBD with the histone chaperone TAF-I. Interaction between these proteins is specific. This interaction inhibits Sp1 DNA binding, and also likely as a result of such, inhibits promoter activation by Sp1; thus TAF-I functions as a negative regulator of Sp1. This novel regulatory interaction between DNA-binding regulatory transcription factor and histone chaperone adds to our understanding of the mechanisms of how DNA-binding regulatory transcription factors are regulated by protein-protein interactions.

EXPERIMENTAL PROCEDURES

Preparation of Recombinant Proteins—The hexahistidine-tagged and GST-fusion Sp1 DBD constructs and purification have been described (22). The purification protocol for the hexahistidine-tagged protein was slightly modified. Bacterial supernatant was applied to HiTrap Heparin column (Amersham Biosciences), washed with buffer A (20 mM Tris-HCl, pH 7.9, 0.2 mM KCl, 20% glycerol, 5 mM 2-mercaptoethanol, 100 μM ZnSO4, 1 mM phenylmethylsulfonfyl fluoride, 0.5 μg/ml leupeptin, and 1 μg/ml pepstatin), and then eluted with a linear salt gradient of 0.2–2 mM KCl. Pooled fractions were next applied to HisTrap column (Amersham Biosciences), washed with buffer A containing 500 mM KCl and 20 mM imidazole, and then eluted with a stepwise gradient up to 0.5 M imidazole. Pooled fractions were dialyzed against buffer B (25 mM HEPES-KOH, pH 7.9, 10% glycerol, 150 mM KCl, 100 μM ZnSO4, 5 mM 2-mercaptoethanol, 1 mM phenylmethylsulfonfyl fluoride, 0.5 μg/ml leupeptin, and 1 μg/ml pepstatin). For GST fusion proteins, glutathione-Sepharose resin (Amersham Biosciences) was added to the lysate, incubated, followed by repeated washes with buffer A, and then eluted using buffer B containing 20 mM reduced glutathione (Wako). The GST fusion full-length Sp1 construct was a gift from Dr. Hans Rotheneder. TAF-Iα, β, and mutant constructs (gifts of Dr. Kyosuke Nagata) were expressed in bacteria and purified using Protobond resin (Invitrogen) with buffer C (20 mM Tris-HCl, pH 7.4, 10% glycerol, 50 mM KCl, 50 mM β-mercaptoethanol, 1 mM phenylmethylsulfonfyl fluoride, 2 μg/ml leupeptin, and 1 μg/ml pepstatin). Washes were done with buffer C containing 20 mM imidazole, and then eluted with buffer C containing 0.2 M imidazole. All protein procedures were done at 4°C.

Preparation of Nuclear Extract—Nuclear extract from HeLa S3 cells was prepared as described (29). Briefly, cells were lysed by glass Dounce homogenizer in Dignam’s buffer (25 mM HEPES-KOH, pH 7.9, 10% glycerol, 150 mM KCl, 100 μM ZnSO4, 5 mM 2-mercaptoethanol, 1 mM phenylmethylsulfonfyl fluoride, 0.5 μg/ml leupeptin, and 1 μg/ml pepstatin). For GST fusion proteins, glutathione-Sepharose resin (Amersham Biosciences) was added to the lysate, incubated, followed by repeated washes with buffer A, and then eluted using buffer A containing 20 mM reduced glutathione (Wako). The GST fusion full-length Sp1 construct was a gift from Dr. Hans Rotheneder. TAF-Iα, β, and mutant constructs (gifts of Dr. Kyosuke Nagata) were expressed in bacteria and purified using Protobond resin (Invitrogen) with buffer C (20 mM Tris-HCl, pH 7.4, 10% glycerol, 50 mM KCl, 50 mM β-mercaptoethanol, 1 mM phenylmethylsulfonfyl fluoride, 2 μg/ml leupeptin, and 1 μg/ml pepstatin). Washes were done with buffer C containing 20 mM imidazole, and then eluted with buffer C containing 0.2 M imidazole. All protein procedures were done at 4°C.

Isolation of Factors Interacting with Sp1 DNA-binding Domain—50 μg of hexahistidine-tagged Sp1 DBD was bound to 50 μl of Protobond resin (Invitrogen), and after washing, incubated with 725 μl of HeLa S3 nuclear extract. Proteins were eluted with buffer B containing 0.5 M imidazole. Samples were resolved by SDS-PAGE analysis and stained with Coomassie Brilliant Blue.

Protein Identification by MALDI/TOF Mass Spectrometry—Protein bands were excised, dehydrated with acetonitrile, and after removing acetonitrile, dried, and then in-gel digested with trypsin in 25 mM ammonium bicarbonate, pH 8. After soaking in 50% acetonitrile/5% tetrahydrofuran, the supernatant was collected, dried, and then reconstituted by adding 50% acetonitrile, 0.1% tetrahydrofuran and mixed with 0.1% cys-[3,5-dimethoxybenzyl]oxycarbonyl acid. Mass spectrometry (MALDI-TOF MS; Voyager-DE STR, Applied Biosystems) was used to analyze proteins. Masses were calibrated internally with peptides derived from trypsin autolysis, and accuracy was within 10 ppm. Data base searches were done against a nonredundant protein sequence data base of NCBI using the Protein Prospector program V.3.2.1 (UCSF mass spectrometry facility).

Protein-Protein Interaction Assays—GST fusion proteins were immobi- bilized to glutathione-Sepharose 4B resin (Amersham Biosciences) and incubated with hexahistidine-tagged proteins in buffer of 20 mM HEPES (pH 7.6 at 4°C), 20% glycerol, 0.2 mM EDTA, 0.1% Triton X-100, 100 mM NaCl, and 100 μM ZnSO4. Reactions were carried out at 4°C for 2 h and washed twice with the same buffer. Bound proteins were resolved on a 10% SDS-PAGE gel, transferred to nitrocellulose membrane, immunoblotted with anti-HIS-probe (G-18) antibody (Santa Cruz Biotechnology), and then visualized by chemiluminescence (ECL, Amersham Biosciences). Commercially available recombinant proteins were done essentially as described (22). Briefly, the Sp1 consensus binding sequence (top 5'-ATTCGATCGGGGCAGGAC-3') was used as the probe (underlined nucleotides GG were substituted by TT for mutant analysis). Annealed double-strand probe was gel-purified, and then kinase-labeled by using [γ-32P]ATP (222 TBq(6000 Ci)/mmol, PerkinElmer Life Sciences) and T4 polynucleotide kinase (Stratagene). Unincorporated radiolabeled ATP was separated by NucTrap purification column (Stratagene). Specific activity of the radiolabeled probe was adjusted by adding cold double-stranded probe. Binding reactions were then incubated at 30°C for 30 min in binding buffer prior to addition of 1.0 × 10^6 cpm (1 ng) of labeled probe, followed by further incubation at 30°C for 15 min before separation on nonde- natured polyacrylamide gels. Gels were dried and analyzed using BAS 1500 (Fuji Photo Film).

Co-transfection Reporter Assay—25,000 HeLa cells were seeded and transfected 24 h later with the SV40 early promoter reporter (100 ng) and effector expression vectors by liposome-mediated transfer (Tfx20; Promega). Full-length human Sp1 cDNA (a gift of Dr. James Kadonaga) was inserted into the expression vector pCMV3 (Invitrogen). pCHA-TAF-Iα and β were gifts of Dr. Kyosuke Nagata. p53-Luc, which contains 15 copies of a p53-binding sequence upstream of the luciferase reporter, and the expression vector pPC-p53, were purchased from Stratagene. The total effector DNA amount in transfection reactions was corrected to 1 μg by addition of empty vector. Cells were harvested after 48 h and assayed for luciferase activity (luciferase assay system, Promega). Luciferase activity was normalized against protein concentration of cell lysates. Protein expression was examined by Western blot using anti-HA antibody (Roche Diagnostics) for TAF-Iα, and anti-Sp1 antibody (PEP2, Santa Cruz Biotechnology) for Sp1. Error bars denote S.E.
Functional Interaction between Sp1 and TAF-I

Identification of proteins interacting with Sp1 DBD. A, TOF-MS analysis/charts of tryptic fragments of p41. B, TOF-MS analysis/charts of tryptic fragments of p39. C, primary amino acid sequences of TAF-Iα and β with identifying tryptic fragments (a) and (b)boxed.

Expressed and purified to near homogeneity using heparin and Ni²⁺-chelating resins (Fig. 1A). HeLa S3 nuclear extract was applied to hexahistidine-tagged Sp1 DBD immobilized on Ni²⁺-chelating resin, and then proteins bound to the peptide were eluted with imidazole (Fig. 1B).

Coomassie Brilliant Blue (CBB) staining of the bound proteins as resolved by SDS-gel electrophoresis revealed several bands spanning molecular mass from 30 to 200 kDa, which were seen only when the binding reaction between the Sp1 DBD and HeLa nuclear extract was done (Fig. 1C, lane 2) and not when Sp1 DBD alone (lane 1) or HeLa nuclear extract alone (lane 3) was reacted with resin. Two major bands, which were the most abundant on CBB staining were of apparent molecular masses of 41 and 39 kDa and are hereafter referred to as p41 and p39, respectively.

To identify the proteins, the p41 and p39 bands were excised, trypsinized, and then subjected to MALDI-TOF mass spectrometry. The proteins were identified by peptide mass fingerprinting with a computer search of the nonredundant protein sequence NCBI data base as available for the mammalian proteome, and then further subjected to post-source decay peptide sequencing. Surprisingly, mass spectra of p41 and p39 identified them to be the products of a single gene, template-activating factor-I (TAF-I) (Fig. 2, A–C) (30, 31). p41 and p39 were TAF-Iα and β, respectively. TAF-Iα and β are the alternative splicing products of a single gene and differ only in a short region of their amino-terminal ends (Fig. 2C) (31).

TAF-Iα was originally identified as a cellular factor which stimulates adenovirus core DNA replication (30, 31), and has been shown to be a histone chaperone which is a factor which can displace and/or assemble nucleosomal histones in an ATP-independent manner (32–35). TAF-Iβ is identical to the SET oncogene whose translocation has been implicated in leukemia (36).

Sp1 and TAF-I Interact in Vitro and in Vivo—To examine whether Sp1 DBD directly binds the TAF-I proteins, GST pull-down binding assays were done with recombinant TAF-Iα, β, and Sp1 DBD (Fig. 3A). Under the described binding conditions, both bacterially expressed hexahistidine-tagged TAF-Iα and β (lanes 1 and 2) bound to GST fusion Sp1-DBD (lanes 4 and 6) but not to GST alone (lanes 3 and 5) showing that Sp1 DBD directly binds both TAF-Iα and β. We have reproducibly seen that TAF-Iβ binds Sp1 DBD with a slightly higher affinity than TAF-Iα (lanes 4 and 6).

To next see if TAF-I also binds full-length Sp1, GST pull-down binding assays were done with recombinant TAF-Iβ and full-length Sp1 (Fig. 3B). Under the described binding conditions, bacterially expressed hexahistidine-tagged TAF-Iβ (lane 1) bound to GST fusion full-length Sp1 (lane 3) but not to GST alone (lane 2) showing that full-length Sp1 directly binds TAF-I. TAF-I can therefore directly bind Sp1.

To further see whether these proteins interact in the cell, immunoprecipitation was done using specific antibodies against TAF-Iα, β, and Sp1 (Fig. 3C). Sp1 was immunoprecipitated from HeLa cells followed by immunoblotting using TAF-Iα and β-specific antibodies. TAF-Iα and β (lane 1) both bound Sp1 (lane 3) as compared with the control immunoprecipitation
using normal IgG antibody (lane 2) confirming that these proteins do indeed interact in the cell. Collectively, TAF-I interacts with Sp1 in vitro and in vivo.

Specific Interaction Between Sp1 and TAF-I—To see whether interaction of Sp1 DBD with TAF-I is specific or common for histone chaperones, a GST pull-down binding assay of Sp1 DBD was done with the histone chaperone CIA (also known as ASF1 in Saccharomyces cerevisiae and RCAF in Drosophila complexed with histones) (37–39) (Fig. 4A). Under conditions in which Sp1 DBD bound TAF-I (lanes 4 and 5), Sp1 DBD did not bind CIA (lanes 1–3) showing that interaction between Sp1 DBD and TAF-I is specific. Therefore, interaction between Sp1 DBD and TAF-I is a specific property of these factors, and not a property common for histone chaperones.

To next see whether interaction with TAF-I is specific for Sp1 or common for DNA-binding factors, a GST pull-down binding assay with the DNA-binding transcription factors p53, MyoD, and NFκB was performed (Fig. 4B). Under conditions in which Sp1 DBD bound TAF-I (lanes 1–3), TAF-I did not bind p53, MyoD, or NFκB (lanes 4–6) thus showing that interaction between Sp1 DBD and TAF-I is specific. A CBB stain of the GST fusion proteins is shown (Fig. 4C). Therefore, interaction between Sp1 DBD and TAF-I is a specific property of these two factors.

Effects of the Acidic Carboxyl-terminal Regions of TAF-I in Interaction with Sp1—Histone chaperones including TAF-I have in common an acidic region (32, 34, 38, 40), but as Sp1 DBD did not bind CIA (Fig. 4A), we thought that the acidic region may not mediate interaction between TAF-I and Sp1 DBD. To address the effects of the acidic region of TAF-I on interaction with Sp1 DBD, GST pull-down binding assays using mutants of TAF-IA and β, which lack the common acidic carboxyl-terminal end, TAF-IαC and TAF-IβC, respectively, were done (Fig. 5A). We have reproducibly seen that TAF-IαC binds Sp1 DBD with less affinity than TAF-IαC (Fig. 5B, lanes 3 and 6), which may suggest that the acidic carboxyl-terminal end participates in regulation of binding of TAF-I with Sp1 DBD greater than for TAF-Iβ. The acidic region of TAF-I may therefore be involved in modulation of binding affinity.

TAF-I Inhibits DNA Binding Activity of Sp1—As TAF-I binds the DNA-binding domain of Sp1, we examined the effects of TAF-I proteins on the DNA binding activity of Sp1 by gel mobility shift analysis (Fig. 6A). Under conditions in which TAF-IA and β did not show DNA binding activity (lanes 2 and 3), incubation of TAF-I with Sp1 DBD resulted in inhibition of specific DNA binding activity of Sp1 DBD to its cognate binding sites (lanes 4–6) as shown by the dose-dependent decrease in intensity of the shifted DNA-protein complex for TAF-IA (lanes 7 and 8) and TAF-Iβ (lanes 9 and 10). TAF-Iβ inhibited the DNA binding activity of Sp1 DBD to a slightly greater extent than TAF-IA (lanes 7 and 8 versus 9 and 10). Under identical conditions, the acetyltransferase p300 stimulates DNA binding activity of Sp1 DBD and thus this is not a non-specific effect of TAF-I (data not shown and Ref. 22). Therefore, TAF-I inhibited the DNA binding activity of Sp1 DBD.

TAF-I Inhibits Sp1-dependent Promoter Activation—As interaction of TAF-I proteins and Sp1 DBD inhibited the DNA binding activity of Sp1 DBD, we examined whether TAF-I would inhibit Sp1-dependent promoter activation as would be expected as a secondary result. Co-transfection analysis was performed with a luciferase-reporter construct harboring the SV40 early promoter which contains six Sp1 binding sites (Fig. 7A). As expected, under conditions in which transfection of an expression plasmid harboring full-length Sp1 showed dose-de-
p40 mono-specific DNA binding by Sp1 DBD to the consensus binding site probe (DNA-protein complex indicated by arrow; Sp1 DBD 1.9 pmol; lane 1, probe alone) as shown by competition experiments with 100-fold excess wild-type binding site (WT, lane 5) and mutant site (Mut, lane 6) oligonucleotides is seen, and TAF-Ia and b do not bind DNA (lanes 2 and 3; 2.5 pmol) under these conditions. TAF-Ia (lanes 7 and 8; lane 7, 0.83 pmol; lane 8, 2.5 pmol) and TAF-Ib (lanes 9 and 10; lane 9, 0.83 pmol; lane 10; 2.5 pmol) were reacted with Sp1 DBD and then subjected to gel-shift analysis.

FIG. 6. Effect of TAF-I on Sp1 DNA binding activity. Gel-shift analysis for effects of TAF-I on DNA binding activity of Sp1. Sequence-specific DNA binding by Sp1 DBD to the consensus binding site probe (DNA-protein complex indicated by arrow; Sp1 DBD 1.9 pmol; lane 1, probe alone) as shown by competition experiments with 100-fold excess wild-type binding site (WT, lane 5) and mutant site (Mut, lane 6) oligonucleotides is seen, and TAF-Ia and b do not bind DNA (lanes 2 and 3; 2.5 pmol) under these conditions. TAF-Ia (lanes 7 and 8; lane 7, 0.83 pmol; lane 8, 2.5 pmol) and TAF-Ib (lanes 9 and 10; lane 9, 0.83 pmol; lane 10; 2.5 pmol) were reacted with Sp1 DBD and then subjected to gel-shift analysis.

DISCUSSION

Here we have described a novel interaction between a DNA-binding transcription factor and histone chaperone. Specifically, we have described functional interaction between the DNA-binding transcription factor Sp1 and the histone chaperone TAF-I.

Novel Activity of Histone Chaperone—Histone chaperones are a class of factors that possess activity to mediate assembly and/or disassembly of nucleosomal histones in an ATP-independent manner and include the factors TAF-I, CIA, nucleoplasmin, and NAP-1 among others (30, 31, 38–43). While interaction with histones and their activity to assemble/disassemble nucleosomal histones has been well addressed, their interaction with DNA-binding transcription factors has not been explored. The present study shows that the histone chaperone TAF-I functionally interacts with the DNA-binding transcription factor Sp1 (Figs. 3, 6, and 7). This interaction is specific as another histone chaperone examined did not bind Sp1, and as other tested DNA-binding transcription factors did not bind TAF-I (Fig. 4). TAF-I acts to negatively regulate the DNA binding and likely as a result of such also promoter activation by Sp1 (Figs. 6–8). TAF-I has been similarly shown to negatively regulate promoter activation by the retinoic acid receptor using cell co-transfection studies (43). Importantly, TAF-I may act to negatively regulate a subset of DNA-binding transcription factors that includes at least the zinc finger-type factors, which both Sp1 and nuclear receptors are.

We note that TAF-I has been shown to stimulate transcription from in vitro chromatin template (35). The cell co-transfection assay used by us and others differs from the in vitro transcription reaction as the former is a cellular experiment in which a transfected episomal plasmid reporter is activated by forced expression of a transcription factor likely in the cyto-
plasm, in contrast to the latter biochemical study, which uses reconstituted components. The latter better reflects the involved fundamental reactions and allows for dissection of mechanisms of action; however, the former in contrast better reflects the collective surrounding regulatory reactions as seen in the cell albeit possible inherent limitations associated with compartmentation (e.g. cytoplasmic reaction) and concentration (e.g. effect of forced expression in contrast to basal endogenous levels). The apparent discrepancy in results between in vitro and in vivo experiments will be a topic needed to be addressed in further studies.

The mechanism of how histone chaperones are involved in reactions associated with specific promoters has also remained elusive. Functional interaction between DNA-binding transcription factor and histone chaperone may play a role in specifying the site of the reaction as dictated by the gene- and site-specific targeting properties of the DNA-binding factor. In reference, the centromeric proteins (CENP-A, B, C) through its DNA-binding component (CENP-B) shows centromere sequence-specific binding and localization allowing the CENP complex to modulate centromeric nucleosomes (44). This is one example in which concerted action between sequence-specific DNA-binding factor with histone-associated catalytic protein(s) results in specific and localized chromosomal/nucleosomal processes. Functional sequelae of the interaction between DNA-binding transcription factor and histone chaperone may be dictated of site-specificity by DNA-binding transcription factors for catalytic events to be mediated by the histone chaperone (e.g. nucleosome assembly/disassembly).

**Regulatory Role of the DNA-binding Domain**—Of additional importance, interaction of Sp1 with TAF-I was mediated through the DBD. Much focus on regulation of Sp1 through protein-protein interaction has focused on the role of the activation domain (e.g. interaction with the basal machinery dTAF110/hTAF130 and transcriptional complex ARC) (8, 23) in contrast to the role of the DNA-binding domain, which has been poorly addressed. However, past studies by ourselves and others have shown that the DBD of Sp1 mediates important regulatory interactions such as with the cell cycle regulator E2F (24, 25), the acetyltransferase p300 (22), the histone deacetylase HDAC1 (26), the ATP-dependent nucleosomal remodeling enzyme SWI/SNF (27) as well as other zinc finger transcription factors including Krüppel-like factors (28).

Interestingly, the Sp1 DBD interacts with all three major chromatin-related factors consisting of chemical modification enzymes (e.g. acetyltransferase p300), ATP-dependent nucleosome assembly factor (e.g. SWI/SNF) and histone chaperone (e.g. TAF-I), which is a finding which has only been shown for histones. This finding is of particular interest because it implicates the DBD to play a likely role in mediating transcriptional regulatory processes in eukaryotes at the chromatin level.

Combined regulation of the transcription factor by interaction with chromatin-related complexes through its activation domain (e.g. ARC, DRIP, TRAP) and the three factors through the DBD likely results in coordinated transcriptional regulation at the chromatin level. Importantly, as the DBD specifies the target gene or DNA sequence, selective and ordered interaction of chromatin-related factor with the DBD of the transcription factor may play a role in gene- and factor-selective regulation. Selective interaction between histone chaperones with DNA-binding factors such as interaction of TAF-I with zinc-finger type transcription factors is further suggestive of a specific regulatory role in transcription.

Cooperative Interaction of Histone Chaperone and DNA-binding Transcription Factor—Functional interaction between DNA-binding factor and histone chaperone is the most noteworthy new molecular mechanism, which results from our present study. As the interaction is specific, and as the histone chaperone negatively regulates activities of the DNA-binding factor, it is tempting to envision that TAF-I plays an important role to negatively regulate subsets of DNA-binding factors to affect selective gene expression. We, however, do not rule out the possibility that TAF-I may also participate in activation processes under certain regulatory conditions in consideration of the fact that TAF-I has been shown to possess stimulatory effects on transcription in vitro (35).

The next important questions which need to be answered are whether TAF-I contributes to continuous regulation/inactivation or if this is a triggered event, and how histones which also bind histone chaperones contribute to this process. It is noteworthy that the chaperone proteins including the Hsp90-co-chaperone p23, Hsp90, and Hsp70 modulate assembly as well as disassembly of transcriptional complexes as shown for nuclear receptors (45–47). It is tempting to envision that histone chaperones also contribute to DNA-binding transcription factor regulation by mediating inactivation processes. Although the mechanisms of interaction of histone chaperone on DNA-binding factor are yet unclear, TAF-I may inhibit the activities of Sp1 by competitive interaction with the DNA-binding surface, but alternatively binding to the non-DNA-binding surface of Sp1 DBD may induce an allosteric/conformational change to Sp1 DBD making it transcriptionally incompetent/competent for further regulatory interactions (e.g. DNA binding, transcriptional activation).

Based on our data centered on Sp1 DBD, we have shown in the past that p300 acetyltransferase facilitates promoter access (22), therefore TAF-I as a negative regulator may act in concert with the acetyltransferase to mediate a balance of promoter activation and inactivation. Given that acetyltransferase and TAF-I have been shown to regulate acetylation and its inhibition on histones (43), respectively, this may be one of the signal modifications regulated by this concerted interaction (22, 43). As we have shown that Sp1 DBD is acetylated (22), further experiments to investigate whether TAF-I regulates inhibition of acetylation of DNA-binding transcription factor will also add to our understanding of transcriptional regulation. Further, although it would seem that inactivation/activation is an energy-consuming process, as histone chaperones are essentially non-ATP-dependent factors, their contribution would likely facilitate this process and allow for efficient transcriptional regulation.

Collectively, we have shown that the histone chaperone TAF-I negatively regulates a DNA-binding transcription factor. Our results provide an initial step in understanding the role of histone chaperones in the regulation of DNA-binding transcription factors.

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**Functional Interaction of the DNA-binding Transcription Factor Sp1 through Its DNA-binding Domain with the Histone Chaperone TAF-I**

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