The Basic Leucine Zipper Domain of c-Jun Functions in Transcriptional Activation through Interaction with the N Terminus of Human TATA-binding Protein-associated Factor-1 (Human TAF1250)*

Received for publication, January 27, 2004, and in revised form, April 14, 2004
Published, JBC Papers in Press, April 15, 2004, DOI 10.1074/jbc.M400892200

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We previously reported that c-Jun binds directly to the N-terminal 163 amino acids of Homo sapiens TATA-binding protein-associated factor-1 (hsTAF1), causing a derepression of transcription factor IID (TFIID)-driven transcription (Lively, T. N., Ferguson, H. A., Galasinski, S. K., Seto, A. G., and Goodrich, J. A. (2001) J. Biol. Chem. 276, 25582–25588). This region of hsTAF1 binds TATA-binding protein to repress TFIID DNA binding and transcription. Here we show that the basic leucine zipper domain of c-Jun, which allows for DNA binding and homodimerization, is necessary and sufficient for interaction with hsTAF1. Interestingly, the isolated basic leucine zipper domain of c-Jun was able to derepress TFIID-directed basal transcription in vitro. Moreover, when the N-terminal region of hsTAF1 was added to in vitro transcription reactions and overexpressed in cells, it blocked c-Jun activation. c-Fos, another basic leucine zipper protein, did not interact with hsTAF1, but c-Fos/ c-Jun heterodimers did bind the N terminus of hsTAF1. Our studies show that, in addition to dimerization and DNA binding, the well characterized basic leucine zipper domain of c-Jun functions in transcriptional activation by binding to the N terminus of hsTAF1 to derepress transcription.

Transcription of mRNA is a complex process that is tightly regulated and involves the assembly of RNA polymerase II, its associated general transcription factors (TFIIB, TFIID, TFIIE, TFIIF, and TFIIH), and other factors such as the mediator into preinitiation complexes at promoters (1–3). Among the general transcription factors, TFIID plays a central role in promoter recognition and preinitiation complex assembly (4–9). TFIID is composed of TATA-binding protein (TBP) and at least 12 TBP-associated factors (TAFs) (10, 11). TBP recognizes and binds the TATA box in the minor groove (12, 13). The TAFs function in DNA binding and serve as coactivators by interacting with transcriptional activators (14, 15).

hsTAF1 (previously termed human TAF1250) (16), the largest TAF, is thought to function as a scaffolding protein for TFIID assembly through its interactions with TBP and the other TAFs (14, 15). The binding of TFIID to the TATA box is internally repressed through the concave surface of TBP interacting with the N terminus of hsTAF1 (amino acids 1–163) (17–20). The N terminus of hsTAF1 can be divided into two smaller subdomains, subdomain I (amino acids 1–87) and subdomain II (amino acids 88–163), which bind the concave and convex surfaces of TBP, respectively (17, 21). The NMR structure of subdomain I of the Drosophila homolog of hsTAF1 (dmTAF1) complexed with the core domain of TBP revealed that dmTAF1 resembles the minor groove surface of partially unwound DNA and contacts TBP in its concave surface (22). Within subdomain II, dmTAF1 has a core domain (amino acids 118–143) that is required for interaction with the convex surface of TBP (23). hsTAF1, dmTAF1, and the Saccharomyces cerevisiae homolog of TAF1 all inhibit TBP DNA binding, demonstrating that repression of TBP DNA binding by the N terminus of TAF1 is conserved (17–19).

AP-1 is a collection of transcriptional activators with a basic leucine zipper domain that includes the Jun, Fos, and activating transcription factor families (24, 25). Many of the activators in these families can homo- and heterodimerize, and the dimers can bind DNA (25). Dimerization among AP-1 proteins occurs through the leucine zippers, and the basic regions of the dimers bind DNA in a site-specific manner (26, 27). Although Jun and activating transcription factor family members can homodimerize, the Fos family members cannot. Fos proteins can heterodimerize with Jun proteins, and the heterodimers play important biological roles (25). Transcriptional activation is mediated through activation domains that are unique to the different family members (28–30). Deletion analysis has shown that the activation domains of c-Jun and c-Fos are required for activated transcription (28–30). The structures of the leucine zipper of the c-Jun homodimer and the basic leucine zipper of the c-Fos/c-Jun heterodimer have been solved (27, 31); however, structures of activation domains are not available. We have previously shown that c-Jun interacts with the N-terminal 163 amino acids of hsTAF1 and blocks hsTAF1 from inhibiting TBP basal transcription and DNA binding (19). A more
detailed understanding of the c-Jun/hsTAF1 interaction could provide a more complete picture of the mechanisms by which c-Jun activates transcription.

Here we investigated the molecular interactions that function in derepression of transcription by c-Jun. Both c-Jun homodimers and c-Fos/c-Jun heterodimers were found to bind hsTAF1. The regions of c-Jun and hsTAF1 that mediate the interaction were identified, and the functions of these regions in transcriptional activation were studied. Like full-length c-Jun, the DBD vector c-Jun depended on c-Jun for derepressed TFII-D basal transcription. The ability of the N terminus of hsTAF1 to act as a dominant inhibitor of c-Jun activation was tested in a reconstituted transcription system and in cells. These studies revealed that the basic leucine zipper of c-Jun binds to the N terminus of hsTAF1 to derepress TFII-D-directed transcription. Moreover, the inhibition of c-Jun-activated transcription by the N terminus of hsTAF1 is consistent with a model in which the basic leucine zipper of c-Jun participates directly in transcriptional activation when bound to promoter DNA.

EXPERIMENTAL PROCEDURES

Construction of Plasmids—Plasmid pET-Jun for expressing full-length human c-Jun was a gift from T. Hoey (Tularik, Inc.). In creating this plasmid, a Ncol site was generated that changed the 4th base pair in the c-Jun coding region from A/T to G/C, resulting in a point mutation in the 2nd amino acid of c-Jun (Thr to Ala). In addition, this plasmid differs from the published cDNA sequence at the 32nd base pair in the c-Jun coding region (AT to GC), changing the 11th amino acid of c-Jun from Asp to Gly. To make pGEX-c-Jun-(1–254) and pGEX-c-Jun-(255–317), regions of the c-Jun cDNA were amplified by PCR using pET-Jun as a template, and PCR products were digested with Ncol and EcoRI and ligated into the Ncol and EcoRI sites of pGEX2TK (a gift from S. Ruppert and R. Tjian). To make pGEX-c-Jun-(274–317), a region of the c-Jun cDNA was generated by PCR, digested with Ncol and EcoRI, and ligated into the Ncol and EcoRI sites of pGEX2TK. To make pGEX-hsTAF1-125, pGEX-hsTAF1-146–163, and pGEX-hsTAF1-163), the desired region of hsTAF1 was amplified by PCR, and PCR products were digested with Ncol and EcoRI and ligated into the Ncol and EcoRI sites of pGEX2TK.

To make pET-His6-c-Jun-(274–317), oligonucleotides encoding a His6 tag (with Ncol and NdeI ends) were annealed and ligated together with a DNA fragment encoding c-Jun-(274–317) (with Ncol and EcoRI ends) and the pET-19B vector digested with Ncol and EcoRI. pET-c-Jun-N5B was generated via a triple ligation of 1) a modified pET vector (containing a unique EcoRI site) cut with Ncol and EcoRI; 2) the region of the c-Jun cDNA encoding amino acids 1–276 cut with Ncol and Tth111I; and 3) a PCR product encoding amino acids 293–347 of JunB cut with Tth111I and EcoRI. Constructs for expressing proteins in mammalian cells by transient transfection were made using the pcDNA3.1+ vector (Invitrogen).

The pAD-AP1, c-Jun, and c-Fos plasmids were a kind gift from L. Chen (32).

Protein Expression and Purification—Full-length c-Jun, c-Jun-(1–317), and the c-Jun-JunB fusion protein were expressed and purified as described previously for c-Jun (19, 34). c-Jun-(254–317) was expressed and purified as described previously (32, 35). His-c-Jun-(274–317) was resuspended in 10 ml of 5 mM DTT and sonicated two times for 30 s. Samples were centrifuged in a JA20 rotor at 18,000 rpm for 30 min at 4 °C. Precipitated material (inclusion bodies containing GST-c-Fos) was resuspended in 5 mM DTT and sonicated for 95 min before harvesting. The cells were resuspended in 20 mM Tris–HCl (pH 7.9), 0.1 mM EDTA, 10% glycerol, and 0.2 mM phenylmethylsulfonyl fluoride and sonicated three times for 30 s. Samples were centrifuged in a JA20 rotor at 18,000 rpm for 30 min at 4 °C. Precipitated material (inclusion bodies containing GST-c-Fos) was resuspended in 5 mM DTT and sonicated for 95 min before harvesting. The cells were resuspended in 20 mM Tris–HCl (pH 7.9), 1 mM EDTA, 0.1 mM NaCl, 1 mM DTT, and 0.2 mM phenylmethylsulfonyl fluoride and sonicated three times for 30 s. Samples were centrifuged in a JA20 rotor at 18,000 rpm for 30 min at 4 °C.
6 μl of Plus reagent (Invitrogen), 500 ng of p(AP-1)–E1b-CAT, 25 ng of pRL-TK-Renilla luciferase (Promega), and, where indicated, 1 μg of pcDNA-c-Jun(1–331) plus pcDNA3.1(+)(+) parental vector (see Fig. 7B for amounts). Cells were left in serum-free Dulbecco’s modified Eagle medium for 5 h, and then 20% Dulbecco’s modified Eagle’s medium was added. After a 24-h incubation at 37 °C, the cells were lysed with 250 μl of passive lysis buffer (Promega). A CAT assay was used to determine reporter gene activation. Briefly, 30 μl of extract was heated at 65 °C for 10 min, and then 70 μl of reaction mixture was added to make final concentrations of 1.6 mM chloramphenicol, 75 mM Tris-HCl, 1 μCi/ml [14C]acetyl-CoA, and 0.09 mM unlabeled acetyl-CoA. Reactions were incubated at 37 °C for 1 h, cooled on ice, and extracted twice with cold ethanol acetate. Extracts were added to 4 ml of ScintiVerse and measured using a Packard 1600TR liquid scintillation analyzer. Renilla luciferase activity was determined by the Dual-Luciferase assay (Promega) and used to normalize CAT activity.

**RESULTS**

The Basic Leucine Zipper Domain of c-Jun Is Necessary and Sufficient for hsTAF1 Interaction—Previously, we found that c-Jun binds the N terminus of hsTAF1 to derepress transcription (19). We hypothesized that the activation domains of c-Jun would be required for interaction with hsTAF1. This was based on previous observations that activator/TAF interactions that function in transcriptional activation involve interaction between the activation domains of transcriptional activators and TAF coactivators (15, 37). To test this hypothesis, we assessed the interaction of full-length hsTAF1 with the activation domain region of c-Jun and with the DNA-binding/dimerization domain of c-Jun. As shown in Fig. 1A, the basic leucine zipper domain of c-Jun lies C-terminal of the activation domain region. The activation domain (amino acids 1–254) and the basic leucine zipper domain (amino acids 255–331) were expressed as fusions to GST. The GST-c-Jun affinity resins were incubated with insect cell extract containing HA-hsTAF1, and bound protein was analyzed by Western blotting. Surprisingly, the region of c-Jun that interacted with hsTAF1 was not the region containing the activation domains, but instead was the basic leucine zipper domain, which is responsible for dimerization and DNA binding. As shown in Fig. 1B, HA-hsTAF1 bound GST-c-Jun(255–331) (lane 3); in contrast, GST-c-Jun(1–254) did not bind HA-hsTAF1 (lane 2). Thus, the basic leucine zipper region of c-Jun is necessary and sufficient for interaction with hsTAF1.

In performing protein/protein interaction assays, we are always concerned with the possibility that observed interactions are not direct, but instead are mediated by nonspecific nucleic acids containing protein preparations and extracts (39). This concern is especially important when working with DNA-binding proteins. To eliminate problems due to contaminating nucleic acid, we pretreat all protein preparations and crude extracts with micrococcal nuclease, an endonuclease that cleaves single- and double-stranded DNA and RNA before starting the interaction assay. Indeed, the interaction assay described above was performed with micrococcal nuclease-treated samples. We were interested in determining whether micrococcal nuclease treatment affected the interaction between c-Jun and hsTAF1 in any way. We found that omitting the micrococcal nuclease treatment of immobilized GST-c-Jun (255–331) and insect cell extract decreased the amount of hsTAF1 that bound GST-c-Jun (Fig. 2A, compare lanes 1 and 2). This indicates that nonspecific nucleic acid bound to either c-Jun or hsTAF1 inhibits the interaction between these two proteins rather than mediating it.

The basic leucine zipper domain of c-Jun has a calculated pI of 11.3, whereas the N terminus of hsTAF1 is very acidic (pI 3.8). To determine whether the interaction between c-Jun and hsTAF1 is entirely ionic in character, we tested the sensitivity of the complex to increasing salt washes. Weakly interacting proteins can often be disrupted with increasing salt concentrations. The GST-c-Jun–hsTAF1 complex was preformed on beads, and aliquots of the beads were incubated with buffer containing different concentrations of NaCl for 10 min. As shown in Fig. 2B, the GST-c-Jun–hsTAF1 complex resisted the high salt washes, with a significant portion of hsTAF1 remaining bound to GST-c-Jun after the 1 M NaCl wash. These results suggest that the interaction between c-Jun and hsTAF1 involves contacts that are not entirely ionic in nature.

Since functionally relevant interactions between c-Jun and hsTAF1 are likely to occur with c-Jun bound to promoters at AP-1 sites, we tested whether c-Jun bound to an AP-1 element could bind hsTAF1 (Fig. 2C). HA-hsTAF1 interacted with GST-c-Jun(255–331) bound to the AP-1 column (lane 1), but not with control resin lacking GST-c-Jun(255–331) (lane 2). These results show that a specific interaction between the basic leucine zipper domain of c-Jun and hsTAF1 does occur when c-Jun is bound to DNA.

hsTAF1 Interacts with c-Fos/c-Jun Heterodimers, but Not with c-Fos Monomers—Mapping the c-Jun/hsTAF1 interaction to the basic leucine zipper domain raised the question of whether hsTAF1 interacts with other basic leucine zipper proteins (three of which are aligned in Fig. 3A). We tested the interaction between hsTAF1 and c-Fos, a basic leucine zipper protein that is unable to homodimerize with itself, but can form heterodimers with c-Jun. As shown in Fig. 3B, GST-c-Fos did not interact with hsTAF1 (lane 1). We also tested GST-c-Fos/c-Jun heterodimers for interaction with hsTAF1. To form GST-c-Fos/c-Jun heterodimers, extract containing GST-c-Fos was incubated with purified c-Jun before the addition of glutathione-Sepharose beads. As a control, an extract containing GST was also incubated with c-Jun. The immobilized GST-c-Fos/c-
Jun heterodimers bound hsTAF1 (lane 4). Control GST that had been incubated with c-Jun did not bind hsTAF1 (lane 5). Therefore, hsTAF1 does not interact with all basic leucine zipper proteins, but it does bind the biologically important c-Fos/c-Jun heterodimer.

The basic leucine zipper of JunB has 81% sequence identity to that of c-Jun. To determine whether the JunB leucine zipper can mediate interaction with the N terminus of hsTAF1, we expressed and purified a fusion protein consisting of amino acids 1–276 of c-Jun fused to the leucine zipper of JunB. The c-Jun-JunB fusion protein and c-Jun were tested for interaction with immobilized GST-hsTAF1-(1–163) and control GST. The c-Jun-JunB fusion protein bound the N terminus of hsTAF1 similarly to c-Jun (Fig. 3C, lanes 1 and 2). Therefore, the interaction with hsTAF1 is conserved between at least two of the Jun family members.

Both the Basic Region and the Leucine Zipper of c-Jun Are Required for Interaction with hsTAF1—We next tested whether the basic region (amino acids 255–276) or the leucine zipper (amino acids 274–317) of c-Jun is sufficient to interact with hsTAF1. GST-c-Jun-(255–276), GST-c-Jun-(274–317), GST-c-Jun-(255–331), and control GST affinity resins were incubated with insect cell extract containing HA-hsTAF1. Neither GST-c-Jun-(255–276) nor GST-c-Jun-(274–317) showed significant interaction with HA-hsTAF1 as shown by Western blotting (Fig. 4A, lanes 2 and 3). To further test for an interaction between the leucine zipper and the N terminus of hsTAF1, we expressed and purified the leucine zipper (amino acids 274–317) and incubated it with immobilized GST-hsTAF1-(1–163). Control reactions contained the basic leucine zipper of c-Jun (amino acids 254–317). Only c-Jun-(254–317) significantly interacted with GST-hsTAF1-(1–163) (Fig. 4B, compare lanes 1 and 4). Therefore, we conclude that the leucine zipper of c-Jun is not sufficient to interact with hsTAF1. Hence, both the basic region and the leucine zipper are required to mediate the interaction between c-Jun and hsTAF1.

The Basic Leucine Zipper of c-Jun Can Derepress TFIIID-directed Transcription—Finding that the basic leucine zipper domain of c-Jun is necessary and sufficient for hsTAF1 interaction led us to question whether the basic leucine zipper of c-Jun could derepress TFIIID transcription. We have shown that full-length c-Jun can derepress TFIIID basal transcription on a template containing no known AP-1-binding sites (19). Previous work has shown that the c-Jun basic leucine zipper domain is not sufficient for activated transcription (28, 29, 39, 40). We tested purified c-Jun-(254–317) in our in vitro transcription assay to confirm that it does not activate transcription in an AP-1 site-dependent manner. Increasing amounts of c-Jun-(254–317) were added to the p(AP-1)_5-E1b-G-less template (five AP-1 sites upstream of the E1b TATA box fused to a 377-bp G-less cassette). As shown in Fig. 5A, c-Jun-(254–317) did not activate transcription from the five-AP-1 site template, whereas an equimolar amount of full-length c-Jun activated transcription (compare lanes 4 and 5).

To test for derepression, we used a DNA plasmid that lacks known AP-1 sites, p(GAL4)_5-AdMLP-G-less and contains the adenovirus major late core promoter (positions −53 to +10) upstream of a 380-bp G-less cassette. TFIIID and TBP were incubated separately on ice with increasing concentrations of c-Jun-(254–317) and then added to transcription reactions with p(GAL4)_5-AdMLP-G-less. As shown in Fig. 5B, c-Jun-(254–317) stimulated TFIIID transcription (lanes 9–14), whereas c-Jun-(254–317) preincubated with TBP slightly repressed transcription at the highest amounts (lanes 2–7). The reason for this repression of TBP-driven transcription is not understood and was not observed in previous studies with full-length c-Jun (19). Quantitation of the data revealed that TFIIID transcription was increased 2.4-fold (lane 14), whereas TBP transcription was decreased 2.3-fold (lane 7). The plot in Fig. 5C demonstrates the effect of increasing amounts of c-Jun-(254–317) on TFIIID transcription relative to TBP transcription levels. As shown by the plot, c-Jun-(254–317) derepressed TFIIID-driven transcription to a level similar to that of TBP-directed basal transcription. Interestingly, the amount of c-Jun-(254–317) required to fully derepress transcription (~400 nM) was much higher than the amount of full-length c-Jun required to activate transcription from a template containing AP-1 sites (25 nM) (data not shown).

The Two Subdomains in the N Terminus of hsTAF1 Both Contribute to c-Jun Interaction—To determine which regions of the N terminus of hsTAF1 are important for TBP binding, we generated five deletions (Fig. 6A). Two of the deletions isolated the two known subdomains of hsTAF1 (amino acids 1–87 and 88–163). Previous work on the Drosophila and yeast homologs of hsTAF1 identified two subdomains in the N terminus of...
hsTAF1 that bound two different surfaces of TBP. Subdomain I (consisting of amino acids 1–87 of hsTAF1) binds to the concave surface of TBP, preventing DNA binding (17). Subdomain II (consisting of amino acids 88–163 of hsTAF1) binds to the convex surface of TBP and helps stabilize the hsTAF1/TBP interaction (21, 23).

All of the deletions were tested for the ability to bind c-Jun. GST fusion proteins and control GST were immobilized on glutathione-Sepharose beads and incubated with purified c-Jun-(1–317), and after extensive washing, bound protein was analyzed by Western blotting with anti-HA antibody. C, the JunB leucine zipper mediates interaction with hsTAF1, c-Jun and a c-Jun-JunB fusion protein (consisting of amino acids 293–347 of JunB fused to amino acids 1–276 of c-Jun) were incubated with immobilized GST-hsTAF1-(1–163) (lanes 1 and 2) and control GST (lanes 3 and 4). Bound protein was analyzed by SDS-PAGE and stained with Coomasie Blue. 20% input c-Jun and c-Jun-JunB fusion protein were loaded in lanes 5 and 6, respectively. Purified GST-hsTAF1-(1–163) and GST are shown in lanes 7 and 8, respectively.

The hsTAF1 N-terminal Peptide Can Block Transcriptional Activation by c-Jun—Our data are consistent with a model that the interaction between the basic leucine zipper of c-Jun and the N terminus of hsTAF1 derepresses TFIID DNA binding, thereby contributing to c-Jun transcriptional activation. A prediction of this model is that a hsTAF1 N-terminal peptide added in excess would bind c-Jun and block the interaction with hsTAF1 in TFIID, resulting in dominant inhibition of c-Jun activation. To test this, we preincubated GST-hsTAF1-(1–87) with full-length c-Jun before the addition of template DNA (containing AP-1 sites) and the general transcription factors. As shown in Fig. 7A, GST-hsTAF1-(1–87) repressed c-Jun-activated transcription ~5-fold (lanes 6–10). Under the same conditions, GST-hsTAF1-(1–87) repressed basal transcription ~2-fold (lanes 1–5), likely due to interaction with the concave surface of TBP in the TFIID complex. The net result was a decrease in c-Jun activation from 3.8- to 1.6-fold. GST alone did not affect either c-Jun-activated or basal transcription (data not shown). Thus, the exogenous hsTAF1 N terminus can partially block c-Jun activation in vitro.

As a further test of the inhibitory effect of the hsTAF1 N terminus on c-Jun activation, we performed transient transfection experiments. HA-tagged human c-Jun was overexpressed in COS-7 cells in the presence of a reporter plasmid containing one AP-1 site upstream of the E1b TATA box and the CAT gene. To control for transfection efficiency, a Renilla luciferase re-
porter was included in all transfections. As shown in Fig. 7B, overexpressed HA-c-Jun caused a 9-fold increase in CAT expression. In pilot experiments, we were unable to detect expression of HA-hsTAF1-(1-163); however, HA-hsTAF1-(1-163) expressed well. When the plasmid encoding HA-hsTAF1-(1-163) was titrated into the transfection assay, c-Jun activation was decreased >3-fold. The specificity of this repression for c-Jun-activated transcription is demonstrated in three controls: 1) HA-hsTAF1-(1-163) did not repress the level of Renilla luciferase expressed from the highly pRL-TK-Renilla luciferase plasmid (Fig. 7B, upper panel, solid line); 2) HA-hsTAF1-(1-163) did not inhibit expression of c-Jun from the cytomegalovirus promoter as shown in the anti-HA Western blot (Fig. 7B, lower panel); and 3) HA-hsTAF1-(1-163) did not repress the low level of CAT expression in the absence of c-Jun (Fig. 7B, upper panel, compare the first and second bars). Thus, the hsTAF1 N-terminal peptide specifically inhibits c-Jun activation when overexpressed in cells.

**DISCUSSION**

Having previously shown that TAFs are required for c-Jun activation in our highly purified RNA polymerase II transcription system and that c-Jun interacts with hsTAF1 (19), we wanted to identify the region of c-Jun that binds hsTAF1. To our surprise, the basic leucine zipper domain, and not the transcriptional activation domains, of c-Jun interacted with hsTAF1. c-Fos/c-Jun heterodimers interacted with hsTAF1, but c-Fos monomers did not. The minimal region of c-Jun that interacted with hsTAF1 did not activate transcription in an AP-1 site-dependent manner in vitro, but was able to derepress TFIID-directed basal transcription when added to reactions at relatively high concentrations. Of importance, an N-terminal piece of hsTAF1, when preincubated with c-Jun, lowered c-Jun activation in our purified in vitro transcription system and blocked activation by c-Jun in cells. These results show that the interaction between the basic leucine zipper region of c-Jun and the hsTAF1 subunit of TFIID plays a direct role in transcriptional activation by c-Jun.
resins were incubated with purified c-Jun. Bound protein was analyzed for maximal c-Jun interaction. A region overlapping these two clones of the hsTAF1 N terminus for maximal interaction. This interaction interface between c-Jun and hsTAF1 might be dependent on large surface, possibly utilizing the two subdomains of the hsTAF1 N terminus for maximal interaction. This is also the case with the interaction between TBP and the N terminus of hsTAF1 as well as the similar proteins in Drosophila and yeast (17, 21, 23). Division of the N-terminal 163 amino acids into two subdomains (positions 1–87 and 88–163) increases diversity in transcriptional regulation in cells. As such, we tested the ability of c-Fos/c-Jun heterodimers to bind hsTAF1 and found that they could do so. In contrast, c-Fos monomers did not bind hsTAF1. These results indicate that only one molecule of c-Jun is required for the interaction with hsTAF1 and raise the question of whether a c-Jun monomer can interact with hsTAF1 on its own. Testing this will require utilizing a dimerization mutant.

The hsTAF1 N-terminal deletion mutants suggest that the DNA-binding domains function in transcriptional activation beyond simply bringing the activators to the DNA-binding/dimerization domain and a TFIID TAF is rather unique. Most other activator/TAF interactions have mapped to the activation domain regions of activators (15, 37). For example, the glutamine-rich activation domain of SP1 interacts with dmTAF4 and hsTAF4 (41). E1A interacts with hsTAF1 and dmTAF4 through its C-terminal activation domain (42). cAMP-responsive element-binding protein has also been shown to interact, through its activation domain, with dmTAF4 and its homolog hsTAF4 (43, 44). In our laboratory, the activation domain of the transcriptional activator NFAT (nuclear factor of activated T cells) was shown to interact with both hsTAF4 and dmTAF4 (45). By contrast, Chiang and Roeder (46) found that hsTAF7 (human TAFII55) binds the DNA-binding domain of SP1. Recently, a number of studies have found functional interactions between the DNA-binding domains of activators and coactivators (47–50) as well as TBP (51). Hence, emerging evidence indicates that DNA-binding domains function in transcriptional activation beyond simply bringing the activators to promoter DNA.

The observation that the DNA-binding/dimerization domain rather than an activation domain of c-Jun binds hsTAF1 raises the possibility that other DNA-binding domains might also interact with the N-terminal inhibitory domain of this TFIID subunit. Indeed, the leucine zipper region of JunB, which is highly similar to that of c-Jun, can mediate interaction with hsTAF1. Interestingly, we found that the minimal domain of c-Jun required for interaction with hsTAF1 is the complete basic leucine zipper domain since separating the basic and leucine zipper regions abolished the interaction (Fig. 4). Hence, both the dimerization domain and the region that contacts DNA are required for binding hsTAF1. The ability of AP-1 proteins to heterodimerize increases diversity in transcriptional regulation in cells. As such, we tested the ability of c-Fos/c-Jun heterodimers to bind hsTAF1 and found that they could do so. In contrast, c-Fos monomers did not bind hsTAF1. These results indicate that only one molecule of c-Jun is required for the interaction with hsTAF1 and raise the question of whether a c-Jun monomer can interact with hsTAF1 on its own. Testing this will require utilizing a dimerization mutant.

The hsTAF1 N-terminal deletion mutants suggest that the interaction interface between c-Jun and hsTAF1 might be dependent on a large surface, possibly utilizing the two subdomains of the hsTAF1 N terminus for maximal interaction. This is also the case with the interaction between TBP and the N terminus of hsTAF1 as well as the similar proteins in Drosophila and yeast (17, 21, 23). Division of the N-terminal 163 amino acids into two subdomains (positions 1–87 and 88–163) has been previously characterized for their ability to interact with TBP (17, 21) resulted in decreased interaction with c-Jun, as well as the deletion mutants tested for interaction with c-Jun. B, GST-tagged hsTAF1 affinity resins were incubated with purified c-Jun. Bound protein was analyzed by Western blotting with anti-c-Jun antibody.

**Fig. 6.** The N-terminal 163 amino acids of hsTAF1 are required for maximal c-Jun interaction. A, the schematic shows the two subdomains of the N terminus of hsTAF1 as well as the deletion mutants tested for interaction with c-Jun. B, GST-tagged hsTAF1 affinity resins were incubated with purified c-Jun. Bound protein was analyzed by Western blotting with anti-c-Jun antibody.

**Fig. 7.** The N-terminal region of hsTAF1 inhibits c-Jun transcriptional activation in vitro and in cells. A, hsTAF1(1–87) represses c-Jun-activated transcription in vitro. GST-hsTAF1(1–87) was incubated alone (lanes 2–5) or with c-Jun (lanes 7–10) before the addition of the p(AP-1), E1b-G-less template (containing five AP-1-binding sites) and the general transcription factors. The amount of c-Jun added to the transcription reaction was 50 ng (lanes 6–10). The amount of transcript was quantitated and normalized to the amount of product in lane 1, and relative transcription levels are indicated above the lanes. B, overexpressed hsTAF1(1–163) represses c-Jun transcriptional activation in cells. Cells were transfected with a CAT reporter construct containing a single AP-1 site upstream of the E1b TATA box and pRL-TK-Renilla luciferase to control for transfection efficiency. Plasmids for expression of HA-c-Jun and HA-hsTAF1(1–163) were transfected where indicated. For each sample, CAT activity was normalized by Renilla luciferase activity, and the normalized numbers were then divided by that measured in the absence of HA-c-Jun and HA-hsTAF1(1–163) (upper panel). Each bar in the plot represents the average of at least three data points, and the error bars represent 1 S.D. The solid line across the plot shows the relative Renilla luciferase level observed under each of the conditions. Levels of protein expression in cells were analyzed by anti-HA Western blotting, and representative data are shown (lower panel).
c-Jun. Hence, both regions 1–45 and 126–163 of hsTAF1 are important for interaction with c-Jun.

When we initially found that the basic leucine zipper bound the N terminus of hsTAF1, we were concerned that the interaction might be specific and not simply a nonspecific charge-charge attraction. Increasing salt washes would disrupt a complex formed via ionic interactions, but the c-Jun-hsTAF1 complex was stable to 0.5 M NaCl. By contrast, c-Jun could be eluted from a column containing an AP-1 site oligonucleotide using 0.5 M salt (data not shown). Deletion analysis of the c-Jun basic leucine zipper and the hsTAF1 N-terminal region further supported the conclusion that the interaction between c-Jun and hsTAF1 is not simply ionic. Neither the basic region (pl 12) nor the leucine zipper (pl 9.4) was able to bind hsTAF1. Moreover, region 46–125 of hsTAF1 (pl 3.9) did not bind to c-Jun. Together, our results show that the interaction between the basic leucine zipper domain of c-Jun and the N-terminal region of hsTAF1 is a direct protein/protein interaction that is not completely ionic in nature.

Model for Transcriptional Derepression and Activation by c-Jun—The basic leucine zipper domain of c-Jun was not sufficient for activation of transcription from a promoter containing upstream AP-1 sites (Fig. 5A) (28–30). When added at high concentrations, however, the basic leucine zipper domain of c-Jun (amino acids 254–317) was able to derepress TFIIID-driven transcription from a promoter lacking AP-1 sites (Fig. 5B). Given that this region of c-Jun binds the N terminus of hsTAF1 and that this region of hsTAF1 acts as an internal damper to decrease TFIIID binding to DNA and basal transcription (17–20), we propose that c-Jun mediates derepression through interaction with the N terminus of hsTAF1. This eliminates the interaction of hsTAF1 with the DNA-binding surface of TBP and contributes to transcriptional activation by c-Jun. Consistent with this proposal, we find that the N terminus of hsTAF1 can inhibit c-Jun-activated transcription when added to an in vitro transcription system and when overexpressed in cells.

Fig. 8 depicts a working model for activation by c-Jun through the TFIIID complex. The activation domain(s) of c-Jun that is bound to an AP-1 site makes yet uncharacterized interactions with subunits of TFIIID, another general transcription factor, or a coactivator that ultimately results in recruiting TFIIID to the promoter. This brings the N terminus of hsTAF1 in close range to the basic leucine zipper domain of c-Jun, enabling the c-Jun-hsTAF1 interaction. This interaction destabilizes the repressive effect of the N terminus of hsTAF1 on the TBP subunit within TFIIID, thereby allowing TBP to bind to the TATA element in the promoter. This initiates the formation of functional preinitiation complexes and hence increases the levels of transcription. Our studies show that the c-Jun basic leucine zipper domain is sufficient for interaction with hsTAF1 and can derepress TFIIID-mediated transcription. Additional work must be done to identify and characterize the protein targets of the c-Jun activation domains in our reconstituted in vitro transcription system, which responds to c-Jun.

Acknowledgments—We thank Natalie Ahn, Lin Chen, Judith Jaehning, Jennifer Kugel, and Irene Ota for helpful discussions and comments. We are grateful to Amy Buck for pilot studies with JunB. We thank W. Lee Kraus for bringing to our attention a difference in sequence between the c-Jun cDNA in the pET-Jun plasmid and the published cDNA sequence.

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