

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 TAF_{II}250)*

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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 (TFIIA,¹ TFIIB, TFIID, TFIIIE, 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 TAF_{II}250) (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

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¹ The abbreviations used are: TF, transcription factor; TBP, TATA-binding protein; TAF, TBP-associated factor; hsTAF and dmTAF, *Homo sapiens* and *Drosophila melanogaster* TBP-associated factor, respectively; AP-1, activator protein-1; HA, hemagglutinin; CAT, chloramphenicol acetyltransferase; DTT, dithiothreitol; GdnHCl, guanidine hydrochloride; GST, glutathione *S*-transferase.

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 basic leucine zipper domain of *c-Jun* was found to derepress TFIID basal transcription. The ability of the N terminus of hsTAF1 to act as a dominant inhibitor of *c-Jun* activation in a reconstituted transcription system and in cells was tested. These studies revealed that the basic leucine zipper of *c-Jun* binds to the N terminus of hsTAF1 to derepress TFIID-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, an NcoI 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 (A/T to G/C), changing the 11th amino acid of *c-Jun* from Asp to Gly. To make pGEX-*c-Jun*-(1–254) and pGEX-*c-Jun*-(255–331), regions of the *c-Jun* cDNA were amplified by PCR using pET-Jun as a template, and PCR products were digested with NdeI and EcoRI and subcloned into the NdeI and EcoRI sites of pGEX-2TKN (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 NdeI and EcoRI, and ligated into the NdeI and EcoRI sites of pGEX-2TKN. To make pGEX-hsTAF1-(1–87), pGEX-hsTAF1-(88–163), pGEX-hsTAF1-(1–125), pGEX-hsTAF1-(46–163), and pGEX-hsTAF1-(46–125), the desired region of hsTAF1 was amplified by PCR, and PCR products were digested with NdeI and EcoRI and ligated into the NdeI and EcoRI sites of pGEX-2TKN.

To make pET-His₆-*c-Jun*-(274–317), oligonucleotides encoding a His₆ tag (with NcoI and NdeI ends) were annealed and ligated together with a DNA fragment encoding *c-Jun*-(274–317) (with NdeI and EcoRI ends) and the pET-19B vector digested with NcoI and EcoRI. pET-*c-Jun*-JunB was made via a triple ligation of 1) a modified pET vector (containing a unique EcoRI site) cut with NcoI and EcoRI, 2) the region of the *c-Jun* cDNA encoding amino acids 1–276 cut with NcoI and TfiI, and 3) a PCR product encoding amino acids 293–347 of JunB cut with TfiI and EcoRI. Constructs for expressing proteins in mammalian cells by transient transfection were made using the pcDNA3.1(+) vector (Invitrogen). pcDNA-*c-Jun*-(1–317) encodes amino acids 1–317 of *c-Jun* with an N-terminal hemagglutinin (HA) tag. pcDNA-hsTAF1-(1–163) encodes amino acids 1–163 of hsTAF1 with an N-terminal HA tag. pET-*c-Jun*-(254–317) was a kind gift from L. Chen (32).

The p(AP-1)₅-E1b-G-less template was described previously (19). Plasmid p(AP-1)₁-E1b-CAT was created by inserting one copy of a double-stranded oligonucleotide containing the AP-1 element from the human metallothionein IIA promoter (positions –105 to –96) into the XbaI site of plasmid pE1b-CAT (33).

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 expressed in BL21 cells in LB medium containing 0.3 mM ampicillin at 37 °C until A_{600 nm} = 0.4. Expression was induced with 0.5 mM isopropyl-β-D-thiogalactopyranoside for 2 h at 37 °C. Cells were harvested; resuspended in 15 ml of solution containing 20 mM Tris-HCl (pH 7.9), 100 mM NaCl, 10% glycerol, and 5 mM β-mercaptoethanol; and sonicated four times for 20 s. Samples were centrifuged in a JA20 rotor at 18,000 rpm for 30 min at 4 °C. Precipitated material (inclusion bodies) containing *c-Jun*-(274–317) was resuspended in 10 ml of 5 mM dithiothreitol (DTT) and sonicated two times for 30 s. Samples were centrifuged in a JA20 rotor at 15,000 rpm for 10 min at 4 °C. Insoluble

material was washed three more times by resuspension in 10 ml of 5 mM DTT, followed by centrifugation. Pellets from the final wash were resuspended in 3 ml of buffer containing 20 mM Tris-HCl (pH 7.9), 0.1 M NaCl, 6 M guanidine hydrochloride (GdnHCl), and 5 mM β-mercaptoethanol and nutated overnight at 4 °C. Soluble protein was loaded on a nickel-nitrilotriacetic acid-agarose column (500 μl; QIAGEN Inc.) and washed with 10 column volumes of buffer containing 6 M GdnHCl, 0.1 M NaH₂PO₄, and 10 mM Tris-HCl (pH 8.0). The column was washed with 5 column volumes of buffer A (8 M urea, 0.1 M NaH₂PO₄, and 10 mM Tris-HCl) at pH 8.0. The column was washed with buffer A (pH 6.3) until no protein was detected by the Bio-Rad protein assay. Protein was eluted with buffer A (pH 5.9) and dialyzed sequentially in buffer containing 7 M urea, 10 mM Tris-HCl (pH 8.0), and 0.1 M NaH₂PO₄ and then dialysis buffer I (20 mM Tris-HCl (pH 7.9), 0.1 mM EDTA, and 10% glycerol) with the following additions: 1) 1 M urea and 1 M NaCl, 2) 1 M NaCl, and 3) 0.1 M KCl. After dialysis, the purified His-*c-Jun*-(274–317) was aliquoted and stored at –80 °C.

GST-*c-Jun*-(1–254), GST-*c-Jun*-(255–331), GST-hsTAF1-(1–163), GST-hsTAF1-(1–87), GST-hsTAF1-(88–163), GST-hsTAF1-(1–125), GST-hsTAF1-(46–163), and GST-hsTAF1-(46–125) were expressed in *Escherichia coli* cells and purified as described previously for GST-*c-Jun* (19). GST-*c-Fos* was expressed in XA-90 cells in the presence of the pSBET plasmid (34, 36). First, 50 ml of LB medium with 0.1 mg/ml ampicillin and 10 μg/ml kanamycin was inoculated with a GST-*c-Fos* colony and shaken at 37 °C for ~5 h. 10 ml of this starter culture was added to 500 ml of LB medium with 0.1 mg/ml ampicillin and shaken at 37 °C until A_{600 nm} = 0.45. The cells were induced with 0.5 mM isopropyl-β-D-thiogalactopyranoside (final concentration) and shaken at 37 °C for 95 min before harvesting. The cells were resuspended in 20 mM Tris-HCl (pH 7.9), 1 mM EDTA, 0.1 M NaCl, 1 mM DTT, and 0.2 mM phenylmethylsulfonyl fluoride and sonicated three times for 20 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 two times for 30 s. Samples were centrifuged in a JA20 rotor at 15,000 rpm for 10 min at 4 °C and washed three more times before a final centrifugation in the JA20 rotor at 18,000 rpm for 30 min at 4 °C. The pellet was resuspended in 6 M GdnHCl, 20 mM Tris-HCl (pH 7.9), 1 mM EDTA, and 1 mM DTT and nutated for 3 h at room temperature. The sample was cleared by centrifugation in a JA20 rotor at 18,000 rpm for 30 min at 4 °C. GdnHCl was removed from the sample by sequential dialysis in dialysis buffer II (20 mM Tris-HCl (pH 7.9), 0.1 mM EDTA, 10% glycerol, 0.1 M NaCl, and 1 mM DTT) with 2 M GdnHCl for 2 h, followed by dialysis buffer II with 1 M GdnHCl for 1 h and by dialysis buffer II with 0.5 M GdnHCl for 1 h. The final step in dialysis was performed overnight by pumping a 5-fold excess of dialysis buffer II (no GdnHCl) into dialysis buffer II containing 0.5 M GdnHCl.

In Vitro Protein/Protein Interaction and Transcription Assays—Interaction assays with GST-immobilized proteins were performed as described previously (19). For Western blot analysis, protein was transferred to nitrocellulose membrane, probed with anti-HA monoclonal antibody followed by horseradish peroxidase-conjugated anti-mouse secondary antibody, and visualized with ECL reagents (Pierce). The reaction conditions for the derepression of TFIID transcription by *c-Jun*-(254–317) were as described previously (19).

To prepare DNA affinity resin, two oligonucleotides were annealed (biotin-labeled-5'-AGCGCAGATCTGTGACTCAGCGGATCTGTGACTCAGCGGATCCCGT and 5'-ACGGGATCCGCTGAGTCACAGATCCGCTGAGTCACAGATCTGCGCT) and subsequently incubated with streptavidin-agarose beads overnight in phosphate-buffered saline. GST-*c-Jun*-(255–331) was immobilized on the DNA affinity resin in 20 mM Tris-HCl (pH 7.9), 10% glycerol, 1 mM EDTA, 5 mM MgCl₂, and 1 mM DTT containing 0.1% Nonidet P-40, 0.2 mM phenylmethylsulfonyl fluoride, and 0.1 M NaCl. For the interaction assay, 2 μg/ml sonicated calf thymus DNA was present during the 1-h nutation at 4 °C.

To study the effect of hsTAF1-(1–87) on *c-Jun* transcriptional activation *in vitro*, reaction conditions were the same as described previously (19) with the following changes. *c-Jun*, alone or with hsTAF1-(1–87), was incubated in 3 μl for 10 min on ice before the addition of p(AP-1)₅-E1b-G-less. The reactions were left on ice for an additional 5 min before the addition of general transcription factors. The reactions (20 μl) were transferred to 30 °C for 20 min prior to adding nucleoside triphosphates. RNA synthesis was allowed to proceed for 20 min at 30 °C.

Transient Transfection Assays—COS-7 cells cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum were seeded onto 12-well plates to reach 90% confluence on the day of transfection. Each well received 2 μl of LipofectAMINE (Invitrogen),

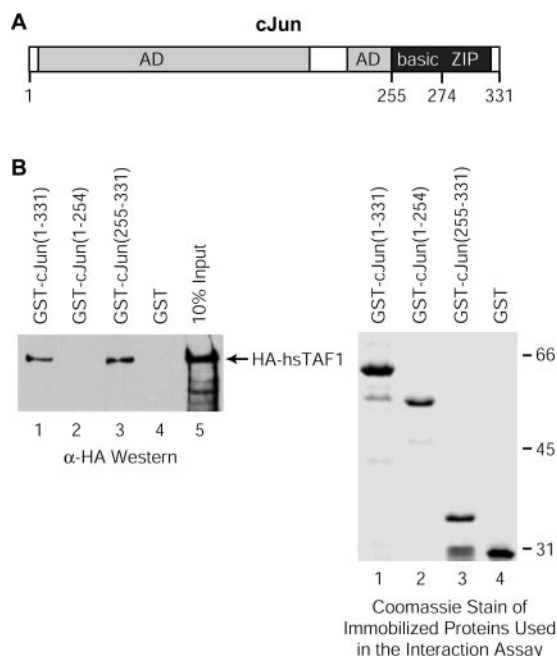


FIG. 1. The basic leucine zipper of c-Jun is necessary and sufficient for interaction with hsTAF1. *A*, schematic of c-Jun. The basic leucine zipper domain of c-Jun is C-terminal of the activation domains (AD). *B*, HA-hsTAF1 interacts with GST-c-Jun-(255–331). Immobilized GST-c-Jun-(1–331), GST-c-Jun-(1–254), GST-c-Jun-(255–331), and GST were incubated with insect cell lysate containing full-length HA-hsTAF1. Protein bound to the affinity resins was analyzed by Western blotting with anti-HA antibody (*left panel*). 10% of the input HA-hsTAF1 is shown in *lane 5* (*left panel*). The Coomassie Blue-stained SDS gel shows the immobilized proteins used in the assay (*right panel*).

6 μ l of Plus reagent (Invitrogen), 500 ng of p(AP-1)₁-E1b-CAT, 25 ng of pRL-TK-*Renilla* luciferase (Promega), and, where indicated, 1 μ g of pcDNA-c-Jun-(1–317) as well as a total of 1.6 μ g of pcDNA-hsTAF1-(1–163) plus pcDNA3.1(+) parental vector (see Fig. 7B for amounts). Cells were left in serum-free Dulbecco's modified Eagle's medium for 5 h, and then 20% Dulbecco's modified Eagle's medium was added. After a 24-h incubation at 37 $^{\circ}$ C, the cells were lysed with 250 μ l of passive lysis buffer (Promega). A CAT assay was used to quantitate reporter gene activation. Briefly, 30 μ l of extract was heated at 65 $^{\circ}$ C for 10 min, and then 70 μ l of reaction mixture was added to make final concentrations of 1.6 mM chloramphenicol, 75 mM Tris-HCl, 1 μ Ci/ml [¹⁴C]acetyl-CoA, and 0.09 mM unlabeled acetyl-CoA. Reactions were incubated at 37 $^{\circ}$ C for 1 h, cooled on ice, and extracted twice with cold ethyl 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 contaminating protein preparations and extracts (38). 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 performed 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-

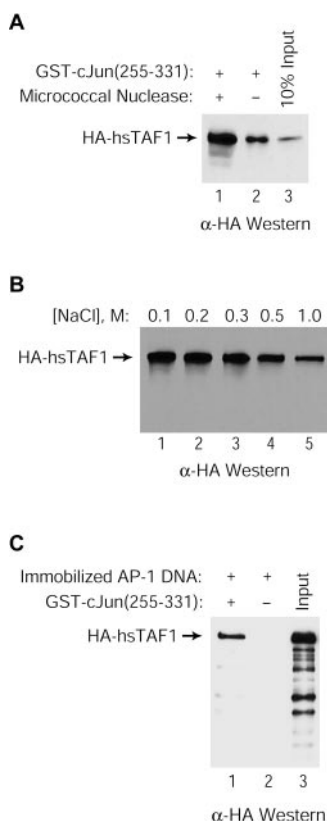


FIG. 2. The *c-Jun*/hsTAF1 interaction is not dependent on nucleic acid and is not disrupted at low salt concentrations. *A*, the *c-Jun*-hsTAF1 complex is not mediated by contaminating nucleic acid. Binding reactions were performed using immobilized GST-*c-Jun*-(255–331) and insect cell extracts containing full-length HA-hsTAF1 that were previously treated with micrococcal nuclease where indicated. Protein bound to the affinity resins was analyzed by Western blotting with anti-HA antibody. *B*, the *c-Jun*-hsTAF1 complex is stable to high salt washes. Full-length HA-hsTAF1 was incubated with immobilized GST-*c-Jun*-(255–331) to form a complex. Portions of the resin were then incubated with 11 volumes of buffer containing the indicated concentrations of NaCl for 10 min at 4 °C with constant nutation. Following two additional washes, protein bound to the affinity resin was analyzed by Western blotting with anti-HA antibody. *C*, DNA-bound *c-Jun* interacts with hsTAF1. GST-*c-Jun*-(255–331) was incubated with AP-1 site DNA affinity resin. Unbound GST-*c-Jun*-(255–331) was washed away, and the resin was incubated with full-length HA-hsTAF1. A control reaction performed with AP-1 DNA-agarose in the absence of GST-*c-Jun*-(255–331) is shown in lane 2. After extensive washing, protein bound to the resin was eluted and analyzed by Western blotting with anti-HA antibody. 10% of the input HA-hsTAF1 is shown in lane 3.

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 TFIID-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 TFIID transcription. We have shown that full-length *c-Jun* can derepress TFIID 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. TFIID 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 TFIID 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 TFIID 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 TFIID transcription relative to TBP transcription levels. As shown by the plot, *c-Jun*-(254–317) derepressed TFIID-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

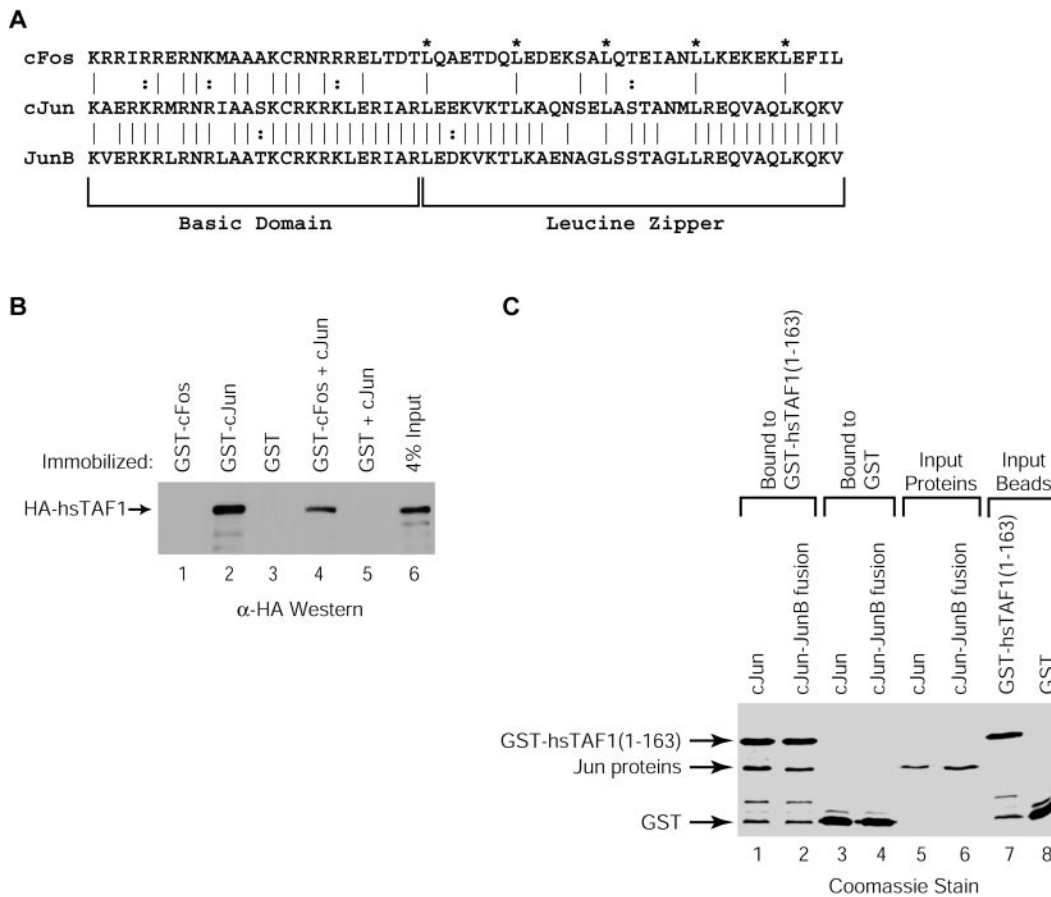


FIG. 3. c-Fos/c-Jun heterodimers and the leucine zipper of JunB interact with hsTAF1, but c-Fos monomers do not. *A*, alignment of the basic leucine zipper regions of c-Fos (amino acids 139–197), c-Jun (amino acids 254–312), and JunB (amino acids 270–328). Vertical lines indicate identity, and colons indicate similarity. Conserved leucines critical for dimerization are indicated with asterisks above the c-Fos sequence. *B*, c-Fos/c-Jun heterodimers bind hsTAF1, whereas c-Fos monomers do not. Immobilized GST-c-Fos (lane 1), GST-c-Jun (lane 2), and GST (lane 3) affinity resins were incubated with HA-hsTAF1. GST-c-Fos extract (lane 4) and GST extract (lane 5) were incubated with purified c-Jun at 4 °C for 1 h. The GST-c-Fos-c-Jun complex and control GST + c-Jun were then incubated with glutathione-Sepharose beads. After washing away non-immobilized protein, these two resins were separately incubated with full-length HA-hsTAF1. 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 Coomassie 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.

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 α -c-Jun. As shown in Fig. 6B, the two subdomains, GST-hsTAF1-(1–87) and GST-hsTAF1-(88–163), interacted with c-Jun (lanes 2 and 3, respectively), but the interaction was weaker than with GST-hsTAF1-(1–163). GST-hsTAF1-(46–163) and GST-hsTAF1-(1–125) bound slightly more c-Jun (lanes 4 and 5, respectively). GST-hsTAF1-(46–125) and control GST did not interact with c-Jun (lanes 6 and 7, respectively). From these results, we conclude that multiple regions within the N-terminal 163 amino acids of hsTAF1 are required for maximal interaction with c-Jun; specifically, both ends of the hsTAF1 N-terminal region are required for maximal binding.

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 show). 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-

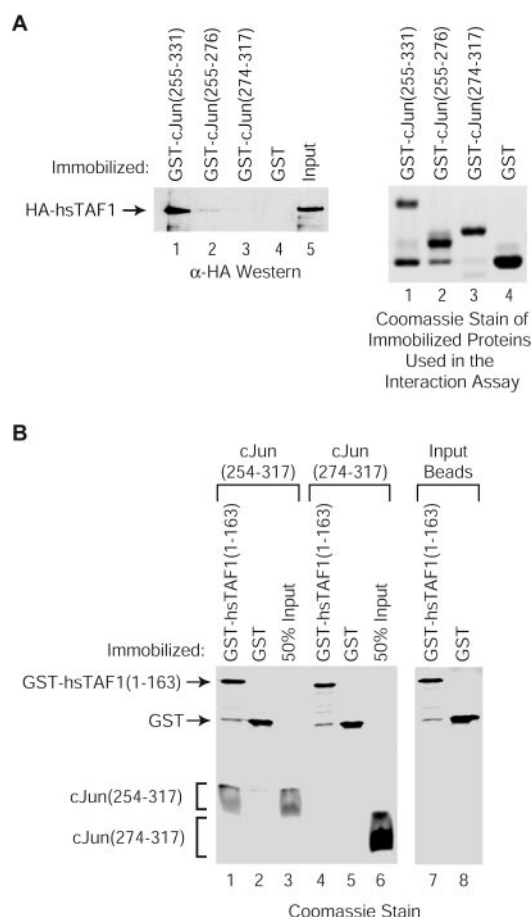


FIG. 4. Both the basic region and the leucine zipper of c-Jun are required for interaction with hsTAF1. A, HA-hsTAF1 does not interact with either the basic region (amino acids 255–276) or the leucine zipper (amino acids 274–317) of c-Jun individually. GST-c-Jun-(255–331), GST-c-Jun-(255–276), and GST-c-Jun-(274–317) affinity resins were incubated with insect extracts containing HA-hsTAF1. Bound protein was analyzed by anti-HA Western blotting (left panel). The Coomassie Blue-stained SDS gel shows the immobilized proteins used in the assay (right panel). B, GST-hsTAF1(1–163) binds the c-Jun basic leucine zipper, but not the leucine zipper alone. c-Jun-(254–317) and c-Jun-(274–317) were incubated with immobilized GST-hsTAF1(1–163) (lanes 1 and 4, respectively) and control GST (lanes 2 and 5, respectively). Bound protein was analyzed by SDS-PAGE and Coomassie Blue-stained. 50% input c-Jun-(254–317) and c-Jun-(274–317) were loaded in lanes 3 and 6, respectively. GST-hsTAF1(1–163) and GST affinity resins used for the interaction assay are shown in lanes 7 and 8, respectively.

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(1–87); 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 >4-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 active 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.

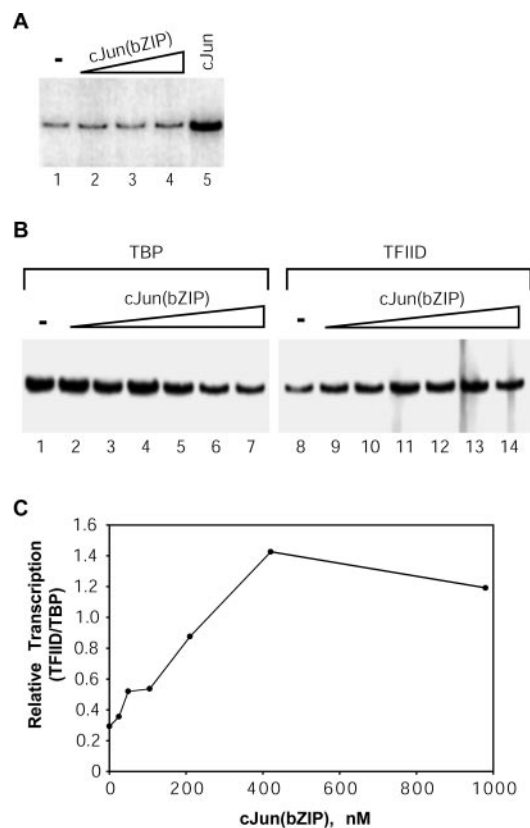


FIG. 5. The basic leucine zipper domain of c-Jun does not activate transcription *in vitro*, but derepresses TFIID-directed transcription from a template that lacks AP-1 sites. A, the basic leucine zipper of c-Jun is not sufficient for activated transcription *in vitro*. Either recombinant c-Jun-(254–317) (*c-Jun(bZIP)*) or full-length c-Jun was added to the p(AP-1)₅-E1b-G-less template (five AP-1 sites upstream of the adenovirus E1b TATA box fused to a 377-bp G-less cassette). The concentrations of c-Jun-(254–317) in the reactions were 24.4 nM (lane 2), 49 nM (lane 3), and 70 nM (lane 4). The concentration of c-Jun-(1–331) was 70 nM (lane 5). B, the c-Jun basic leucine zipper domain derepresses TFIID transcription. Recombinant c-Jun-(254–317) was incubated with TBP (lanes 2–7) or TFIID (lanes 9–14) prior to adding the p(GAL4)₅-AdMLP-G-less template (consisting of the adenovirus major late core promoter upstream of a 380-bp G-less cassette) and the remaining general transcription factors. The concentrations of recombinant c-Jun-(254–317) in the transcription reactions were 24.5 nM (lanes 2 and 9), 49 nM (lanes 3 and 10), 105 nM (lanes 4 and 11), 210 nM (lanes 5 and 12), 420 nM (lanes 6 and 13), and 980 nM (lanes 7 and 14). C, plot of the ratio of TFIID transcription levels to TBP transcription levels versus recombinant c-Jun-(254–317) concentration. The transcription data in B were quantitated by phosphorimaging and plotted.

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.

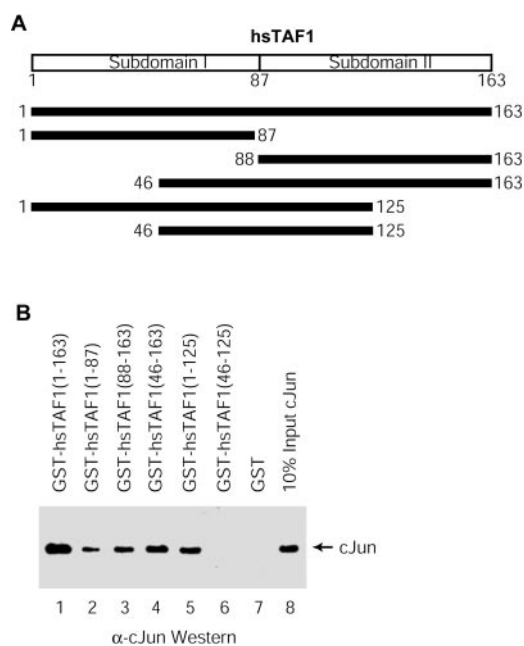


FIG. 6. The N-terminal 163 amino acids of hsTAF1 are required for maximal c-Jun interaction. *A*, the schematic shows the two subdomains of the N terminus of hsTAF1 as well as the deletion mutants tested for interaction with c-Jun. *B*, GST-tagged hsTAF1 affinity resins were incubated with purified c-Jun.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

Interaction between Basic Leucine Zipper Domains and the N-terminal Region of hsTAF1—The interaction between a 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 TAF_{II}55) 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 re-

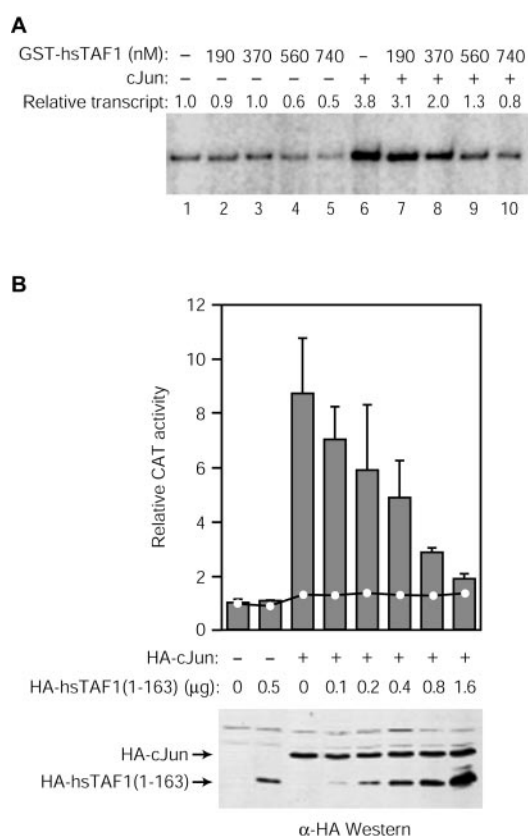


FIG. 7. The N-terminal region of hsTAF1 inhibits c-Jun transcriptional activation in vitro and in cells. *A*, hsTAF1(1–87) represses c-Jun-activated transcription *in vitro*. GST-hsTAF1(1–87) was incubated alone (lanes 2–5) or with c-Jun (lanes 7–10) before the addition of the p(AP-1)₅-E1b-G-less template (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 for 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).

quired 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) that have been previously characterized for their ability to interact with TBP (17, 21) resulted in decreased interaction with c-Jun (Fig. 6). However, the addition of ~40 amino acids to either subdomain (positions 1–125 and 46–163) increased the interaction with c-Jun. A region overlapping these two clones (amino acids 46–125) was not sufficient for an interaction with

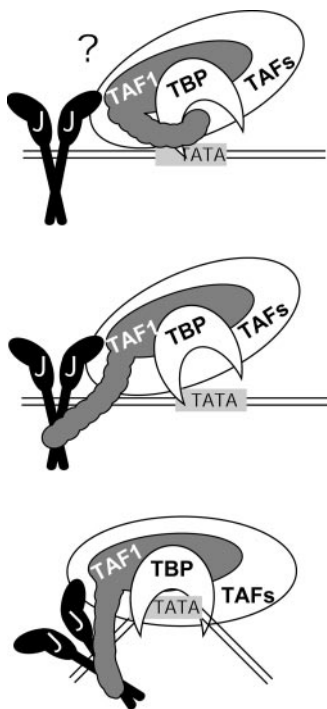


FIG. 8. Model of transcriptional derepression and activation by c-Jun. The activation domains of c-Jun interact with TFIID (or some other unidentified factor bound to TFIID). The interaction of the N terminus of hsTAF1 with TBP, which normally represses TFIID DNA binding, is destabilized by direct interaction between the basic leucine zipper of c-Jun and the N terminus of hsTAF1. TBP is then free to bind to the TATA box, bend DNA, and start preinitiation complex assembly.

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 entirely due to charged residues. The basic leucine zipper domain of c-Jun has a calculated pI of 11.3, whereas the calculated pI of the N-terminal region of hsTAF1 is 3.8. Several experiments were performed with the basic leucine zipper domain of c-Jun and hsTAF1 to ensure that this interaction was specific and not simply a nonspecific charge-charge attraction. Increasing salt washes would disrupt a complex formed via all 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 (pI 12) nor the leucine zipper (pI 9.4) was able to bind hsTAF1. Moreover, region 46–125 of hsTAF1 (pI 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 TFIID-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 TFIID binding to DNA and basal transcrip-

tion (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 found 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 TFIID complex. The activation domain(s) of c-Jun that is bound to an AP-1 site makes yet uncharacterized interactions with subunits of TFIID, another general transcription factor, or a coactivator that ultimately results in recruiting TFIID 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 TFIID, 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 TFIID-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.

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