THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 275, No. 21, Issue of May 26, pp. 15912–15916, 2000

Printed in U.S.A.

Divergent hTAF$_{II}$31-binding Motifs Hidden in Activation Domains*[S]

Received for publication, January 20, 2000, and in revised form, March 21, 2000

Yongmun Choi, Shinichi Asada, and Motonari Uesugi‡

From the Verna and Marrs McLean Department of Biochemistry and Molecular Biology, Baylor College of Medicine, Houston, Texas 77030

Activation domains are functional modules that enable DNA-binding proteins to stimulate transcription. Characterization of these essential modules in transcription factors has been hampered by their low sequence homology. Here we delineate the peptide sequences that are required for transactivation and interaction with hTAF$_{II}$31, a classical target of the acidic class of activation domains. Our analyses indicate that hTAF$_{II}$31 recognizes a diverse set of sequences for transactivation. This information enabled the identification of hTAF$_{II}$31-binding sequences that are critical for the activity of the activation domains of five human transcription factors: NFAT1, ALL1, NF-IL6, ESX, and HSF-1. The interaction surfaces are localized in short peptide segments of activation domains. The brevity and heterogeneity of the motifs may explain the low sequence homology among acidic activation domains.

Transcription factors typically have distinct domains for binding specific DNA sequences and for activating transcription through protein-protein interactions (1–3). Although a large number of activation domains are known, these functional modules share little sequence homology and have only loosely been classified by the preponderance of amino acid residues such as acidic residues, glutamine, and proline (1, 4). This low homology of activation domains has made it difficult to characterize these essential modules in transcription factors. Identification of functional sequence motifs that are hidden in activation domains would dissect the functions of activation domains and help to understand their composite regulations.

Multiple target proteins for each class of activation domains have been proposed, including the basal transcriptional factors, mediators, and chromatin-remodeling factors. One such direct target for acidic activators is hTAF$_{II}$31 (a human TFIID TATA box-binding protein-associated factor) (5–9). Functional inactivation of its yeast homolog, yTAF$_{II}$17, results in the loss of transcription for approximately 67% of the actively expressed yeast genes (10–13). Moreover, hTAF$_{II}$31 has been found in a human histone-acetylase complex in addition to TFIID (14, 15). These previous results collectively suggest a general role of hTAF$_{II}$31 and its homologs in the regulation of eukaryotic gene transcription both at the level of chromatin modification and RNA polymerase recruitment (16).

It has been reported that hTAF$_{II}$31 makes direct contacts with the activation domains of VP16, p53, and NF-κB p65 and that the strength of the interactions correlates with the ability to activate transcription (5–9, 17, 18). NMR and biochemical studies have shown that the activation domains of VP16 and p53 undergo an induced transition from random coil to α-helix upon interaction with hTAF$_{II}$31, with key hydrophobic residues along one face of the nascent helix (17, 18). The pattern of such hydrophobic residues, F*XX*ΦΦ (where *X* represents any residue and Φ represents any hydrophobic residue) is conserved among the activation domains of VP16, p53, and NF-κB p65, suggesting that this sequence represents a recognition element for hTAF$_{II}$31.

Here we delineate the peptide sequences that are required for transactivation and for interaction with hTAF$_{II}$31. Our analyses indicate that hTAF$_{II}$31 recognizes a more divergent set of peptide sequences than F*XX*ΦΦ for the transmission of activation signals. This sequence characterization enabled the identification of hTAF$_{II}$31-binding sequences hidden in the activation domains of NFAT1, ALL1, NF-IL6, ESX, and HSF-1. A combination of mutational studies and NMR analyses indicated that the interaction surfaces comprise short peptide regions containing signature α-helical motifs. Furthermore, the strength of the interactions between these activators and hTAF$_{II}$31 correlates with the ability to activate transcription in human cells, supporting the notion that hTAF$_{II}$31 and its homologs are important targets of eukaryotic transactivators.

EXPERIMENTAL PROCEDURES

Mutant Library Screening—We constructed four small libraries of a mammalian expression vector, each encoding a 17-amino acid peptide from the VP16 activation domain (VP16-(469–485)) fused with the GAL4 DNA-binding domain. Each of the four libraries consists of random point mutants at one of the four positions within the F*XX*ΦΦ sequence of VP16-(469–485) (**Phe**^{479}-**Thr**^{480}-Asp^{481}-**Ala**^{482}-**Leu**^{483}). 192 clones from each library were miniprepped and individually transfected into human Jurkat Tag cells in two 96-well plates (100 ng/well of expression plasmid and ~10^5 cells/well). The reporter construct we used (120 ng/well) was pG5IL2SX in which the secreted alkaline phosphatase (SEAP)[1] gene is controlled by five GAL4-binding sites. After a 48-h incubation, each well was assayed for SEAP activity through fluorescence change of 4-methylumbeliferyl phosphate as described (19). Fluorescence measurements were carried out by a microplate reader, Fluoroskan II (Labsystems). Positive clones were characterized by DNA sequencing, and their activities were quantitatively estimated through repeated transfection experiments in a larger volume.

In Vitro Protein-Protein Interaction Assay—The protein hTAF$_{II}$31-(1–140) was purified as described (18). Glutathione *S*-transferase fusion proteins of activation domains were expressed in BL21(DE3)pLysS and purified by affinity chromatography using glutathione-agarose beads. The beads binding either glutathione *S*-transferase (GST) fusion protein or GST only were incubated with hTAF$_{II}$31-(1–140) in 200 μl of binding buffer containing 25 mM NaCl, 0.005% Nonidet P-40, 10% glycerol, 20 mM Tris-HCl, pH 7.5, 10 mM $MgCl_2$, and 2 mM dithiothreitol

* This work was supported in part by research funds from Yoshitomi Pharmaceutical Industries and the Leukemia Society of America. The 600-MHz NMR spectrometer at the University of Houston is funded by the W. M. Keck Foundation. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[S] The on-line version of this article (available at http://www.jbc.org) contains one table.

‡ To whom correspondence should be addressed. Fax: 713-798-1625; E-mail: muesugi@bcm.tmc.edu.

[1] The abbreviations used are: SEAP, secreted alkaline phosphatase; GST, glutathione *S*-transferase; NOE, nuclear Overhauser effect; NOESY, NOE spectroscopy.

for 1 h at 4 °C. After extensive washing with the same buffer, the bound $hTAF_{II}31$-(1–140) was analyzed by SDS-polyacrylamide gel electrophoresis.

Transcription Assay—The DNA encoding the GAL4 DNA-binding domain (residues 1–94) was subcloned into the *Hin*dIII/*Kpn*I site of pcDNA3 (Invitrogen) and the resultant pcDNAGAL4 plasmid was used to construct mammalian expression vectors for GAL4 fusions of ALL1-(2829–2883), NFIL6-(24–124), ESX-(129–159), HSF-1-(371–430) and NFAT1-(1–96). Jurkat Tag cells ($\sim 2 \times 10^6$) were transfected with 500 ng of each GAL4 fusion construct along with 2 μg of pG5IL2SX. After a 48-h incubation, an aliquot of the culture was removed and assayed for SEAP activity as described (19). The expression levels of the GAL4 fusion proteins were comparable, as judged by Western blot analyses using an antibody against the GAL4 DNA-binding domain.

NMR Studies—Peptides were synthesized using Rink Amide MBHA resin, purified by high performance liquid chromatography, and characterized by NMR, amino acid analyses, and electrospray ionization mass spectroscopy. The peptide was dissolved in 95% H_2O plus 5% ^2H_2O containing 130 mM KCl, 5 mM perdeuterated dithiothreitol, 20 mM perdeuterated Tris-AcOH (pH 6.2), and 10 μM EDTA, and then the pH of the solution was adjusted to ~6.2 by adding dilute KOH. The final concentrations of peptides were determined by UV absorption or amino acid analyses to be ~4 mM. NMR experiments were performed in the absence or presence of $hTAF_{II}31$-(1–140) (240 μM) on a Bruker AMX600 spectrometer. The sequential assignment of the peptide signals was obtained by using a combination of total correlation spectroscopy, DQF-COSY, and nuclear Overhauser effect spectroscopy (NOESY) data sets of a free peptide sample. Sequential $d_{\alpha N}(i, i + 1)$ NOEs, although weak, were observed in the NOESY spectra of the free peptide, which served as a basis for the complete sequential assignment. In the NOESY spectra, 512 free induction decays were recorded at 290 or 300 K with mixing times of 350 ms. The data were processed with the Felix 98.0 software (Biosym Technologies) with appropriate apodization and zero-filling.

RESULTS

Divergent Motif for $hTAF_{II}31$—To delineate the peptide sequences required for transactivation through interaction with $hTAF_{II}31$, we first constructed four small libraries of a mammalian expression vector, each encoding a 17-amino acid peptide from the VP16 activation domain (VP16-(469–485)) fused with the GAL4 DNA-binding domain. VP16-(469–485) was chosen because it is the minimal VP16 peptide that binds a fragment of $hTAF_{II}31$ ($hTAF_{II}31$-(1–140)) and activates transcription (18). Each of the four libraries consists of random point mutants at one of the four positions within the F*XX*ΦΦ sequence of VP16-(469–485) (**Phe**^{479}-**Thr**^{480}-Asp^{481}-**Ala**^{482}-**Leu**^{483}). 192 clones from each library were miniprepped and individually transfected into human Jurkat T cells in 96-well plates. We cotransfected the cells with a reporter containing the SEAP gene under the control of five GAL4-binding sites. This permits convenient detection of transcriptional activation through fluorescence change of a phosphatase substrate.

The DNA sequence analyses of positive clones (>50% SEAP activity of the wild type) revealed that the ability of VP16-(469–485) to activate transcription can endure a variety of amino acid substitutions (Fig. 1). The screen identified Trp, Ile, and Leu at the conserved position of Phe^{479}; substitution of Phe^{479} with any one of the three residues exhibited no substantial loss in its transcriptional activity. The mutation of Phe^{479} with Val abrogated much of its activation potential, thus validating our screen and indicating the presence of a clear boundary between Leu and Val for the activity. At the positions of Thr^{480} and Ala^{482}, we obtained many clones that activate transcription more than 50% of the wild type. Although hydrophobic residues are favored at these two positions, clones with some of hydrophilic residues such as Tyr also had significant activity. The identity of Leu^{483} is more tightly controlled, since we isolated only Trp, Phe, and Leu as positive clones. Clones with the other bulky hydrophobic residues, *i.e.* Ile and Val, at this position exhibited less than 20% activity of the wild type. However, the simultaneous substitution of Ala^{482} with a bulky hydrophobic residue (Trp, Phe, Ile, or Leu) restored the transcriptional level of the Ile and Val mutants to >50% of the wild type, indicative of the complementarity between these two adjacent positions.

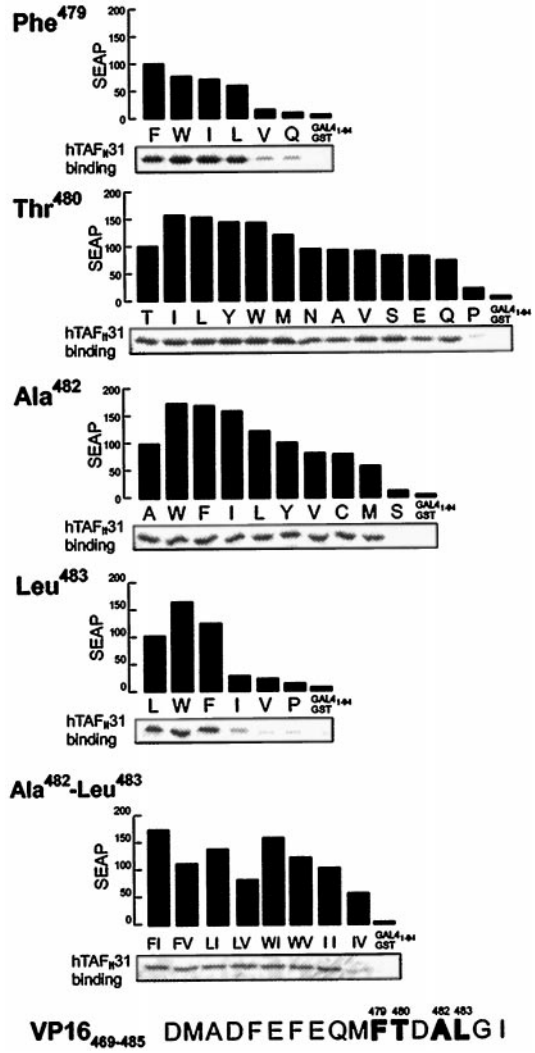

FIG. 1. **Divergent $hTAF_{II}31$-binding motifs.** We constructed four small mutant libraries of a mammalian expression vector of GAL4-VP16-(469–485), each of which consisted of point mutants at one of the four positions within the F*XX*ΦΦ sequence of VP16-(469–485) (**Phe**^{479}-**Thr**^{480}-Asp^{481}-**Ala**^{482}-**Leu**^{483}). 192 clones were miniprepped for each library and individually transfected into human Jurkat T cells in a 96-well format. Each well was assayed for reporter gene activity (SEAP) through fluorescence change. Positive clones were characterized by DNA sequencing, and their activities were quantitatively estimated through repeated transfection experiments in a larger volume (*upper*). The mutants of Val and Glu at Phe^{479}; Pro at Thr^{480}; Ser at Ala^{482}; and Ile, Val, and Pro at Leu^{483} are shown as negative clones. The transcriptional level of mutants of Val and Ile at Leu^{483} is restored to >50% of the wild type when Ala^{482} is simultaneously replaced by a bulky hydrophobic residue as shown at the *bottom* for Ala^{482}-Leu^{483}. The DNAs encoding these mutants were then subcloned into pGEX3X vectors, which provided GST fusions of the mutants. It is evident that activation-positive mutants were able to bind $hTAF_{II}31$-(1–140), whereas activation-deficient mutants had substantially lower affinity (*lower*).

The peptide sequences examined above were then fused with GST and tested for the ability to bind $hTAF_{II}31$-(1–140). As shown in Fig. 1, the activation-positive peptides bound $hTAF_{II}31$-(1–140) to the same extent as the wild type, whereas the binding of activation-deficient peptides was significantly impaired. Thus the strength of the interaction with $hTAF_{II}31$-(1–140) *in vitro* correlates with the ability to activate transcription in transfected cells.

Search for hTAF$_{II}$31-binding Sequences—Guided by the information obtained from the screen, we searched for potential hTAF$_{II}$31-binding sequences in human activation domains. A series of selection steps was carried out on the activation domains of 65 distinct human transcription factors. The amino acid sequences of these 65 activation domains and their original references are available on the World Wide Web and in the supplemental materials. In an initial step, we selected for any activation domains that contain a signature **P1-P2-*X*-P3-P4** sequence (where P1 represents Phe, Trp, Ile, or Leu; P2 represents Ile, Leu, Tyr, Trp, Met, Asn, Ala, Thr, Val, Ser, Glu, or Gln; *X* represents any amino acid; and P3-P4 represents Trp/Phe/Ile/Leu-Trp/Phe/Leu/Ile/Val or Ala/Tyr/Val/Cys/Met-Trp/Phe/Leu) and found 26 that did so. Those activation domains whose signature sequences were not conserved among species and subtypes were eliminated in a second step, and a third elimination step was then run on the remaining candidates by calculating the probability of α-helix formation of their signature sequences. 15 candidates were eliminated by the second and third steps. For example, the activation domain of cell cycle regulator E2F1 was eliminated because its signature sequence, **FS**G**LL**, was not conserved in its chicken homolog (**F*P***G**FL**) and because these sequences had little α-helix probability. The activation domain of MSG1 was also eliminated because its signature sequence, **LMSLV**, was not conserved in a subtype of its mouse homolog (**LTSL*E***). As expected, the activation domains of E2F1 and MSG1 had no detectable affinity to hTAF$_{II}$31, validating our elimination steps (data not shown). 11 activation domains survived all three elimination steps. These are the activation domains of ALL1, NF-IL6, NFAT1, Sox-4, MyoD, c-Jun, HIF-1α, TEF-1, HSF-1, TREB5, and ESX (Fig. 2*A*). The peptide segments that correspond to VP16-(469–485) were fused to GST and assayed for the ability to bind hTAF$_{II}$31. Only the peptides of ALL1, NF-IL6, ESX, HSF-1, and NFAT1 bound hTAF$_{II}$31 as tightly as VP16-(469–485) (Fig. 2*B*).

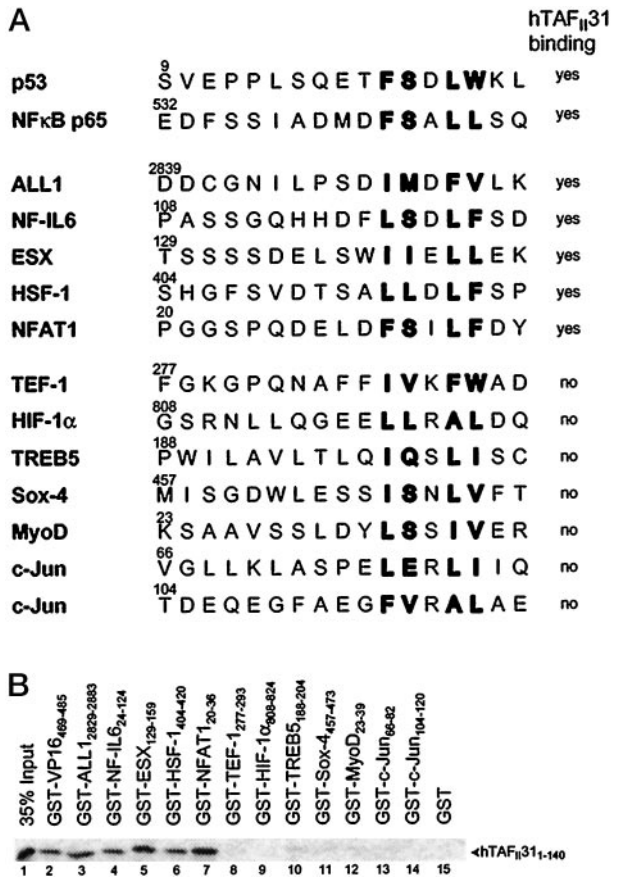

FIG. 2. **Identification of short hTAF$_{II}$31-binding peptides in the activation domains.** *A*, sequence alignment of relevant regions in the activation domains of p53, p65, and the 11 activators that survived all three elimination steps. The signature sequences are *highlighted*. *B*, *in vitro* binding assay. It is evident that GST fusion proteins of the peptide segments of ALL1 (*lane 3*), NF-IL6 (*lane 4*), ESX (*lane 5*), HSF-1 (*lane 6*), and NFAT1 (*lane 7*) bind hTAF$_{II}$31-(1–140), whereas those of TEF-1 (*lane 8*), HIF-1α (*lane 9*), TREB5 (*lane 10*), Sox-4 (*lane 11*), MyoD (*lane 12*), c-Jun (*lanes 13* and *14*), and GST alone (*lane 15*) have no detectable affinity to hTAF$_{II}$31-(1–140).

The full-length activation domains of these factors were further analyzed. NFAT1 (nuclear factor of activated T cells 1) belongs to the NFAT family of transcription factors and plays a central role in inducible gene transcription during the immune response. Whereas the full-length activation domain of NFAT1 (amino acids 1–96) bound hTAF$_{II}$31-(1–140) to the same extent as its peptide version, mutation of Phe^{30} and Phe^{34} with Ala impaired its interaction (Fig. 3*A*), indicative of direct involvement of the signature sequence in the interaction with hTAF$_{II}$31. ALL1 (acute lymphoblastic leukemia gene product; also referred as HRX or MILL) is a human transcription factor that is involved in acute lymphoblastic leukemia (20, 21), and its transcriptional activity is considered to be responsible for malignant transformation (22). We identified the Ile^{2849}-Met^{2850}-Asp^{2851}-Phe^{2852}-Val^{2853} sequence in its activation domain as a hTAF$_{II}$31-binding motif. This was in good agreement with the previous mutational studies showing the importance of these residues in transactivation (22). As shown in Fig. 3*A*, the full-length activation domain of ALL1 (amino acids 2829–2883) bound hTAF$_{II}$31-(1–140) as tightly as its peptide version. Substitution of Ile^{2849} and Val^{2853} by Ala greatly impaired the interaction, indicative of the involvement of the IMDFV sequence in the interaction. NF-IL6 (nuclear factor interleukin-6; also referred as C/EBPβ or LAP) induces cytokine genes and has been implicated as a master regulator of the acute-phase response (23). Its full-length activation domain (amino acids 24–124) (24) bound hTAF$_{II}$31-(1–140) to the same extent as its peptide version. This interaction appears to be mediated by the LSDLF sequence, since substitution of Leu^{118} and Phe^{122} by Ala impairs the interaction. The (L/F)(S/A)DLF sequence is conserved among the activation domains of C/EBPs, and amino acid substitutions in the conserved region in rC/EBPα adversely affect its transactivation potential (25, 26). HSF-1 (heat shock factor 1) responds a multitude of stress conditions and plays an important role in the molecular response to nonnative proteins (27), and its activation domain is known to be highly potent (28). We found a hTAF$_{II}$31-binding motif in its COOH-terminal half, which was consistent with the previous truncation studies (29). Whereas the full-length activation domain of HSF-1 (amino acids 371–430) bound hTAF$_{II}$31-(1–140) to the same extent as its peptide version, mutation of Leu^{414} and Phe^{418} with Ala impaired its interaction (Fig. 3*A*), indicative of direct involvement of the signature sequence in the interaction with hTAF$_{II}$31. Last, we identified a hTAF$_{II}$31-binding sequence in the activation domain of ESX (an epithelium-restricted Ets factor) that regulates the expression of the *HER2/neu* (*c-erbB2*) oncogene in human breast cancer (30) and has been found to be overexpressed at an early stage of human breast cancer development (31). Once again, the ESX activation domain (amino acids 129–159) bound hTAF$_{II}$31-(1–140) as tightly as its peptide version. Substitution of Ile^{139} and Leu^{143} by Ala compromised this interaction, indicating that the interaction is mediated at least in part by the IIELL sequence.

To analyze the ability to activate transcription, each activator was fused with the GAL4 DNA-binding domain, and its expression plasmid was transfected into human Jurkat T cells

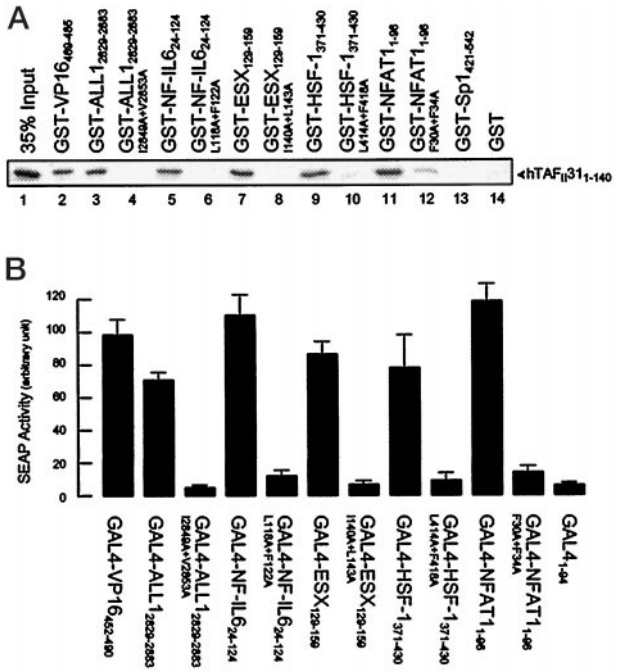

FIG. 3. *A*, *in vitro* binding assay. It is evident that GST fusions of the activation domains of ALL1 (*lane 3*), NF-IL6 (*lane 5*), ESX (*lane 7*), HSF-1 (*lane 9*), and NFAT1 (*lane 11*) bind $hTAF_{II}31$-(1–140), whereas the glutamine-rich activation domain of Sp1 (*lane 13*) and GST alone (*lane 14*) have no affinity to $hTAF_{II}31$-(1–140). Substitutions of key hydrophobic residues in the signature motifs greatly impair interactions (*lanes 4*, *6*, *8*, *10*, and *12*). The position of $hTAF_{II}31$-(1–140) is indicated. *B*, activities of the activation domains of ALL1, NF-IL6, ESX, HSF-1, NFAT1, and their mutants in human cells when fused with the DNA-binding domain of GAL4. The expression construct of each GAL4 fusion was transiently transfected into human Jurkat T cells along with a reporter gene that expresses SEAP under the control of five GAL4-binding sites. SEAP activities were monitored by fluorescence.

along with the reporter plasmid driven by five copies of the GAL4-binding element. As shown in Fig. 3*B*, the interaction-deficient mutants of the activation domains were correspondingly unable to activate transcription of the reporter gene, whereas the wild type proteins activated transcription, similar to the VP16 activation domain ($VP16_{452-490}$). This functional reduction of the mutants is not the result of differences in their expression levels as judged by Western analyses. Thus, the $hTAF_{II}31$-binding sequences of NFAT1, ALL1, NF-IL6, HSF-1, and ESX are critical for their ability to activate transcription; perhaps $hTAF_{II}31$ directly mediates the transcriptional activation by these human factors. However, it is not impossible to imagine that the same amino acids involved in the interaction with $hTAF_{II}31$ may also interact with surfaces in some other co-activators.

Comparison of $hTAF_{II}31$-binding Sequences—Including p53 and p65, we have now obtained a total of seven human activation domains whose activities are critically dependent on their $hTAF_{II}31$-binding sequences. Their amino acid sequences are compared in Fig. 2*A*. Apart from the COOH-terminal signature sequences, the $hTAF_{II}31$-binding peptides have no sequence similarity among themselves. Nonetheless, the NH_2-terminal nonhomology region is necessary for binding because truncation of the NH_2-terminal five residues in ALL1-(2839–2855) and VP16-(469–485) abolish $hTAF_{II}31$ binding (18) (Fig. 4*A*).

To compare the conformations of the $hTAF_{II}31$-binding peptides upon binding to $hTAF_{II}31$, each peptide was chemically synthesized and analyzed by transferred nuclear Overhauser effects (TRNOE), an NMR technique that provides conformational information of a small ligand interacting weakly with its macromolecular receptor (18, 32). Only $ALL1_{2839-2855}$ and NF-

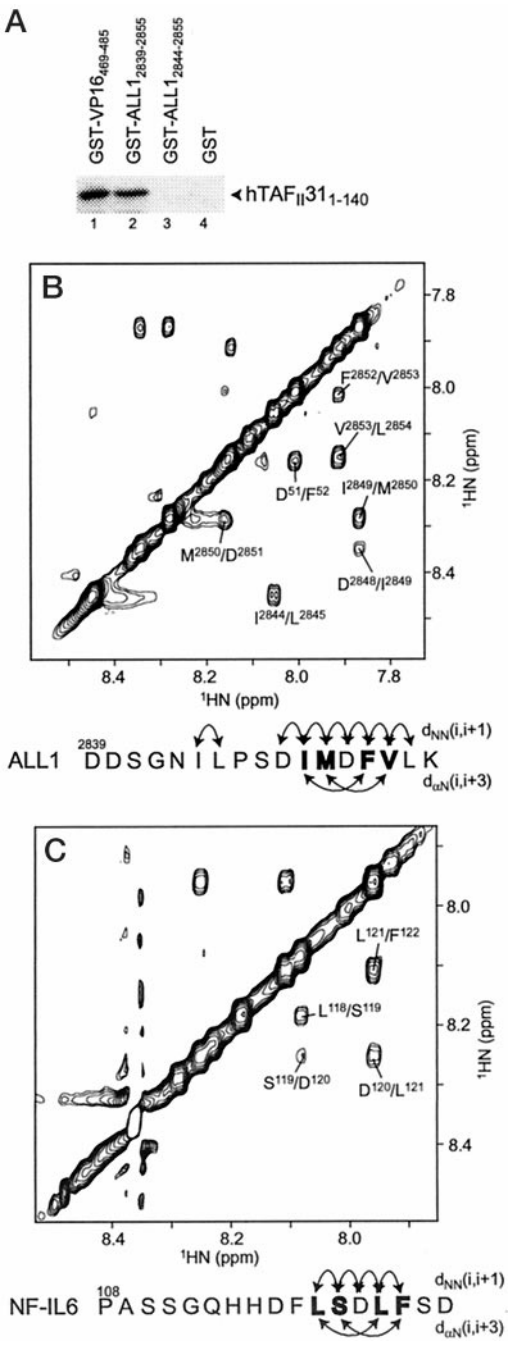

FIG. 4. *A*, truncation study. Deletion of the NH_2-terminal five residues from ALL1-(2839–2855) impaired the interaction with $hTAF_{II}31$-(1–140) (compare *lanes 2* and *3*). *B* and *C*, transferred NOE NMR experiments. Amide regions of 350-ms NOESY spectra of ALL1-(2839–2855) (*B*) and NF-IL6-(108–124) (~4 mM) (*C*) in the presence of $hTAF_{II}31$-(1–140) (240 μM). The identities of residues that exhibit NOE cross-peaks are indicated. Sequential NOEs characteristic of a helix formation are summarized in the *lower panels*. For the NMR analysis of ALL1, the peptide in which Cys^{2841} was substituted with Ser was used for technical convenience. The GST pull-down experiment independently verified that this substitution had no effect on the interaction with $hTAF_{II}31$-(1–140).

$IL6_{108-124}$ showed good physical properties under NMR conditions and were amenable to TRNOE analyses. NOESY spectra of the free peptides exhibited few NOEs, as expected from low molecular weight peptides tumbling freely in solution. In the presence of $hTAF_{II}31$-(1–140), however, numerous TRNOE peaks newly appeared, including those between successive amide protons in the main chain (Fig. 4, *B* and *C*). The pattern of

these cross-peaks and the presence of long range $d_{\alpha N}(i, i + 3)$ and $d_{\alpha\beta}(i, i + 3)$ NOEs suggest the formation of short α-helices encompassing the signature motifs in ALL1-(2839–2855) and NF-IL6-(108–124). No TRNOEs were observed with a control 17-amino acid peptide that has a similar acidicity/hydrophobicity profile but no affinity to $hTAF_{II}31$, indicating that the interaction with $hTAF_{II}31$-(1–140) under the NMR condition is specific (data not shown). These results support the notion that the interactions between activation domains and $hTAF_{II}31$ are mediated generally by short α-helices in the activators.

In ALL1-(2839–2855) and NF-IL6-(108–124), the NH_2-terminal halves appear to be in an extended conformation, and we failed to detect any NOEs that suggest the formation of a folded structure in this region. These nonhomology segments thus may make variable contributions to the association, possibly by lowering the energetic barrier for helix formation or by making additional contacts with the surface of $hTAF_{II}31$, perhaps including those between main chain amide groups in the peptides and chemically complementary functional groups in $hTAF_{II}31$.

DISCUSSION

Our analyses indicate that $hTAF_{II}31$ recognizes a diverse set of peptide sequences in activation domains. There are two advantages for the cells in using such a promiscuous interaction for transactivation. One is the weakness of the interaction; the dissociation constant of the interaction between $hTAF_{II}31$ and the VP16 activation domain is in the high micromolar range, and the weakness of the interactions is often translated to the diversity of binding sequences. Synergism of such weak interactions between activators and co-activators makes transactivation signals diverse and steep enough to emulate a binary switch (3, 33, 34). Low affinity interactions also permit dynamic modulation in response to the alteration of signals that high affinity interactions would be unable to generate (35). Therefore, the coupling of weak interactions with transcriptional activation may be ideal for eukaryotic cells that respond to various signals in a highly tuned manner.

Another advantage is the fact that each one of the binding sequences can be unique enough to be recognized specifically by its regulatory proteins. This permits specific modulation of activity of particular transcription factors in response to the alteration of signals. For example, the MDM2 protein, a cellular attenuator of p53, specifically recognizes and masks the $hTAF_{II}31$-binding motif in the p53 activation domain while exhibiting no detectable affinity to any other activation domains that bind $hTAF_{II}31$ (17). This differential recognition is evidently enabled by the heterogeneity of $hTAF_{II}31$-binding sequences. Many phosphorylation sites can also be arranged in the $hTAF_{II}31$-binding peptides for specific regulation (Fig. 2*A*). Some of the Ser residues are indeed known to be phosphorylated upon particular stimuli. Ser^{15} and Ser^{20} in the p53 activation domain are phosphorylated upon DNA damage, and Ser^{15} is the site of the phosphorylation by the ATM kinase (36–38) and the DNA-activated protein kinase (39). Phosphorylation of Ser^{536} in the activation domain of NF-κB p65 has been detected in TNF-α-induced cells (40). It is also known that the activation potential of rat NF-IL6/LAP is directly enhanced by phosphorylation of the Ser residue that is 13 amino acids away from the $hTAF_{II}31$ motif (41). Phosphorylation of nonconserved Ser residues within or adjacent to $hTAF_{II}31$-binding motifs may be a general strategy for building dynamic and specific characters into the regulation of transcription factors in higher eukaryotes.

The physical association between activation domains and $hTAF_{II}31$ requires a small surface comprising divergent peptide motifs in activation domains. The brevity and heterogeneity of the motifs obscure their existence in transactivators but add flexibility to the functions. It is now clear that $hTAF_{II}31$ is just one of the many targets of activation domains, and other targets may also recognize short, divergent peptide sequences. The combinatorial presence of such motifs in a single activation domain would render a highly mosaic and cryptic nature to the activation domain.

Acknowledgments—We thank Xiaolian Gao for assistance in using the NMR instrument at the University of Houston, Florante A. Quiocho for generous instrumental support, Donghai Yu for technical assistance, and Jun Qin for mass analyses. We are also grateful to Tadamitsu Kishimoto, Gretchen Darlington, Eli Canaani, Anjana Rao, Akira Nakai, Toshi Shioda, and Wade Harper for plasmid constructs, Sheldon Park for helpful comments on the manuscript, and the members of the Wakil laboratory for encouragement and discussion.

REFERENCES

1. Tjian, R., and Maniatis, T. (1994) *Cell* **77,** 5–8
2. Radhakrishnan, I., Perez-Alvarado, G. C., Parker, D., Dyson, H. J., Montminy, M. R., and Wright, P. E. (1997) *Cell* **91,** 741–752
3. Ptashne, M., and Gann, A. (1997) *Nature* **386,** 569–577
4. Triezenberg, S. J. (1995) *Curr. Opin. Genet. Dev.* **5,** 190–196
5. Goodrich, J. A., Hoey, T., Thut, C. J., Admon, A., and Tjian, R. (1993) *Cell* **75,** 519–530
6. Klemm, R. D., Goodrich, J. A., Zhou, S., and Tjian, R. (1995) *Proc. Natl. Acad. Sci. U. S. A.* **92,** 5788–5792
7. Burley, S. K., and Roeder, R. G. (1996) *Annu. Rev. Biochem.* **65,** 769–799
8. Thut, C. J., Chen, J. L., Klemm, R., and Tjian, R. (1995) *Science* **267,** 100–104
9. Lu, H., and Levine, A. J. (1995) *Proc. Natl. Acad. Sci. U. S. A.* **92,** 5154–5158
10. Holstege, F. C., Jennings, E. G., Wyrick, J. J., Lee, T. I., Hengartner, C. J., Green, M. R., Golub, T. R., Lander, E. S., and Young, R. A. (1998) *Cell* **95,** 717–728
11. Moqtaderi, Z., Keaveney, M., and Struhl, K. (1998) *Mol. Cell* **2,** 675–682
12. Michel, B., Komarnitsky, P., and Buratowski, S. (1998) *Mol. Cell* **2,** 663–673
13. Apone, L. M., Virbasius, C. A., Holstege, F. C., Wang, J., Young, R. A., and Green, M. R. (1998) *Mol. Cell* **2,** 653–661
14. Ogryzko, V. V., Kotani, T., Zhang, X., Schlitz, R. L., Howard, T., Yang, X. J., Howard, B. H., Qin, J., and Nakatani, Y. (1998) *Cell* **94,** 35–44
15. Grant, P. A., Schieltz, D., Pray-Grant, M. G., Steger, D. J., Reese, J. C., Yates, J. R., III, and Workman, J. L. (1998) *Cell* **94,** 45–53
16. Hahn, S. (1998) *Cell* **95,** 579–582
17. Uesugi, M., and Verdine, G. L. (1999) *Proc. Natl. Acad. Sci. U. S. A.* **96,** 14801–14806
18. Uesugi, M., Nyanguile, O., Lu, H., Levine, A. J., and Verdine, G. L. (1997) *Science* **277,** 1310–1313
19. Belshaw, P. J., Ho, S. N., Crabtree, G. R., and Schreiber, S. L. (1996) *Proc. Natl. Acad. Sci. U. S. A.* **93,** 4604–4607
20. Tkachuk, D. C., Kohler, S., and Cleary, M. L. (1992) *Cell* **71,** 691–700
21. Gu, Y., Nakamura, T., Alder, H., Prasad, R., Canaani, O., Cimino, G., Croce, C. M., and Canaani, E. (1992) *Cell* **71,** 701–708
22. Prasad, R., Yano, T., Sorio, C., Nakamura, T., Rallapalli, R., Gu, Y., Leshkowitz, D., Croce, C. M., and Canaani, E. (1995) *Proc. Natl. Acad. Sci. U. S. A.* **92,** 12160–12164
23. Akira, S., Isshiki, H., Sugita, T., Tanabe, O., Kinoshita, S., Nishio, Y., Nakajima, T., Hirano, T., and Kishimoto, T. (1990) *EMBO J.* **9,** 1897–1906
24. Trautwein, C., Walker, D. L., Plumpe, J., and Manns, M. P. (1995) *J. Biol. Chem.* **270,** 15130–15136
25. Nerlov, C., and Ziff, E. B. (1995) *EMBO J.* **14,** 4318–4328
26. Williams, S. C., Baer, M., Dillner, A. J., and Johnson, P. F. (1995) *EMBO J.* **14,** 3170–3183
27. Morimoto, R. I. (1998) *Genes Dev.* **12,** 3788–3796
28. Molinari, E., Gilman, M., and Natesan, S. (1999) *EMBO J.* **18,** 6439–6447
29. Newton, E. M., Knauf, U., Green, M., and Kingston, R. E. (1996) *Mol. Cell. Biol.* **16,** 839–846
30. Neve, R., Chang, C. H., Scott, G. K., Wong, A., Friis, R. R., Hynes, N. E., and Benz, C. C. (1998) *FASEB J.* **12,** 1541–50
31. Chang, C. H., Scott, G. K., Kuo, W. L., Xiong, X., Suzdaltseva, Y., Park, J. W., Sayre, P., Erny, K., Collins, C., Gray, J. W., and Benz, C. C. (1997) *Oncogene* **14,** 1617–1622
32. Campbell, A. P., and Sykes, B. D. (1993) *Annu. Rev. Biophys. Biomol. Struct.* **22,** 99–122
33. Frankel, A. D., and Kim, P. S. (1991) *Cell* **65,** 717–719
34. Sauer, F., Hansen, S. K., and Tjian, R. (1995) *Science* **270,** 1783–1788
35. Nguyen, J. T., Turck, C. W., Cohen, F. E., Zuckermann, R. N., and Lim, W. A. (1998) *Science* **282,** 2088–2092
36. Chehab, N. H., Malikzay, A., Stavridi, E. S., and Halazonetis, T. D. (1999) *Proc. Natl. Acad. Sci. U. S. A.* **96,** 13777–13782
37. Banin, S., Moyal, L., Shieh, S., Taya, Y., Anderson, C. W., Chessa, L., Smorodinsky, N. I., Prives, C., Reiss, Y., Shiloh, Y., and Ziv, Y. (1998) *Science* **281,** 1674–1677
38. Canman, C. E., Lim, D. S., Cimprich, K. A., Taya, Y., Tamai, K., Sakaguchi, K., Appella, E., Kastan, M. B., and Siliciano, J. D. (1998) *Science* **281,** 1677–1679
39. Woo, R. A., McLure, K. G., Lees-Miller, S. P., Rancourt, D. E., and Lee, P. W. (1998) *Nature* **394,** 700–704
40. Sakurai, H., Chiba, H., Miyoshi, H., Sugita, T., and Toriumi, W. (1999) *J. Biol. Chem.* **274,** 30353–30356
41. Trautwein, C., Caelles, C., van der Geer, P., Hunter, T., Karin, M., and Chojkier, M. (1993) *Nature* **364,** 544–547