Promoter-specific transcription by RNA polymerase II depends on the general initiation factors TFIIA,2 -B, -D, -E, -F, and -H, which are the minimal set of factors needed for assembly of the preinitiation complex and subsequent initiation of transcription (1–4). The activity of RNA polymerase II and the general transcription factors is controlled by a host of DNA-binding transcription factors and coregulators (1, 8, 9). TBP has also been shown to be an essential component of SL1 and TFIIIB, which are multisubunit general transcription factors for RNA polymerases I and III, respectively (10–16).

Because of its central role in transcriptional regulation, there has been considerable interest in defining the repertoire of TBP-interacting proteins. Biochemical studies that led to definition of TFIIID, SL1, TFIIIB, and other TBP-associated proteins have typically used conventional chromatography and/or immunoaffinity purification methods to purify TBP-containing complexes (17), followed by protein isolation by reverse-phase chromatography or SDS-PAGE and identification by Edman sequencing or mass spectrometry (e.g. Refs. 11 and 18–26). Although these methods were very successful in identifying stoichiometric components of TBP-containing complexes, they are likely to have missed proteins that interact only weakly or transiently with TBP. Here, we report a comprehensive analysis of TBP-interacting proteins using MudPIT (multidimensional protein identification technology) mass spectrometry. MudPIT is a multidimensional chromatography-based proteomics method in which a mixture of proteins is digested into peptides and analyzed by tandem mass spectrometry without prior isolation of individual proteins. MudPIT has proven to be an unbiased and exquisitely sensitive method for identifying proteins in complex mixtures because it does not suffer from the inevitable losses associated with the identification and elution of proteins from polyacrylamide gels or reverse-phase resins.

The results of these experiments identified as TBP-interacting proteins most subunits of the previously known TBP-containing complexes. In addition, we identified a number of proteins not previously identified as TAFs, including several DNA-binding transcription factors. Among these was the DNA-binding transcription factor and orphan nuclear receptor HNF4α (27), which plays an important role in early development and in hepatocyte and intestinal differentiation (28, 29). In the adult mammal, HNF4α is expressed in liver, intestine, and pancreas, where it is responsible for the expression of genes that control glucose and lipid metabolism (30, 31). Mutations in
the human HNF4α gene cause maturity onset diabetes in the young (type 1), a rare form of non-insulin-dependent diabetes mellitus (32). Like other members of the nuclear receptor superfamily, HNF4α possesses a DNA-binding domain with a conserved double zinc finger motif, an N-terminal transactivation domain called AF-1 (activation function 1), and a C-terminal domain called AF-2, which in other nuclear receptors functions as a ligand-dependent activation domain. Although HNF4α AF-2 is structurally homologous to other receptors and several reports have indicated that fatty acids or fatty acyl-CoA associates with HNF4α, no definitive ligand has been identified (33–35).

In this study, we focused on the newly identified interaction between TBP and HNF4α, which we demonstrate is mediated via the HNF4α DNA-binding domain. Through this interaction, HNF4α can target the TFIIID complex to promoters containing HNF4α-binding sites. In vitro, HNF4α recruits TFIIID or TBP to an immobilized template through direct physical interactions between its DNA-binding domain and TBP. An HNF4α mutant that interferes with TBP binding also interferes with recruitment of TBP or TFIIID to promoters in vitro and with HNF4α-dependent gene activation in cells. Taken together, these observations define a novel role for the HNF4α DNA-binding domain in mediating key regulatory interactions and are consistent with the model that the interaction of HNF4α with TBP contributes to HNF4α-induced transcription.

EXPERIMENTAL PROCEDURES

Cell Culture—Parental HeLa S3 cells and their derivatives were maintained in Dulbecco’s modified Eagle’s medium with 5% glucose, 10% fetal bovine serum, 2 mM GlutaMAX, and 100 μg/ml streptomycin (Invitrogen). For large scale cultures, HeLa cells were grown in spinner culture in Joklik medium with 5% calf serum. Full-length cDNAs encoding human TAF7 (GenBank™ accession number BC032737) and mouse HNF4α (GenBank™ accession number BC039220) were subcloned with FLAG tags into pQCXIH (Clontech). Recombinant retroviruses were generated by transfection into Plat E packaging cells (36) and used to infect a HeLa S3 cell line stably expressing the mouse ecotropic retrovirus receptor (mCAT-1) (37).

Antibodies, Affinity Purification, and Immunoprecipitation—Anti-FLAG (M2) and anti-HA (HA-7) antibodies and anti-FLAG (M2)-agarose were from Sigma; anti-TBP, anti-TAF6, and anti-TAF7 monoclonal antibodies were from Abcam; anti-TAF1 and anti-TAF4 monoclonal antibodies and goat anti-HNF4α polyclonal antibody C-19 were obtained from Santa Cruz Biotechnology. Anti-GST monoclonal antibody was obtained from Cyclo Therapeutic. Mouse anti-HA monoclonal antibody 12CA5 was a gift from Michael Carey (Department of Biological Chemistry, UCLA School of Medicine). Anti-HA antibody 12CA5 was bound to protein A-Sepharose (Repligen Corp.). Anti-FLAG and anti-HA affinity purifications were performed essentially as described except that proteins were eluted in buffer containing 0.2 mg/ml FLAG or 3×HA peptide in Buffer A (20 mM HEPES-KOH (pH 7.9), 0.1 mM EDTA, and 20% glycerol) plus 0.05% Triton X-100 (38). Mediator complex was purified as described (39).

Mass Spectrometry—Identification of proteins was accomplished using a modification of the MudPIT procedure (40, 41). Trichloroacetic acid-precipitated proteins were urea-denatured, reduced, alkylated, and digested with endoproteinase Lys-C (Roche Applied Science) followed by modified trypsin (Roche Applied Science) as described (40). Peptide mixtures were loaded onto 100-μm fused silica microcapillary columns packed with 5-μm C18 reverse-phase (Aqua, Phenomenex), strong cation-exchange (PartiSphere SCX, Whatman), and reverse-phase particles (42). Loaded microcapillary columns were placed in-line with an Agilent 1100 series quaternary high pressure liquid chromatography pump and an LTQ ion trap mass spectrometer equipped with a nano-liquid chromatography-electrospray ionization source (Thermo Finnigan). Fully automated 10-step MudPIT runs were carried out on the electrosprayed peptides as described (41). Tandem mass spectra were interpreted using SEQUEST (43) against a data base of 61,427 sequences consisting of 37,742 human proteins (downloaded from NCBI on 03/04/2008), 177 usual contaminants (such as human keratins, IgGs, and proteolytic enzymes), and, to estimate false discovery rates, 30,713 randomized amino acid sequences derived from each non-redundant protein entry. Peptide/spectrum matches were sorted and selected using DTASelect (44) with the following criteria set: peptide/spectrum matches were retained only if they had a DeltaCn of at least 0.08 and minimum XCorr of 1.8 for singly, 2.0 for doubly, and 3.0 for triply charged spectra. In addition, peptides had to be fully tryptic and at least 7 amino acids long. Combining all runs, proteins had to be detected by at least two such peptides or one peptide with two independent spectra. With these criteria, the final false discovery rates at the protein and peptide levels were 0.47 and 0.063 ± 0.026%, respectively. Peptide hits from multiple runs were compared using CONTRAST (44). To estimate relative protein levels, normalized spectral abundance factor values were calculated for each detected protein essentially as described (45–47), with the following exception. To deal with peptides shared between multiple isoforms, distributed normalized spectral abundance factor values were calculated based on distributed spectral counts, in which shared spectral counts are distributed according to the proportion of spectral counts unique to each isoform (48).

Production of Recombinant Proteins in Bacteria—HA-tagged human TBP and FLAG-tagged mouse HNF4α (GenBank™ accession number AAH39220) and derivatives were expressed from pET30 (Novagen, Madison, WI) with an N-terminal His6 tag in Escherichia coli BL21(DE3)-CodonPlus cells (Stratagene). N-terminally GST-tagged human HNF4α and derivatives were expressed in E. coli and purified essentially as described (38).

Solution Binding Assays—Anti-FLAG (M2) beads were pre-equilibrated in binding buffer (150 mM NaCl, 20 mM HEPES-KOH (pH 7.9), 1.5 mM MgCl2, and 0.2% Triton X-100). 2 μg of purified, bacterially expressed His-FLAG-HNF4α or HNF4α derivatives were incubated with 20 μl of anti-FLAG beads for 30 min at 4°C in 400 μl of binding buffer, washed three times, and resuspended in 400 μl of binding buffer. Purified recomb-
nant HA-TBP was added to the beads and incubated for an additional 30 min at 4 °C. After the beads were washed four times with binding buffer, bound proteins were eluted by incubation with 0.2 mg/ml FLAG peptide in binding buffer and subjected to SDS-PAGE and immunoblot analysis with anti-HA and anti-FLAG antibodies. Alternatively, 2 μg of GST-tagged HNF4α or HNF4α derivatives were bound to 20 μl of glutathione-Sepharose 4B beads in 400 μl of binding buffer plus 1 mM dithiothreitol. The beads were washed three times with binding buffer, resuspended in 400 μl of binding buffer, and then incubated with HA-tagged TBP for an additional 30 min at 4 °C. The beads were washed four times, and proteins were eluted with 20 mM glutathione in 100 mM Tris-HCl (pH 7.9), 150 mM NaCl, and 0.1% Triton-X-100 and subjected to SDS-PAGE and immunoblot analysis with anti-HA and anti-GST antibodies.

**Promoter Binding Assays**—Immobilized templates were generated essentially as described (49). To construct pRE×4-E4T and pRE×4-MLT, a double-stranded oligonucleotide containing four tandemly repeated copies of the HNF4α-responsive element from the apolipoprotein A-I enhancer element (TGAAACCCTTGACCCCTGC) (50, 51) was introduced between the PstI and BamHI sites of pG5-E4T and pG5-MLT (52). The BamHI site is located 13 or 27 bp upstream of the TATA boxes of the adenovirus major late (AdML) or E4 core promoter, respectively. Biotinylated fragments of pRE×4-E4T or pRE×4-MLT were amplified using a 27-nucleotide biotinylated primer positioned 204 bp upstream of the HNF4α-binding sites and a primer positioned 260 bp downstream of the transcription initiation site. Biotinylated TATA mutant DNA fragments were generated from pRE×4-E4T by two-step PCR mutagenesis. Conversion of TATAATAA to the NotI recognition site GCGGCCGCG was confirmed by digestion with NotI. Biotinylated PCR products were fractionated on agarose gels, purified using a QIAquick gel extraction kit (Qiagen), and bound to streptavidin-coupled Dynabeads™ M-280 (Dynal). The beads were resuspended in Buffer A to contain ~90 fmol/μl DNA fragment.

Promoter binding assays were performed as follows. Various combinations of proteins were incubated with 2.5 μl of Dynal bead suspension in 62 mM KCl, 12.5% glycerol, 12.5 mM HEPES (pH 7.9), 20 mM Tris-HCl (pH 7.9), 0.06 mM EDTA (pH 8.0), 7.5 mM MgCl₂, 0.5 mg/ml bovine serum albumin, 0.3 mM dithiothreitol, and 0.025% Nonidet P-40 in a total volume of 60 μl. After 30 min at 30 °C, the beads were collected using a magnetic particle concentrator (Dynal), washed three times with the same buffer without bovine serum albumin, and resuspended in SDS-PAGE loading buffer. Bound proteins were identified by Western blotting.

**Luciferase Reporter Assays**—293T cells were cotransfected with 1 μg of pG5-Luc (Promega), which encodes firefly luciferase driven by five Gal4-responsive elements upstream of the AdML core promoter; 100 ng of the control plasmid pRL-tk (Promega), which encodes Renilla luciferase under the control of the thymidine kinase promoter; and varying amounts of effecter plasmid expressing wild-type or mutant Gal4-HNF4α using FuGENE 6 reagent (Roche Applied Science). Total effector plasmid in each transfection was adjusted to 1 μg with empty vector. After 48 h, Gal4-HNF4α transactivation activity was determined by measuring firefly and Renilla luciferase activities using the Dual-Luciferase reporter assay kit (Promega) and normalizing firefly to Renilla luciferase.

**RESULTS**

**MudPIT Analysis of TBP-associated Proteins**—To define TBP-interacting proteins, HA epitope-tagged TBP and associated proteins were subjected to anti-HA immunopurification from a cell line stably expressing HA-TBP and analyzed by MudPIT mass spectrometry. This analysis identified many of the known TBP-associated proteins (supplemental Table 1). Among these were subunits of TBP-containing complexes needed for transcription by polymerases I, II, and III, respectively (8–10, 12–16), including all of the known TFID subunits (TAF11s); the SL1 subunits TAF1A, TAF1B, and TAF1C; and the TFIIIB subunit Brf1. We also detected large amounts of B-TAF1, the mammalian ortholog of Saccharomyces cerevisiae Mot1, an Snf2 family ATPase that uses the energy of ATP to displace TBP from DNA and has been proposed to play a key role in the dynamics of TBP in cells (53–57).

The proteasomal ATPases, but not other subunits of the 19 S regulatory particle of the proteasome, have been identified in previous proteomic analyses as TBP-interacting proteins (25). Consistent with these observations, all of the proteasomal ATPases copurified with our preparations of HA-TBP. In addition, however, we also identified all other subunits of the 19 S regulatory particle except for the human deubiquitinating enzyme UCH37 and human Rpn13 (also known as ADRM1), which links UCH37 to the 19 S regulatory particle (38, 58, 59).

Prior proteomic analyses using TBP as a bait have not led to the identification of sequence-specific DNA-binding transcription factors, most likely because only a small fraction of TFIIID or other TBP-containing complexes are associated with any one of the thousands of these transcription factors in the cell. Consistent with these prior results, we found very few DNA-binding transcription factors in our MudPIT data sets. Surprisingly, however, we did identify four DNA-binding transcription factors as abundant components of our preparations of TBP-associated proteins. These included a homeobox-containing protein (HMBOX1), the glucocorticoid receptor, a steroid-responsive nuclear receptor, and two closely related orphan nuclear receptors (HNF4α and HNF4γ). In the remainder of this work, we explored the functional consequences of the interaction of TBP with HNF4α.

**HNF4α Binds TFIIID and TBP**—To confirm the interaction of HNF4α with TBP, nuclear extracts from HeLa cells stably expressing HA-tagged TBP were subjected to immunoprecipitation with anti-HA or control antibodies, and TBP and HNF4α were detected by Western blotting. As shown in Fig. 1A, endogenous HNF4α specifically coprecipitated with HA-TBP.

In complementary experiments, we used anti-FLAG antibodies to immunoprecipitate proteins from nuclear extracts from parental HeLa cells or a HeLa cell line stably expressing FLAG-tagged HNF4α. As shown in the Western blots of Fig. 1B, TBP and the TFIIID subunits TAF1, TAF4, TAF6, and TAF7 all specifically coprecipitated with FLAG-HNF4α, indicating that HNF4α binds endogenous TBP and the TFIIID complex.
Transactivation domain. Regions C and D include the zinc finger and hinge regions (60), which together make up the HNF4α DNA-binding domain (61). Region E contains the ligand-binding, dimerization, and ligand-dependent AF-2 transactivation domains, whereas region F is a C-terminal negative regulatory domain that appears to modulate HNF4α interaction with coregulators and/or fatty acid ligands (Fig. 2A) (62).

To identify the region of HNF4α responsible for TBP binding, we performed in vitro binding assays with recombinant wild-type FLAG-HNF4α or a series of FLAG-tagged HNF4α deletion mutants (Fig. 2B). In these assays, the binding of HNF4α deletion mutants either lacking the AF-1 transactivation domain (HNF4α CDEF) or lacking both AF-1 and C-terminal regulatory domains (HNF4α CDE) to TBP was indistinguishable from that of full-length HNF4α, whereas a mutant also lacking the zinc fingers (HNF4α DE) did not bind well (Fig. 2B). To define further the region of HNF4α needed for TBP binding, we produced a series of N- or C-terminal deletion mutants of recombinant GST-tagged HNF4α and assayed them for their abilities to bind TBP. GST-HNF4α ABCD, which lacks region F and the ligand-binding, AF-2, and dimerization domains, bound TBP as well as did full-length HNF4α; however, a mutant lacking most of region D (HNF4α ABC-(1–115)) exhibited substantially reduced TBP binding, and the TBP-binding activity of a mutant including only regions A–C (residues 1–108) was barely detectable. Finally, HNF4α CD-(42–165) was sufficient for binding to TBP (Fig. 2B). Thus, residues 42–165, which fall within the DNA-binding domain, contribute to TBP interaction, whereas residues 108–115 are particularly important. Residues 108–115 correspond to conserved α-helix III, which is immediately C-terminal to the second zinc finger of the DNA-binding domain (61). Notably, the DNA-binding domains are almost identical in HNF4α and HNF4γ, which we also identified as a TBP-interacting protein in our MudPIT analyses, whereas the AF-1 regions of HNF4α and HNF4γ are highly divergent.

We next tested the TBP-binding activity of a series of HNF4α mutants in which alanine was substituted for charged residues or serines falling within the region important for TBP interaction. Consistent with our observation that sequences within helix III are particularly important for TBP binding, we observed that mutation of Lys109 and Lys110 to Ala or of Arg104, Arg107, Lys109, and Lys110 to Ala interfered with TBP binding, whereas mutation of residues outside this helix had little or no effect (Fig. 2C).

**HNF4α Recruits TBP and TFIID to Promoters in Vitro**—Our observation that HNF4α binds directly to TBP and TFIID raised the possibility that HNF4α might activate transcription in part by stabilizing the binding of TBP or TFIID to promoters. We therefore asked whether HNF4α could stimulate binding of TBP or TFIID to bead-bound DNA fragments containing four HNF4α-responsive elements upstream of the core promoters of the AdML (pRE×4-MLT) or adenovirus E4 (pRE×4-E4T) gene (Fig. 3A). Although TBP alone can bind both core promoters (Ref. 9 and data not shown), we observed that binding of recombinant TBP to both templates was strongly stimulated by recombinant HNF4α when binding assays were performed with limiting concentrations of TBP (Fig. 3B). This stimulation was dependent on sequence-specific interaction of both HNF4α and TBP with their cognate binding sites because mutation of the TATA box sequence or deletion of the HNF4α-
responsive elements resulted in a dramatic decrease in HNF4α-dependent TBP recruitment to immobilized DNA (Fig. 3).

To determine whether HNF4α can also recruit TFIIID to a promoter, we performed binding assays with HNF4α and TFIIID that had been purified by FLAG immunoprecipitation from a HeLa cell line stably expressing FLAG-tagged TAF7. The binding of TFIIID subunits TAF1 and TAF6 and TBP to both the adenovirus E4 and AdML core promoters was increased in the presence of HNF4α (Fig. 3).

**Mutation of the HNF4α DNA-binding Domain Interferes with TBP or TFIIID Recruitment by a Gal4-HNF4α Fusion Protein—** We next wished to determine whether mutations that interfere with the ability of HNF4α to bind directly to TBP interfere with its ability to recruit TBP and TFIIID to a promoter. Because these mutations fall in a region immediately adjacent to the zinc finger portion of the DNA-binding domain, we expected that they might also interfere with HNF4α DNA-binding activity. In preliminary experiments, we found that this was indeed the case (data not shown). For this reason, we generated Gal4-HNF4α fusion proteins in which the Gal4 DNA-binding domain was fused either to full-length wild-type HNF4α or to the TBP binding-defective HNF4α mutant in which Arg104, Arg107, Lys109, and Lys110 were changed to Ala. We then tested their abilities to recruit TBP or TFIIID to an immobilized DNA fragment bearing Gal4 DNA-binding sites upstream of the AdML core promoter (Gal4-5-MLT) (Fig. 4A). As shown in Fig. 4 (B and C), we observed that wild-type Gal4-HNF4α increased TBP or TFIIID recruitment to the promoter, whereas a Gal4 fusion protein containing an HNF4α mutant defective in TBP binding did so less effectively, consistent with the model that direct binding of HNF4α to TBP contributes to recruitment of TBP or TFIIID to the promoter. We noted that, in some experiments, the mutation appeared to have a smaller effect on recruitment of TAF6 than TBP (Fig. 4C). Whether this reflects additional interaction(s) between one or more of the TAF sub-units with HNF4α remains to be determined.
**Physical and Functional Link between HNF4α and TFIID**

**FIGURE 3. HNF4α recruits TBP and TFIID to promoters.** A, left panel, shown are the immobilized templates used in the binding assays. HNF4α–RE4, four tandemly repeated HNF4α-responsive elements; TATA, TATA box; GCCG, TATA box mutated to GC-rich NotI site; E4T, adenovirus E4 core promoter; MLT, AdML core promoter; mut, mutant. Right panel, loading of biotinylated DNA onto streptavidin beads was assessed by analysis of DNA released from beads by BamHI digestion. B, 320 ng of FLAG-HNF4α (F-HNF4α) were prebound to pRE4×4-E4T or pRE×4-MLT on beads and incubated with 20 ng of HA-TBP. Bound proteins were analyzed by immunoblotting with anti-FLAG or anti-HA antibodies. 

**FIGURE 4. TFIID (TBP) recruitment and transactivation activities of Gal4-HNF4α fusion proteins.** A, shown is a schematic diagram of the Gal4–5-MLT promoter. B–D, 200 ng of wild-type (WT) or mutant (mut) Gal4–HNF4α (Gal4-HNF4α) were prebound to immobilized Gal4–5-MLT and incubated with HA-TBP (B), TFIID (C), or the Mediator complex (D), and bound proteins were analyzed by Western blotting (WB). GAL4–RE4×5, five tandemly repeated Gal4-responsive elements. E, the reporter plasmid p65-Luc, in which luciferase expression is driven by five Gal4-responsive elements upstream of the AdML core promoter, was transiently transfected into 293T cells with the control plasmid pRL-tk (which encodes Renilla luciferase under the control of the thymidine kinase promoter) and 1 μg of plasmid encoding Gal4-HNF4α (Gal4-HNF4α WT), 125 ng (1x) or 250 ng (2x) of plasmid encoding Gal4-HNF4α(R104A/R107A/K109A/K110A) (Gal4-HNF4α (mut)), or empty vector (mock). Left panel, expression of Gal4-HNF4α fusion proteins in transfected cells. Right panel, average of the relative luciferase activities obtained in three independent transfection experiments; error bars represent S.D. IB, immunoblot; RLU, relative units. Med6, Mediator subunit 6.

Mutation of the HNF4α DNA-binding Domain Interferes with Gene Activation by a Gal4-HNF4α Fusion Protein —To test the possible contribution of the HNF4α-TBP interaction to HNF4α transactivation activity in cells, we tested the ability of the Gal4–HNF4α fusion proteins to stimulate luciferase expression driven by five Gal4-responsive elements upstream of the AdML promoter. As expected because both include intact AF-1 and AF-2 transactivation domains, we observed that Gal4–HNF4α fusion proteins containing both wild-type and mutant versions of HNF4α could stimulate reporter expression. Importantly, however, the fusion protein containing wild-type HNF4α had about four times greater transactivation activity compared with the mutant (Fig. 4E).

Activation of transcription by HNF4α has been shown previously to depend strongly on an intermediary coregulator referred to as the Mediator complex. Although substantial evidence argues that Mediator functions via interactions with AF-2 (63, 64), we wished to rule out the possibility that DNA-binding region mutations that interfere with interactions of HNF4α with TBP also affect its interactions with the Mediator complex. Accordingly, we asked whether the Gal4–HNF4α mutant was defective in its ability to recruit Mediator to a promoter in vitro. As shown in Fig. 4D, Gal4–HNF4α fusion proteins containing wild-type and mutant HNF4α recruited the Mediator complex to the promoter equally well. Taken together, these results are consistent with the model that HNF4α activates transcription in part via interaction of its DNA-binding region with TBP.

**DISCUSSION**

Activation of transcription in vitro by HNF4α has been shown to depend strongly on intermediary coactivators
referred to as PC4 (positive cofactor 4) and the Mediator complex, which function via interactions with the ligand-dependent activation domain AF-2 (63, 64). The results of a recent study indicate that HNF4α can also stimulate basal transcription via a Mediator-independent mechanism in a highly purified, reconstituted transcription system containing RNA polymerase II, general transcription factors, and PC4 (65), a finding we have confirmed in our laboratory (supplemental Fig. 1). However, the mechanism by which it does so has remained obscure. Here, we have presented evidence that supports a possible mechanism for Mediator-independent stimulation of basal transcription by HNF4α. Our findings indicate that HNF4α binds directly to TBP via its DNA-binding domain and, through this interaction, can recruit TBP or the larger TFIIID complex to HNF4α-responsive promoters in vitro. We observed that a mutation in the HNF4α DNA-binding domain that blocks recruitment of TBP or TFIIID but does not affect interaction with Mediator also blocks HNF4α-dependent transactivation activity in cells. These observations are consistent with the model that HNF4α-dependent recruitment of TBP or TFIIID contributes to transcription activation.

Our observation that the HNF4α DNA-binding domain interacts with TBP was unexpected in light of previous evidence that TBP can interact with several nuclear receptors through the well-characterized AF-1 or AF-2 activation domains (66). The AF-1 domains of both the glucocorticoid and estrogen receptors have been shown to bind TBP (67, 68). In contrast, retinoid X receptor α is capable of interacting directly with TBP through its ligand-dependent AF-2 transactivation domain. Mutation of a highly conserved lysine residue in retinoid X receptor α AF-2 to glutamic acid reduces its ability to bind TBP and to activate a reporter gene (69). Although the AF-2 domain of HNF4α is similar in amino acid sequence to that of retinoid X receptor α (33), we observed that deletion of AF-2 or mutation of the corresponding glutamic acid residue (data not shown) had no effect on the HNF4α-TBP interaction. Thus, HNF4α interacts with TBP by a different mechanism compared with other nuclear receptors. While this work was in progress, Lu et al. (61) reported the crystal structure of the HNF4α DNA-binding domain bound to DNA. According to this structure, lysines 109 and 110 are solvent-exposed and therefore would be available for interaction with TBP. In addition, PRMT1 has been reported to interact with the DNA-binding domain of HNF4α and regulate the affinity of HNF4α for its binding site (70). These studies and our data strongly suggest that the HNF4α DNA-binding domain can provide surfaces for binding not only DNA but also other transcriptional regulatory proteins.

In the TFIIID complex, TBP directly contacts other TFIIID subunits, including TAF1, TAF2, TAF11, TAF12, and TAF13 (71–73), whereas the other TAFs are thought to associate with TBP indirectly. Furthermore, in the preinitiation complex, TBP also binds directly to DNA as well as the general transcription factors TFIIA and TFIIIB (74–77). In light of all these interactions, it is perhaps surprising that there is room on the TBP surface for functional interactions with HNF4α. Nevertheless, structural studies indicate that TBP resides not in the middle but rather at the surface of both TFIIID and larger transcription intermediates, including the TFIIA-TFIIID-TFIIIB complex (78–80). Thus, in the preinitiation complex, surface(s) of TBP likely remain available for interaction with the HNF4α DNA-binding domain as well as other DNA-binding transcription factors and coregulators.

Our observation of a physical and functional link between HNF4α and TFIIID is intriguing in light of a recent study indicating that TFIIID is required for initial activation of HNF4α-dependent transcription during proliferation or differentiation of hepatoblasts but is dispensable for maintenance of transcription in adult hepatocytes (1, 81). Thus, it is possible that the HNF4α-TFIIID interaction we have defined makes its greatest contribution to initial activation of transcription in proliferating cells, such as hepatoblasts or the proliferating tissue culture cells used in our studies, whereas mechanisms involving Mediator and other coregulators are sufficient to maintain transcription in non-proliferating adult hepatocytes.

In conclusion, HNF4α and other nuclear receptors have been shown previously to function through interactions between their AF-1 or AF-2 activation domains and various general transcription factors or coregulators. Our evidence that interactions of the HNF4α DNA-binding domain with TBP can direct recruitment of TFIIID to promoters adds yet more complexity to the network of interactions by which this orphan nuclear receptor activates transcription.

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