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THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 275, No. 14, Issue of April 7, pp. 10064–10071, 2000

Printed in U.S.A.

The Human Transcription Factor IID Subunit Human TATA-binding Protein-associated Factor 28 Interacts in a Ligand-reversible Manner with the Vitamin D_3 and Thyroid Hormone Receptors*

(Received for publication, September 28, 1999, and in revised form, January 5, 2000)

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Using coexpression in COS cells, we have identified novel interactions between the human TATA-binding protein-associated factor 28 (hTAF$_{II}$28) component of transcription factor IID and the ligand binding domains (LBDs) of the nuclear receptors for vitamin D3 (VDR) and thyroid hormone (TRα). Interaction between hTAF$_{II}$28 and the VDR and TR LBDs was ligand-reversible, whereas no interactions between hTAF$_{II}$28 and the retinoid X receptors (RXRs) or other receptors were observed. TAF$_{II}$28 interacted with two regions of the VDR, a 40-amino acid region spanning α-helices H3–H5 and α-helix H8. Interactions were also observed with the H3–H5 region of the TRα but not with the equivalent highly related region of the RXRγ. Fine mapping using RXR derivatives in which single amino acids of the RXRγ LBD have been replaced with their VDR counterparts shows that the determinants for interaction with hTAF$_{II}$28 are located in α-helix H3 and are not identical to those previously identified for interactions with hTAF$_{II}$55. We also describe a mutation in the H3–H5 region of the VDR LBD, which abolishes transactivation, and we show that interaction of hTAF$_{II}$28 with this mutant is no longer ligand-reversible.

Transcription factor IID (TFIID)[1] is one of the general factors required for accurate and regulated initiation by RNA polymerase II. TFIID comprises the TATA-binding protein (TBP) and TBP-associated factors (TAF$_{II}$s; Refs. 1–5). A subset of TAF$_{II}$s are present not only in TFIID but also in the complexes (Refs. 6–10; for review, see Refs. 11–14).

TAF$_{II}$ function has been studied genetically in yeast and by transfection experiments in mammalian cells. In yeast, a variable requirement for TAF$_{II}$s has been found. Temperature sensitive mutations in yeast TAF$_{II}$145 (yTAF$_{II}$145) result in cell cycle arrest and lethality, but the expression of only a small number of genes is affected (15). In contrast, tight temperature-sensitive mutations in yTAF$_{II}$17, yTAF$_{II}$25, yTAF$_{II}$60, yTAF$_{II}$61/68, and the TFIID-specific yTAF$_{II}$40 strongly affect the transcription of the majority of yeast genes (16–21).

An increasing body of results also shows that human TAF$_{II}$28 (hTAF$_{II}$28), hTAF$_{II}$135, and hTAF$_{II}$105 can act as specific transcriptional coactivators in mammalian cells. For example, distinct domains of hTAF$_{II}$135 interact specifically with Sp1, cAMP response element-binding protein, and E1A, and coexpression of these hTAF$_{II}$135 derivatives has a dominant negative effect on the activity of these activators (22–25), whereas coexpression of hTAF$_{II}$135 strongly potentiates transcriptional activation by several nuclear receptors (26). Similarly, hTAF$_{II}$105 interacts specifically with the p65 subunit of nuclear factor-κB, and TAF$_{II}$105 expression potentiates activation by nuclear factor-κB in mammalian cells (27).

The viral protein Tax interacts directly with hTAF$_{II}$28, and coexpression of hTAF$_{II}$28 strongly potentiates activation by Tax (28). Expression of hTAF$_{II}$28 also potentiates activation by the ligand-dependent activation function-2 of the nuclear receptors (NRs) for vitamin D_3 (VDR), 9-cis retinoic acid (RXR), and estrogen (29, 30). Coexpression of TBP and hTAF$_{II}$28 shows that they act synergistically to potentiate activation by the VDR or estrogen receptor. This synergism requires specific amino acids of the hTAF$_{II}$28 histone fold domain located in the conserved C-terminal half of the protein and can also be abolished by a mutation in the H1′ helix of TBP (31, 32).

Despite its ability to act as a transcriptional coactivator for several NRs, we previously reported that no ligand-dependent interactions with the RXR could be observed (29). In this study, we show that hTAF$_{II}$28 does selectively interact with the VDR and thyroid hormone receptor (TRα) but does so in a ligand-reversible manner. Analysis of hTAF$_{II}$ interaction with VDR deletion mutants shows that hTAF$_{II}$28 interacts with a 40-amino acid region spanning α-helices H3–H5 and containing the NR signature and previously shown also to interact with hTAF$_{II}$55 (33). Interaction was also seen with the H3–H5 region of the TRα but not with the analogous highly related region of the RXRγ. Substituting single amino acids of the RXRγ H3–H5 region with their counterparts from the VDR induced interactions with hTAF$_{II}$28, but comparison with the results previously obtained with hTAF$_{II}$55 indicates that the determinants for the interaction of these TAF$_{II}$s with this re-

* This work was supported in part by grants from the CNRS, INSERM, the Hôpital Universitaire de Strasbourg, the Ministère de la Recherche et de la Technologie, the Association pour la Recherche contre le Cancer, the Ligue Nationale contre le Cancer, and the Human Frontier Science Program. 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.

‡ Supported by fellowships from the Ligue Nationale contre le Cancer and the Association pour la Recherche contre le Cancer.

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[1] The abbreviations used are: TFIID, transcription factor IID; TBP, TATA-binding protein; TAF$_{II}$, TATA-binding protein-associated factor; yTAF$_{II}$, yeast TAF$_{II}$; hTAF$_{II}$, human TAF$_{II}$; NR, nuclear receptor; VDR, vitamin D_3 receptor; RXR, retinoid X receptor; TRα, thyroid hormone receptor; CAT, chloramphenicol acetyltransferase; mAb, monoclonal antibody; LBD, ligand binding domain; G4, GAL4.

gion are not identical. We also show that exchange of amino acids in the H3–H5 region between the VDR and RXR leads to a loss of activity in the case of the VDR and a gain of activity in the case of the RXR.

MATERIALS AND METHODS

Construction of Recombinant Plasmids—The hTAF$_{II}$ expression vectors are as described previously (29, 33–35). The mouse RXR, mouse retinoic acid receptor, Sp1, and AP-2 expression vectors are also as described (26, 29, 33, 36, 37). All the G4-VDR, TRα, and RXR chimeras were constructed by PCR using the appropriately designed oligonucleotides with restriction sites and cloned into the vector pXJ440 encoding the DNA binding domain of the yeast activator GAL4 (38). All plasmids were verified by automated DNA sequencing. Further details of constructions are available on request.

Transfection of COS Cells and Immunoprecipitations—COS cells were transfected by the calcium phosphate coprecipitation technique, and immunoprecipitations were performed as described previously (33–35). 48 h after transfection the cells were harvested by three cycles of freeze-thaw in buffer A (50 mM Tris-HCl, pH 7.9, 20% glycerol,. 1 mM dithiothreitol, and 0.1% Nonidet P-40) containing 0,5 M KCl. The expression of the transfected proteins was verified on Western blots. For immunoprecipitations cell extracts were incubated for 1 h at 4 °C with 1–2 μg of the indicated monoclonal antibodies after which time 50 μl of protein G-Sepharose was added, and incubation was continued for another 2 h. After extensive washing the precipitated proteins were detected by immunoblotting with the indicated antibodies using an ECL kit (Amersham Pharmacia Biotech). Where indicated, ligands were added (50 nM all-*trans*-retinoic acid, 9-*cis*-retinoic acid, and 3,5,3′-triiodo-L-thyronine and 100 nM 1,25-dihydroxyvitamin D$_3$) at the same time as the DNA-calcium phosphate coprecipitate. For chloramphenicol acetyltransferase (CAT) assays, 3 μg of the 17 m5-TATA-CAT reporter plasmid was cotransfected with 2 μg of a β-galactosidase reporter as an internal control, along with the indicated concentrations of the G4-RXRγ expression vectors. After correction for transfection efficiency using β-galactosidase assays, CAT assays were performed by standard protocols, and the percentage of acetylated chloramphenicol was determined by quantitative phosporimager analysis on a Fujix BAS 2000 apparatus.

Antibody Preparation—Monoclonal antibodies (mAbs) against hTAF$_{II}$18 (16TA), hTAF$_{II}$20 (22TA), hTAF$_{II}$28 (15TA and 1C9) the B10 epitope, and the G4 DBD (3GV2) were as described previously (29, 34, 35, 39, 40).

RESULTS

Selective Ligand-reversible Interactions between hTAF$_{II}$28 and the Ligand Binding Domains of the VDR and TR—We have previously reported that although expression of hTAF$_{II}$28 strongly potentiates activation by several NR activation function-2s, no ligand-dependent interactions with NRs could be observed that would account for its coactivator activity (29). In the course of these experiments we did, however, observe two hTAF$_{II}$28-NR interactions. These interactions were seen when vectors expressing chimeras comprising the VDR or TRα ligand binding domains (LBDs) (or full-length VDR) fused to the DNA binding domain of the yeast activator GAL4 (GAL4 (1–147), G4) were cotransfected into COS cells along with vectors expressing native or B10-tagged hTAF$_{II}$28 (the expression vectors are schematized in Fig. 1).

When coexpressed with the G4 DNA binding domain (G4 (1–147)), B10-hTAF$_{II}$28 was precipitated only by mAb B10, and G4 (1–147) was precipitated only by the anti-G4 antibody (Fig. 2*A, lanes 1–3*). However, when coexpressed with B10-hTAF$_{II}$28, G4-VDR was coimmunoprecipitated by mAb B10 (Fig. 2, *A, lanes 4* and *5*, and *B, lanes 1* and *2*). Most strikingly, G4-VDR could be coprecipitated with B10-hTAF$_{II}$28 when the transfection was performed in the absence of ligand, whereas no detectable G4-VDR was coprecipitated in the presence of ligand (Fig. 2*B*, compare *lanes 1* and *2* and *3* and *4*). Thus, hTAF$_{II}$28 interacts in a ligand-reversible manner with the VDR in transfected COS cells.

A series of deletion mutants (34) was used to determine the region of hTAF$_{II}$28 required for interaction with the VDR. G4-VDR coprecipitated with wild-type hTAF$_{II}$28 (1–211) and

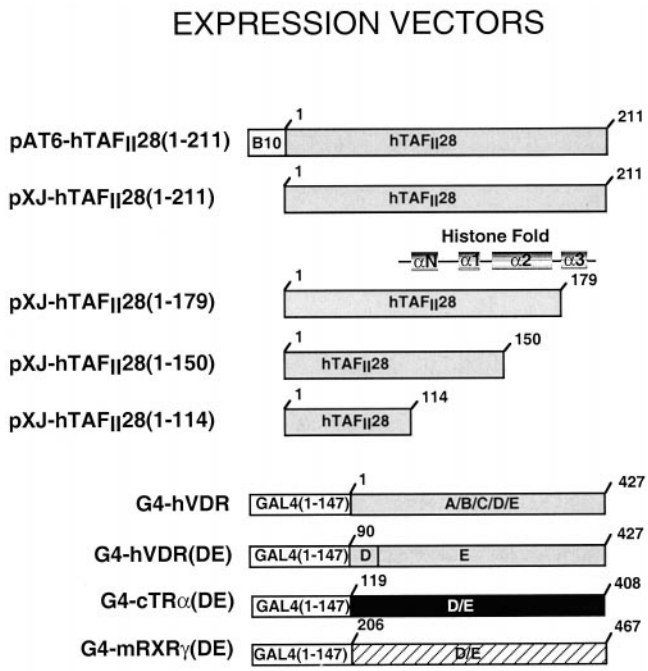

FIG. 1. **Structure of the hTAF$_{II}$s and nuclear expression vectors.** The pAT6 vectors contain the epitope for mAb B10 at the N terminus. The amino acid coordinates of the N- and C-terminal boundaries in each construct are shown. The locations of the αN-α3 helices of the hTAF$_{II}$28 histone fold are indicated. All the NR expression vectors are cloned in the pXJ440 vector in which the NR sequences are fused to the GAL4 DNA binding domain (G4 (1–147)). *h*, human; *m*, mouse; *c*, chicken.

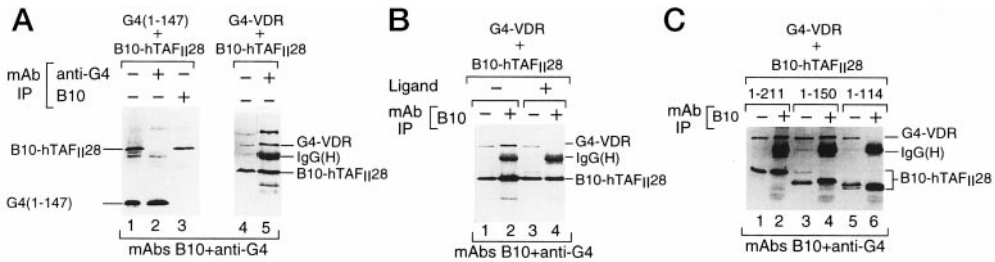

FIG. 2. **Ligand-reversible interaction between hTAF$_{II}$28 and the VDR LBD.** The transfected expression vectors are shown above each panel along with the antibodies used in the immunoprecipitations (*IP*) and in *B* the presence or absence of ligand. The antibodies used to reveal each blot are indicated at the *bottom*. *Lanes* where no antibody was used in the immunoprecipitation (*e.g. A*, *lanes 1* and *4*) show aliquots (10 μl) of the transfected cell extracts used for the immunoprecipitations shown in the adjacent lanes. The locations of the relevant proteins are shown along with the location of the heavy chain of the immunoprecipitating antibody (*IgG(H)*) revealed upon incubation with the peroxidase-conjugated secondary antibody.

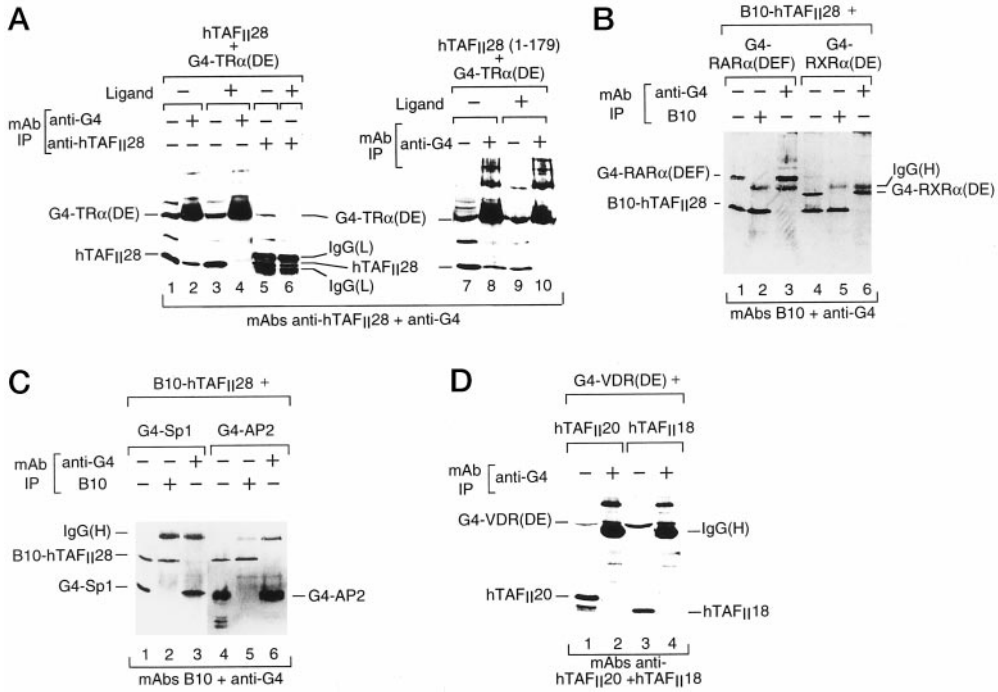

FIG. 3. *A*, ligand-reversible interactions of hTAFII28 with the TR. The layout is as described in Fig. 2. Because the coprecipitated G4-TRα(DE) is masked by the IgG(H), *lanes 5* and *6* have been revealed with peroxidase-conjugated secondary antibody directed against the light chain (*IgG(L)*). TAF$_{II}$28 can be seen between the two signals generated in this region of the blot using this conjugated antibody. In subsequent figures either the light chain or the heavy chain of the immunoprecipitating antibody is indicated, depending on which peroxidase-conjugated secondary antibody was used. *B* and *C,* no interactions between hTAF$_{II}$28 and the retinoic acid receptor RXR Sp1 or AP-2 activation domains. *D,* no interactions between the VDR LBD and hTAF$_{II}$18 and hTAF$_{II}$20. The layout is as described for the other figures.

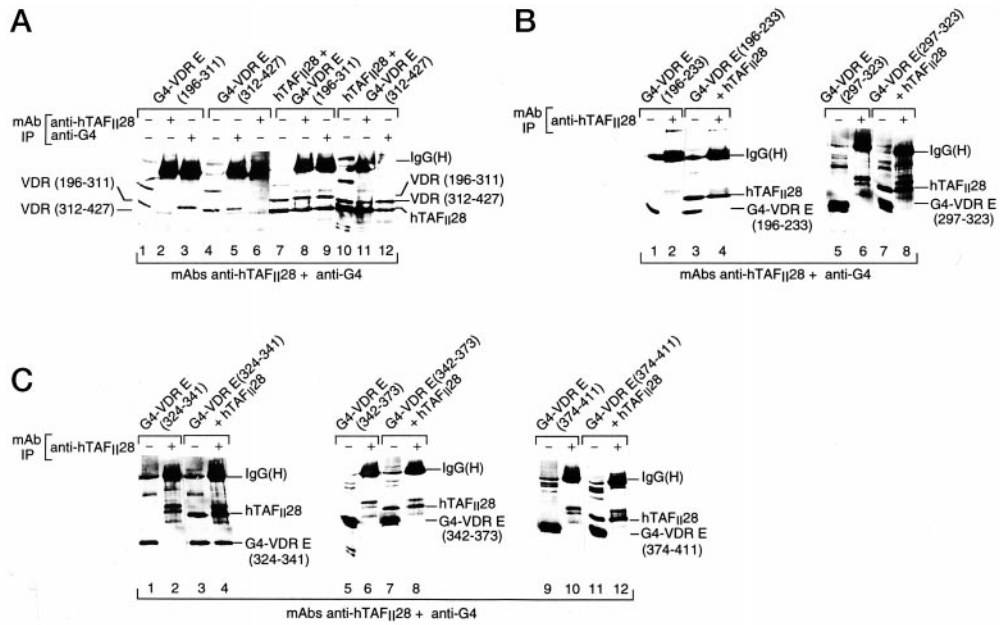

FIG. 4. **Mapping of the hTAF$_{II}$28 interaction domains of the VDR LBD.** The deletion constructs used are indicated above each *lane*. The *lanes* without cotransfected hTAF$_{II}$28 (*e.g. A, lanes 1–6,* and *B, lanes 1* and *2*) are control immunoprecipitations showing that the subsequent coprecipitation is hTAF$_{II}$28-dependent.

with the C-terminal deletion mutant (1–150) (Fig. 2*C, lanes 1–4*). In contrast, G4-VDR was not coprecipitated with hTAF$_{II}$28 (1–114) (Fig. 2*C, lanes 5* and *6*). Therefore, a region within or overlapping with amino acids 114–150, which encode the N-terminal portion of the hTAF$_{II}$28 histone fold, is required for interaction with the VDR.

The ability of hTAF$_{II}$28 to interact with other NRs was investigated. As observed with the VDR, hTAF$_{II}$28 could be coprecipitated by the anti-G4 mAbs in the presence of coexpressed G4-TRα(DE), and conversely G4-TRα(DE) was coprecipitated by the anti-hTAF$_{II}$28 antibodies (Fig. 3*A, lanes 1, 2,* and *5*). A similar result was observed with the C-terminal deletion mutant hTAF$_{II}$28 (1–179) (Fig. 3*A, lanes 7* and *8*). Strikingly, as observed with the VDR, this interaction is destabilised in the presence of ligand (Fig. 3*A*, compare *lanes 2, 4, 5, 6, 8,* and *10*). Thus, the presence of ligand dramatically reduces

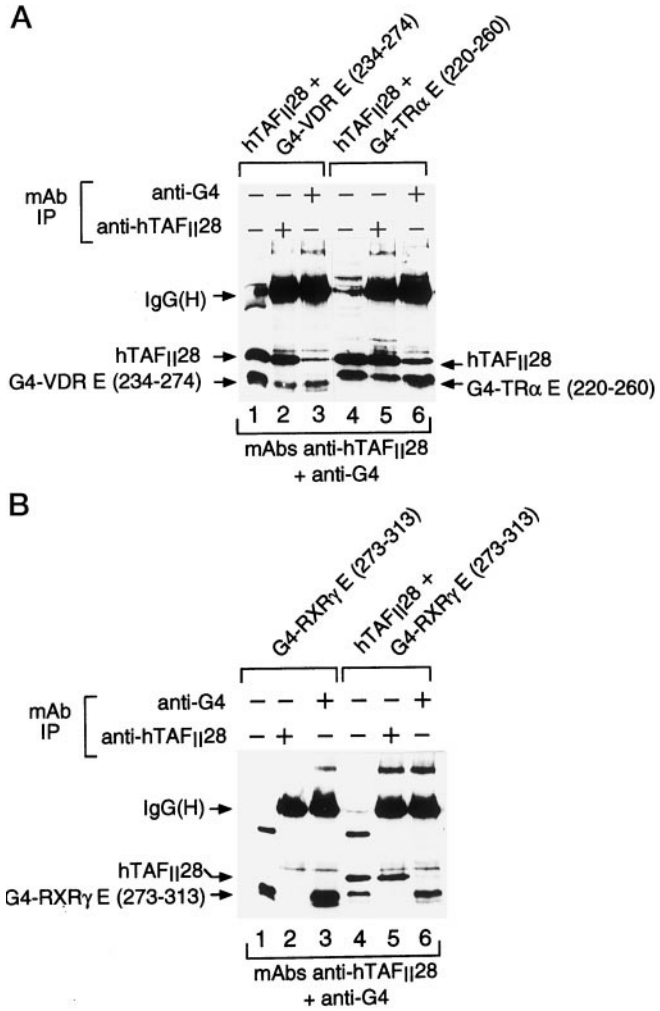

FIG. 5. **Selective interaction of hTAF$_{II}$28 with the H3–H5 regions of the VDR and TR.** The layout is as described in the previous figures.

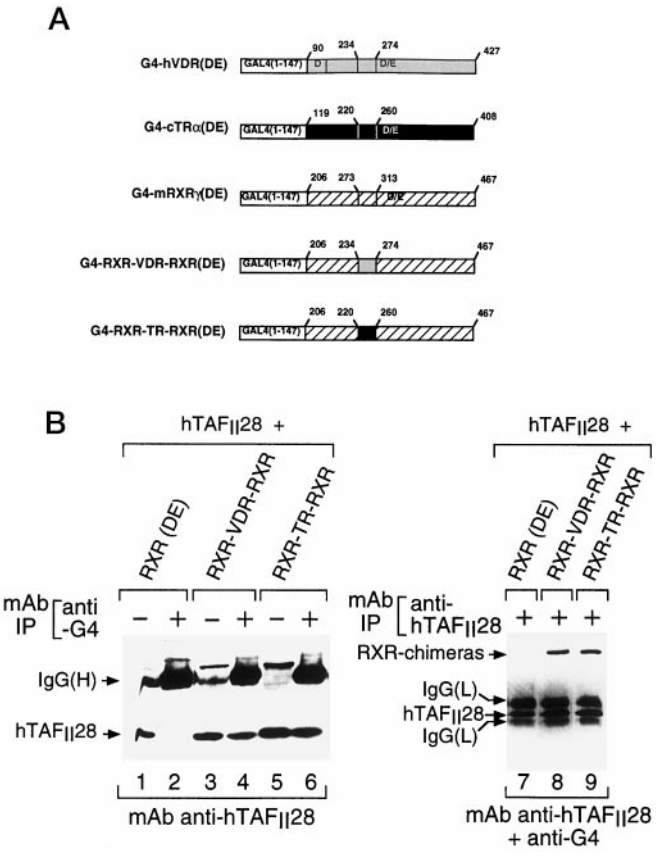

FIG. 6. *A*, schematic structure of the chimeric RXRγ LBDs. *B*, immunoprecipitations using the RXR LBD chimeras. The peroxidase-conjugated antibody against IgG(L) is used in *lanes 7–9*.

the interactions between hTAF$_{II}$28 and the TRα and the VDR LBDs in transfected COS cells.

In contrast to the above, no interactions were observed with G4-RXRα(DE) or G4-retinoic acid receptor-α(DEF) in either the absence or presence of ligand (see Fig. 3*B*, *lanes 1–6*; Ref. 29; data not shown) or with several activators that do not belong to the NR superfamily (see Fig. 3*C*, *G4-Sp1* and *G4-AP2*).

As we have previously observed interactions of hTAF$_{II}$55 with the VDR and TR (33), we also verified the specificity of these interactions with respect to the TAF$_{II}$s. For this, the VDR was cotransfected with hTAF$_{II}$20 or hTAF$_{II}$18. In contrast to hTAF$_{II}$28 and hTAF$_{II}$55, no significant interactions were observed between the VDR and hTAF$_{II}$20 or hTAF$_{II}$18 (Fig. 3*D*).

The region of the VDR required for interaction with hTAF$_{II}$28 was next determined using the previously described series of G4-VDR LBD deletion mutants (33). G4-VDR DE (90–195) was not coprecipitated with B10-hTAF$_{II}$28 (data not shown). In contrast, both the N-terminal half of the E region present in G4-VDR E (196–311) (Fig. 4*A, lanes 1–3* and *7–9*) and the C-terminal half of the E region in G4-VDR E (312–427) (Fig. 4*A, lanes 4–6* and *10–12*) were coprecipitated with hTAF$_{II}$28. Therefore, hTAF$_{II}$28 interacts with two distinct sites in the VDR E domain but not with the VDR D domain.

The hTAF$_{II}$28 interaction regions in the N-, and C-terminal moieties of the VDR LDB were more precisely mapped. After cotransfection, no interaction was observed between hTAF$_{II}$28 and G4-VDR E (196–233), (275–311), (297–323), (342–373), or (374–411) (Fig. 4, *B, lanes 1–8,* and *C, lanes 5–8* and *9–12*; data not shown). In contrast, hTAF$_{II}$28 interacted selectively with G4-VDR E (234–274) (Fig. 5*A, lanes 1–3*) and with G4-VDR E (324–341) (Fig. 4*C, lanes 1–4*). Thus, hTAF$_{II}$28 interacts with the helix H3–H5 NR signature-containing region and with the helix H8 region of the VDR.

Selective Interaction of hTAF$_{II}$28 with the H3–H5 NR Signature-containing Regions of the VDR and TRα—The H3–H5 region of the NRs contains the NR signature, which is a group of well conserved amino acids involved in intramolecular interactions required for stabilization of the LBD fold (41, 42). As a consequence the H3–H5 region is one the most highly conserved regions within the NR LBDs. Based on the above results, we next investigated hTAF$_{II}$28 interactions with the related H3–H5 regions of the chicken TRα and the mouse RXRγ. G4 chimeras containing these H3–H5 regions (G4-TR E (220–260) and G4-RXRγ E (273–313)) were coexpressed along with hTAF$_{II}$28. G4-TRα E (220–260) was specifically precipitated along with hTAF$_{II}$28 (Fig. 5*A, lanes 4–6*). In contrast, no coprecipitation of G4-RXRγ E (273–313) was observed (Fig. 5*B*). The selective interaction of hTAF$_{II}$28 with the H3–H5 region of the VDR and TRα, but not the RXRγ, therefore mimics the specificity seen using the complete LBDs of these NRs.

The above result was confirmed using chimeric RXRγ(DE)s in which amino acids 273–313 of RXRγ containing the H3–H5 region have been replaced by the equivalent amino acids of the VDR or TRα E domains (G4-RXR-VDR-RXR and G4-RXR-TR-RXR; see Fig. 6*A*). As seen above, no coprecipitation of hTAF$_{II}$28 with G4-RXRγ(DE) was observed (Fig. 6*B, lanes 1, 2*, and *7*). In contrast, the RXR-VDR-RXR and RXR-TR-RXR chi-

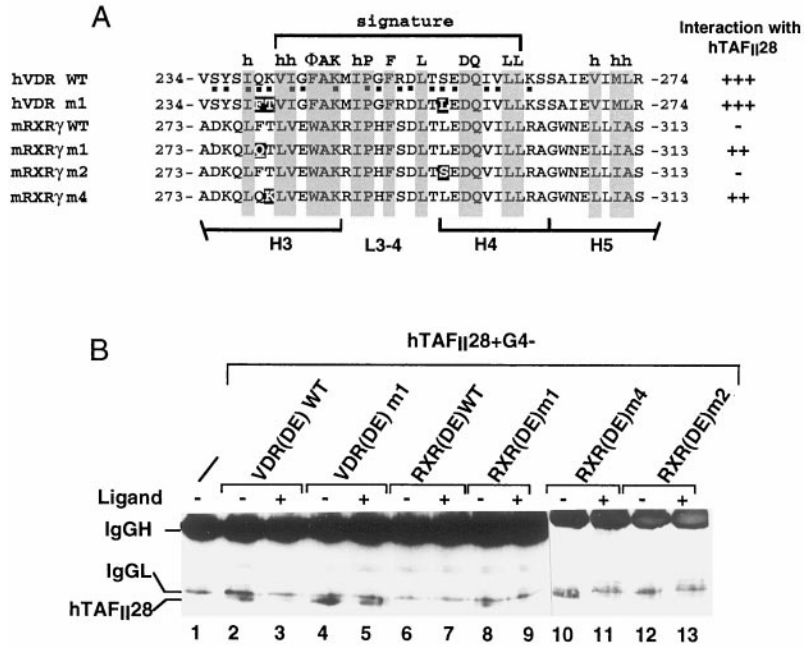

FIG. 7. *A*, sequences of the H3–H5 regions in each G4-NR(DE) chimera. The amino acids mutated in G4-RXR(DE) m1, m2, and m4 and G4-VDR(DE) m1 are highlighted. *B,* immunoprecipitation of the RXR and VDR mutants with hTAF$_{II}$28. The presence of ligand in the transfections is shown above each *lane*. All immunoprecipitations were performed using the anti- G4 antibody. A weak signal for IgG(L) is seen in this series of experiments migrating just above the hTAF$_{II}$28 (see control *lane 1*).

meras were both coprecipitated with hTAF$_{II}$28 (Fig. 6*B, lanes 3–6, 8*, and *9*). Thus, the VDR or TR H3–H5 regions can mediate interactions with hTAF$_{II}$28 in the context of the RXRγ DE region.

Determinants in the α3 Helix of the VDR Are Required for Interactions with hTAF$_{II}$28—The above results show that hTAF$_{II}$28 interacts with the H3–H5 regions of the VDR and TRα but not the RXRγ. We have previously shown that hTAF$_{II}$55-RXRγ LBD interactions can be induced by exchanging solvent-exposed amino acids of the RXRγ H3–H5 region by their VDR equivalents. We next determined which if any of these mutations (Fig. 7*A*) could induce hTAF$_{II}$28-RXR interactions.

hTAF$_{II}$28 was precipitated with the wild-type VDR in a ligand-reversible manner, whereas no coprecipitation with the wild-type RXRγ was observed (Fig. 7*B, lanes 2, 3, 6*, and *7*). In contrast to wild-type RXRγ, hTAF$_{II}$28 was coprecipitated in a ligand-reversible manner with G4-RXRγ(DE) m1 (F278Q) and m4 (T279K) (Fig. 7*B, lanes 8–11*), whereas no significant coprecipitation with m2 (L295S) was observed (Fig. 7*B, lanes 12* and *13*). Therefore, exchange of single amino acids between the RXRγ and VDR LBDs in the α3-helix can induce interactions with hTAF$_{II}$28.

In a converse experiment we simultaneously introduced the above three RXRγ amino acids into the equivalent positions of the VDR LBD to generate a triply mutated G4-VDR(DE) m1 (Q239F,K240T,S256L; Fig. 7*A*). This triple mutation did not abolish interaction with hTAF$_{II}$28, as expected from the fact that hTAF$_{II}$28 interacts also with the VDR H8 region. Interestingly, however, the interaction with the mutated VDR LBD was no longer ligand-reversible (Fig. 7*B, lanes 4* and *5* compared with *2* and *3*).

We next determined whether the triple mutation had affected the ability of the VDR to activate transcription. To do this, increasing quantities of vectors expressing the wild-type or mutated G4-VDR(DE) were cotransfected along with a G4-responsive CAT reporter (see "Materials and Methods" and Refs. 26, 29). The wild-type VDR LBD strongly activated transcription from this promoter (Fig. 8*A, lanes 2–4*), whereas the mutated VDR was unable to activate transcription (Fig. 8*A, lanes 5–7*). As described previously (29, 33, 43), little or no activation of transcription from this promoter is observed with the wild-type G4-RXRγ(DE), whereas the mutant bearing the three VDR amino acids strongly activates transcription (Fig. 8*A, lanes 8–10* and *11–13*, respectively). Equivalent expression of each of the chimeras was detected by immunoblotting (Fig. 8*B*). Consequently, replacing the three amino acids of the VDR with their RXRγ equivalents leads to a loss of function, whereas in the converse experiment there is a gain of function. These three amino acids therefore play a critical role in transactivation.

DISCUSSION

Ligand-reversible hTAF$_{II}$28-NR Interactions—We describe here novel interactions between the LBDs of the VDR and the TRα and the hTAF$_{II}$28 component of transcription factor TFIID. After coexpression in COS cells, hTAF$_{II}$28 could be specifically coprecipitated with the LBDs of the VDR and TRα in the absence of the appropriate ligands, whereas coprecipitation is dramatically reduced in the presence of ligand. The selectivity of the interactions is shown by the observation that under the same conditions no interactions between hTAF$_{II}$28 and other NRs were observed, nor did hTAF$_{II}$20 or hTAF$_{II}$18 interact with the VDR or TR LBDs.

Interaction with the VDR requires the evolutionary conserved C-terminal domain of hTAF$_{II}$28, which contains the histone fold motif (31). Interaction is seen with a deletion mutant (1–150) in which the α2- and α3-helices of the histone fold are deleted, but not with (1–114), in which the αN- and α1-helices are deleted. Therefore, interaction with the NRs does not require the integrity of the histone fold domain but does require determinants in the αN-α1 region. In support of this, interaction with the VDR is abolished by mutation of exposed residues in the α1-helix, further suggesting that this helix plays an important role in the interaction.[2]

hTAF$_{II}$28 interacts with two independent sites in the VDR LBD. We have characterized in detail the interaction of hTAF$_{II}$28 with one of these sites located between amino acids 234 and 274 spanning α-helices H3–H5 and containing the NR signature. The equivalent region of the chicken TRα also interacts with hTAF$_{II}$28, whereas no interaction is seen with the

[2] G. Mengus, Y. G. Gangloff, L. Carré, A.-C. Lavigne, and I. Davidson, unpublished observations.

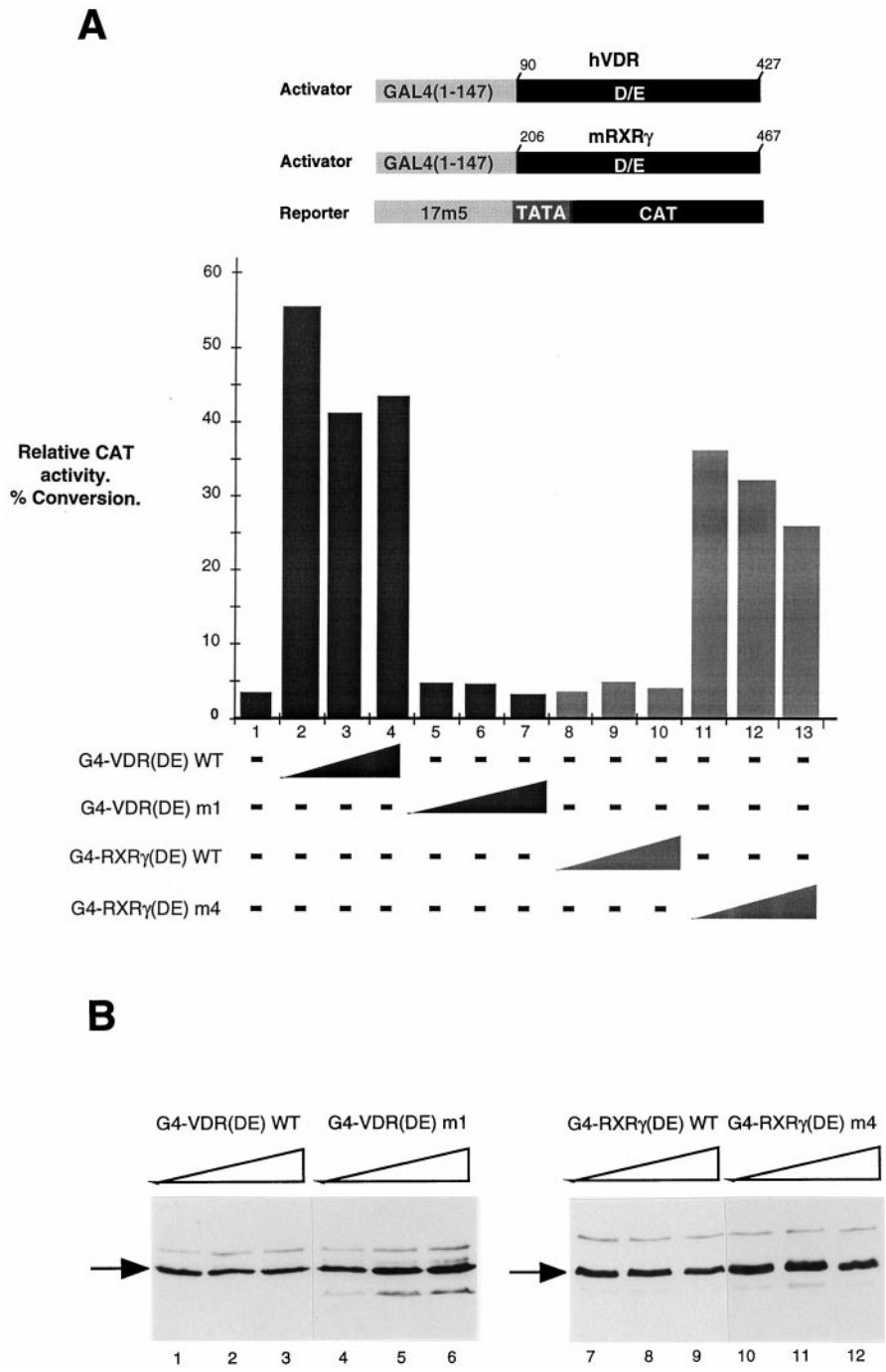

FIG. 8. *A*, graphic representation of CAT assays. The structures of the activator and reporter constructs used are shown schematically at the *top*. The transfected expression vectors used are shown below each *lane*. Transfections contained 0, 0.25, or 0.5 μg of the expression vectors as indicated. All transfections were performed in the presence of ligand. *B,* expression of the wild-type and mutated RXR and VDR chimeras. Cells were transfected with 0.1, 0.25, or 0.5 μg of the expression vectors indicated above each *lane,* and the expressed proteins were detected using the anti-G4 antibodies. The *arrows* indicate the expressed G4-NR chimeras.

equivalent region of the RXRs. Two amino acids in helix H3 are critically involved in the ability of $hTAF_{II}28$ to discriminate between the VDR and RXRγ LBDs. In the context of the full RXRγ DE region, replacement of Phe^{278} or Thr^{279} with Gln or Lys, respectively, of the VDR induces a ligand-reversible interaction with $hTAF_{II}28$. No significant interaction, however, was seen with mutant L295S located in helix H4.

We have previously shown that position Leu^{295} is an important determinant for interaction with $hTAF_{II}55$, which also interacts with the H3–H5 region of the VDR and TR. The differential interaction of $hTAF_{II}55$ and $hTAF_{II}28$ with the L295S mutation indicates that, although both TAF_{II}s interact with the H3–H5 region, the determinants for their respective interactions are not identical. $TAF_{II}28$ may interact only with the H3 helix, whereas $hTAF_{II}55$ requires determinants in both the H3 and H4 helices.

As discussed previously (33), the $hTAF_{II}28$ and $hTAF_{II}55$ interaction sites are close to, but not identical to, with those required for interaction with the L*XX*LL motif in several transcriptional intermediary factors (44–46). The close proximity of these two interaction sites may nevertheless explain the ligand reversibility observed with the $hTAF_{II}28$ interaction, because there may be steric hindrance between $hTAF_{II}28$ and the TIF(s) whose association with the LBD is ligand-induced (see below). It is not clear, however, how the ligand-induced conformational change in the LBD, which brings helix H12 in proximity to H3 and H4 to create the hydrophobic TIF interaction surface, would affect the interaction of $hTAF_{II}28$ with its second interaction site in helix H8.

Possible Roles of the VDR/TR-$hTAF_{II}28$ Interaction—Our previous results indicated that there is a correlation between

transcriptional activation potential and interaction with $hTAF_{II}55$. The wild-type RXR LBD, which has negligible transactivation potential on a minimal promoter, does not interact with $hTAF_{II}55$. In contrast, the single amino acid changes in the RXRγ LBD induce weak interactions with $hTAF_{II}55$ and an increase in transactivation, whereas the F278Q,L295S double mutation induces a strong interaction with $hTAF_{II}55$, and this derivative activates transcription to a level equivalent to that seen with the VDR itself (33).

On the other hand, the NR interaction with $hTAF_{II}28$ does not correlate with activation, since it is ligand-reversible. We therefore do not suggest any obvious role for the NR-$hTAF_{II}28$ interaction in the transcriptional activation described here. Ligand-reversible interactions with NRs are not observed with transcriptional coactivators but are characteristic of those seen with the corepressors N-Cor and SMRT (47, 48). At present we have no direct evidence that NR-$hTAF_{II}28$ interactions are involved in the transcriptional repression seen with the unliganded TR or VDR, and the RXR mutants that interact with $hTAF_{II}28$ do not gain repressor properties, at least not on the promoters that we have tested.

Transcriptional activation is thought to involve both the recruitment of histone acetylase complexes and interactions with the basal transcription apparatus. It is therefore tempting to speculate that, in addition to recruitment of histone deacteylase complexes, interactions such as those described here between the VDR and TR and the basal transcription apparatus may contribute to transcriptional repression. Indeed, previous *in vitro* experiments have suggested that the unliganded TR may target TBP itself and block preinitiation complex formation leading to transcriptional repression (49, 50). It has also been shown that TBP/TFIID may be a target of other repressors such as the homeodomain protein even-skipped (51, 52). Furthermore, ligand-reversible interactions between the TR and TFIIB have also been suggested to contribute to transcriptional silencing (53)

Our results identify amino acids in helices H3 and H4 critical for transcriptional activation by the VDR. These amino acids, which all lie on the surface of the receptor and are not involved in ligand binding (54), cannot be replaced by their RXR homologues, whereas their incorporation into the RXR yields a potent transcriptional activator. Because the interaction of $hTAF_{II}28$ with the VDR m1 mutant, which does not activate transcription, is ligand-independent rather than ligand-reversible, this would suggest that the introduction of the RXR amino acids has abrogated the ability of the VDR to interact with coactivators (one of which may be $TAF_{II}55$), which normally displace $hTAF_{II}28$ in the presence of ligand. Indeed, a similar constitutive interaction is seen when the VDR activation function-2-AD core in helix H12 is deleted.[3] In other receptors deletion of the activation function-2-AD core does not impair ligand binding but does abolish interaction with TIFs. $TAF_{II}28$ interacts with the mutated RXR LBDs in a ligand-reversible manner. Thus, in this context, which is the converse of that observed with VDR m1, it is likely that the introduction of the VDR amino acids allows interactions not only with $hTAF_{II}28$ but also with coactivators that displace $hTAF_{II}28$ in the presence of ligand. Together, these results show that the H3–H5 region contains receptor-specific determinants for transactivation.

Acknowledgments—We thank N. Rochel and J. M. Wurtz for access to the crystal structure of the VDR, S. Vicaire and D. Stephane for DNA sequencing, Y. Lutz and the monoclonal antibody facility, the staff of the cell culture and oligonucleotide facilities, B. Boulay, J. M. Lafontaine, R. Buchert, and C. Werlé for illustrations, and Roussel-Uclaf for providing 1,25-dixydroxyvitamin D_3.

[3] G. Mengus, Y. G. Gangloff, L. Carré, A.-C. Lavigne, and I. Davidson, unpublished data.

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The Human Transcription Factor IID Subunit Human TATA-binding Protein-associated Factor 28 Interacts in a Ligand-reversible Manner with the Vitamin D_3 and Thyroid Hormone Receptors

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J. Biol. Chem. 2000, 275:10064-10071.
doi: 10.1074/jbc.275.14.10064