Identification of a Transactivation Function in the Progesterone Receptor That Interacts with the TAF\textsubscript{II}110 Subunit of the TFIID Complex*

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Transcriptional activation of target genes by the human progesterone receptor is thought to involve direct or indirect protein-protein interactions between the progesterone receptor and general transcription factors. A key role in regulation is played by a transactivation factor TFIID, a multiprotein complex consisting of the TATA-binding protein and several tightly associated factors (TAFs). TAFs have been shown to be required for activated transcription and are, thus, potential targets of activator proteins. Using in vitro interaction assays, we could identify specific interactions between the progesterone receptor and the TATA-binding protein-associated factor dTAF\textsubscript{II}110. The dTAF\textsubscript{II}110 domain responsible for the interaction is distinct from that reported to suffice for binding to Sp1. Somewhat surprisingly, deletion analysis indicated that the previously identified activation functions 1 and 2 of the progesterone receptor are not required for this interaction but pointed to an important role of the DNA binding domain. In cotransfection experiments and in an in vitro transcription assay, the DNA binding domain of the progesterone receptor displayed significant activation potential. These findings, taken together, suggest that an interaction between the progesterone receptor and TAF\textsubscript{II}110 may represent an important step in the mechanism of activation.

In eukaryotes, at least seven basal transcription factors (TFIIB, TFIID, TFIIE, TFIIF, TFIH, and TFIJ) are required for basal levels of transcription at promoters by RNA polymerase II in vitro (reviewed in Ref. 1). In mammalian systems, these factors assemble into functional initiation complexes in a highly ordered, stepwise fashion beginning with the binding of TFIID to the TATA box (1–3). Transcription activators, consists of separable domains responsible for DNA binding (DBD) and transcriptional activation (23). The amino-terminal activation function (AF-1) is constitutively active, whereas the activation function located within the carboxy-terminal part (AF-2) requires hormone for its activity (24, 25).

In the present study, possible interactions of hPR with a subunit of the TFIID complex were examined by protein-protein interaction assays. Our results show that the 110-kDa subunit of Drosophila TFIID (dTAF\textsubscript{II}110) is specifically bound by hPR in vitro and that this interaction is mediated by specific domains of both proteins. Transfection studies and in vitro transcription experiments revealed that the part of hPR which mediates the interaction contains a previously unidentified activation function.

 MATERIALS AND METHODS

Baculovirus Expression—cDNAs encoding full-length hPR (form B) and deletions were derived from expression vector hPR0 (26). By insertion into a Bluescript SK derivative encoding the peptide MSHHHHHH-

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1 The abbreviations used are: TBP, TATA-binding protein; TAF, TBP-associated factor; PR, progesterone receptor; PRE, progesterone response elements; DBD, DNA binding domain; hPR, human progesterone receptor; CAT, chloramphenicol acetyltransferase; aa, amino acid(s); PAGE, polyacrylamide gel electrophoresis; PCR, polymerase chain reaction.
HTHSETY, the hPR cDNAs were fused in frame to a His-tag. Two Nhel sites flanking the cDNAs were used to release the tagged fragments by Nhel digest. To generate baculovirus transfer vectors, these Nhel fragments were ligated into the Nhel site of pbBlueac (Invitrogen). In the transfer vector encoding Mh-hPR0, the His-tag is followed by aa 1–933 of the hPR open reading frame (26), in Mh-hPR0Δcore by aa 1–858, and in Mh-hPR0ΔABC by aa 1–795. The deletion core by aa 1–456/538–639 followed by Arg, in Mh−BC by aa 165–639 followed by Arg, in Mh−BCcore by aa 165–456/538–639 followed by Arg, in Mh−BC by aa 538–639 followed by Arg, in Mh−ABC by aa 1–456/538–555 followed by Gln-Asn-Ser, and in Mh−Bacore by aa 165–456/538–555 followed by Gln-Asn-Ser.

hPR-dTAFII110 Interaction

Numbers included in the vector names indicate the amino acids of the dTAFII110 construct, of which the open reading frame extends from amino acid 106 to 567. The following constructs correspond to the following restriction sites derived from cells infected with plaque-purified recombinant viruses.

Expression in Escherichia coli and Protein Purification—Construction of expression vectors encoding amino-terminally de- added following incubation at 4°C for 1 h. Subsequent washing steps were performed using 50 mM imidazole, and buffer D100 plus 20 mM imidazole. MH6-BC

Expression in vitro

Preparation of Sf21 Cell Extracts and Affinity Purification of Recombinant Proteins—Sf21 cell culture, infection with recombinant baculoviruses, extraction of cellular proteins, and affinity purification on Ni-NTA-agarose (Qiagen) was performed as described by Zapp et al. (27). In the MH6 and MH6-BC constructs, the His-tagged vector was cloned in the presence of 5 mM imidazole, and buffer D100 plus 20 mM imidazole. MH6-BC

For protein expression, a 250-ml culture of E. coli TG1 containing pQE-hPR(DBD), which encodes the pQE His-tag followed by Gly-Ser-Thr-Ser and aa 536–664 of human TFIIB, was grown at 37°C in TB medium containing 100 μg/ml ampicillin to an OD 595 of 0.6. Expression was then induced by addition of isopropyl-1-thio-galactopyranoside to a final concentration of 1 mM.

Expression Vectors for in vitro Translation

Transient Transfection and CAT Assay—The reporter plasmids PRE-TATA-CAT and TATA-CAT were constructed by replacing the Sad-Sall fragment containing the G-free cassettes of PRE-TATA-G300 and TATA-G400 (29), respectively, with a Sad-Sall fragment from BgIII containing a CAT cassette. BS-CAT had been constructed by inserting the HindII fragment of pCAT promoter (Promega) into the HindIII site of the multiple cloning site. The HindIII site of the multiple cloning site.

Expression Vectors for in Vitro Translation—Expression vectors for in vitro transcription/translation of dTAFI110 and mutants were derived from pSG5 (30) by inserting an 8-mer linker into the refilled Xho site of pSGN. pSGN had been derived from pSG5 by inserting an appropriate primer and appropriate primers generating 5′-Ndel and 3′-XbaI overhangs. The PCR fragments were reinserted into Ndel/XbaI-cut pTi110, creating the vectors TAF128–921, TAF373–921, and TAF666–921. In TAF128–921 and TAF666–921, naturally occurring ATG codons encoding the methionine residues 128 and 666, respectively, were used as initiation codons within the Ndel site, while in TAF373–921, a methionine codon was introduced upstream of amino acid 373. TAF373–787 was created by cleavage of TAF373–921 with XhoI and XbaI, Klenow treatment, and religation. The construct TAF138–307 was created by cleavage of pTi110 with Clal and SalI, Klenow treatment, and religation of the vector fragment.

Expression in human TFII B a pSG5-based vector containing the complete open reading frame of human TFII B was kindly provided by D. Reinberg (Robert Wood J o h n n School, University of Medicine and Dentistry of New Jersey, Piscataway, N J). Luciferase was expressed from the control template provided with the TNI kit (Promega).

Interaction assays using oligonucleotide-bound hPR were performed as basically as described by Hoey et al. (12). 5′-Biotinylated DNA fragments containing two PREs and a TATA box were synthesized by PCR using the vector PRE-TATA-CAT (see below) as template and the oligonucleotides Bioln-TGGAAAAGCAGGGCGAGCAATCTCCT-CCGACCC as primers. Binding of the 171-base pair fragments to streptavidin-agarose beads (Pierce) was performed by overnight incubation at 4°C in buffer T (25 mM HEPES, pH 7.6, 1 mM EDTA, 0.01 mM ZnSO4, 2 mM dithiothreitol, 10% glycerol, 0.01% Nonidet P-40) containing 1 nM NaCl (buffer T 0.1 μM) with subsequent removal of unbound DNA by three washing steps. 15 μl of beads (50% suspension) were washed four times with 1 ml of buffer T 0.05 M and incubated at 4°C for 1 h at 4°C with the beads, incubation with [35S]methionine-labeled proteins, and analysis were done as described above.

To test for interactions, Cos7 cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum and antibiotics. One day prior to transfection, 105 cells were seeded onto 6-cm dishes. Transfections were performed using the Lipofectin method according to the manufacturer's (Life Technologies, Inc.) recommendation. Briefly, reporter DNA (2 μg)
and expression vector (100 ng) were diluted into 100 μl of OPTIMEM (Life Technologies, Inc.) and made up to a total of 2.5 μg of DNA by the addition of empty expression vector. This solution was mixed with an equal volume of OPTIMEM containing 6 μg of Lipofectin. 15 min after mixing, 1.8 ml of OPTIMEM was added, and the liposome/DNA mixture was poured onto the cells, which had been washed with phosphate-buffered saline and serum-free OPTIMEM. After 4 h, the solution was removed, and 3 ml of phenol-red-free Dulbecco’s modified Eagle’s medium containing 10% fetal calf serum was added. Progesterone treatment was started by adding 3 μl of a 0.1 mM stock in ethanol; controls received 3 μl of ethanol. Cells were harvested 40 h after transfection. Preparation of cell extracts and CAT assays were done as described (31).

In Vitro Transcription—In vitro transcription reactions contained 6 μl of rat liver nuclear extract (6.3 μg of protein) prepared as described by Döbbling et al. (32). E. coli mock extract, recombinant MH₄ₗ-hPR(DBD), and recombinant MH₄₃-hPR0 added to some of the reactions were expressed in E. coli or SF21 cells infected with recombinant baculovirus, respectively, and purified as described above. The supercoiled transcription templates PRE₂-TATA-G300 and TATA-G400 containing G-free cassettes (33), reaction conditions, and analysis of transcripts were as previously described (29, 34, 35). Quantitation of transcripts was performed by autoradiography and subsequent densitometry.

RESULTS

hPR Interacts Specifically with dTAF₁₁₀ in Vitro—It has been shown that various activators can interact through their activation domains with TAFs (11–18). In vitro transcription interference experiments had indicated that steroid receptors and Sp1 might have a common target(s) in the transcription machinery (29). Combined with the report of Hoey et al. (12), which identified dTAF₁₁₀ as a target of Sp1, these findings thus raised the possibility that hPR and dTAF₁₁₀ might interact with each other. To address this question, we performed protein–protein interaction assays with [³⁵S]methionine-labeled dTAF₁₁₀ and the baculovirus expressed Histagged hPR (MH₄₃-hPR0). In initial experiments, partially purified MH₄₃-hPR0 was bound to PRE-containing DNA fragments, which were immobilized to streptavidin-agarose beads via a biotin moiety. After incubation with labeled dTAF₁₁₀, unbound protein was removed by washing with binding buffer, and bound proteins were eluted with buffer containing 1 M NaCl, separated by SDS-PAGE, and visualized by autoradiography. As shown in Fig. 1A, ³⁵S-labeled dTAF₁₁₀ was retained on beads containing DNA-bound MH₄₃-hPR0, whereas no binding was observed on DNA beads lacking MH₄₃-hPR0 (compare lanes 6 and 5), demonstrating that dTAF₁₁₀ does not stably bind to DNA on its own. In contrast, control beads lacking DNA fragments, which did not contain bound PR after washing (data not shown), did not bind dTAF₁₁₀ (lanes 4 and 3). To explore the specificity of the interaction, we tested whether DNA-bound MH₄₃-hPR0 would interact under the same conditions with ³⁵S-labeled luciferase (Fig. 1B) or human TFIIB (Fig. 1C) and found that both proteins were not retained by the receptor. These results demonstrate that the interaction of DNA-bound hPR with dTAF₁₁₀ is specific.

The sensitivity of the assay involving DNA-bound hPR was relatively low, probably due to gradual dissociation of hPR–dTAF₁₁₀ complexes from the DNA during the washing steps. We thus analyzed whether the interaction would also occur with MH₄₃₄-hPR0 immobilized directly to Ni-NTA-agarose beads through the amino-terminal His-tag. As shown in Fig. 2A, dTAF₁₁₀ was indeed bound on beads loaded with MH₄₃₄-hPR0 (lane 4) but not on control beads without MH₄₃₄-hPR0 (lane 3). Furthermore, a Histagged fusion protein representing DCOH (kindly provided by E. Pogge v. Strandmann and G.U. Ryffel), the Xenopus homologue of DCOH (36), did not bind dTAF₁₁₀ (Fig. 2B). These results confirm that specific interactions between hPR and dTAF₁₁₀ can also occur with hPR immobilized to Ni-NTA-agarose beads.
Fig. 2. hPR interacts with dTAFII110 immobilized to a Ni-NTA matrix. A, partially purified MH6-hPR0 (10 μg) was bound to Ni-NTA-agarose beads (lane 4) and tested for binding of [35S]methionine-labeled dTAFII110 as described under “Materials and Methods.” In lane 3, control beads lacking bound protein were used. Lane 1 contains 2% of the input of dTAFII110. Where indicated (lanes 5–8), EtBr was included in the binding reaction. The position of bound dTAFII110 is indicated on the right side of the autoradiograph. B, same as A, except that 5 μg of His-tagged XDCoH were immobilized on the beads. Lane 1 contains 25% of the dTAFII110 input.

Even though the interaction could be observed with hPR immobilized to Ni-NTA-agarose, an involvement of DNA could not be excluded as in vitro transcription/translation lysate programmed with a significant amount of dTAFII110 expression plasmid was used in the experiment. Therefore, the interaction assay was performed in the presence of increasing amounts of the DNA intercalator ethidium bromide (EtBr), which inhibits DNA binding and thus allows to discriminate between DNA-dependent and DNA-independent protein interactions (37). As shown in Fig. 2A, EtBr up to 100 μg/ml (lanes 5 and 6), a concentration shown to inhibit DNA-dependent protein associations (37), had little effect on the interaction, while at relatively high concentrations of 200 and 400 μg/ml (lanes 7 and 8) significant inhibition was obtained. However, even at the highest EtBr concentration used, the association of hPR and dTAFII110 could not be completely inhibited. The partial sensitivity to EtBr suggests that there is a DNA-independent component reflecting bona fide protein-protein interactions, which are possibly further stabilized through DNA.

Amino acids 538–639 of hPR Are Sufficient for Interaction with dTAFII110—As has been demonstrated by the groups of Gronemeyer and Chambon (24, 25), the hPR contains two activation functions (AF-1 and AF-2) located amino- and carboxy-terminally of homology region C, which comprises the DBD (see Fig. 3A). AF-1 has been further dissected into a core region, which mediates transactivation when linked to a GAL4-DBD and a modulatory domain, which exhibits activation potential only when fused to the homologous DBD (38, 39). To determine which domain of the receptor mediates the binding to dTAFII110, we expressed a number of hPR mutants lacking either single or combinations of domains as His-tagged fusion proteins in insect cells (Fig. 3A). The purity of the various receptor proteins after affinity purification on Ni-NTA-agarose was examined by SDS-PAGE and silver staining (Fig. 3B). In addition to the full-size products, MH6-hPR0 and MH6-hPR0core showed a number of smaller polypeptides (lanes 2 and 3), most of which could be identified as carboxyl-terminally truncated hPR molecules by Western blotting with appropriate antibodies (data not shown). In contrast, all proteins lacking the carboxy-terminal AF-2 (MH6-AB, MH6-ABC, MH6-BC, MH6-BCDcore, MH6-C, MH6-ABcore, MH6-Bcore) were isolated as relatively pure full-size products (lanes 4–10).

To analyze the interaction potential of the various hPR mutants, we employed the assay in which the receptor proteins were immobilized to Ni-NTA-agarose because of the advantage that one can also examine mutants, which do not contain a DBD (MH6-ABDcore and MH6-BDcore). Using this set of mutants, we could demonstrate that progressive deletion of AF-1 and AF-2 sequences up to a construct containing only amino acids 538–555 of AF-1 and amino acids 556–639 of the DBD (MH6-C) did not eliminate binding of dTAFII110 (Fig. 4A). In contrast, two different deletion mutants lacking domain C but containing amino acids 538–555 of AF-1 (MH6-ABDcore and MH6-BDcore) showed negligible binding of dTAFII110, even when 6-fold higher amounts of the receptor proteins were tested (Fig. 4B). Thus, our experiments show that homology region C containing the DNA binding domain plays an impor-
Next, we analyzed a number of amino-terminal deletion constructs for their binding to MH₆-hPR0 (Fig. 5B, lower panel). dTAF₁₁₀ constructs lacking amino acids 1–127 (TAF₁₂₈–₉₂₁) or 1–372 (TAF₂₇₃–₉₂₁) associated efficiently with MH₆-hPR0 (lanes 16 and 18). Further truncation up to amino acid 665 created a protein (TAF₆₆₆–₉₂₁) that showed increased binding to the Ni-NTA matrix (lane 19) but no specific association with MH₆-hPR0-loaded beads (compare lanes 19 and 20). Consistently, an internal deletion mutant lacking amino acids 138–307 of dTAF₁₁₀ (TAF₁₁₈–₃₀₇) retained its ability to interact with MH₆-hPR0 (lanes 21 and 22). Thus, the amino-terminal border of the hPR interaction domain maps between amino acids 373 and 666 of dTAF₁₁₀. As summarized in Fig. 5A, using MH₆-C instead of MH₆-hPR0 to determine the carboxyl- and amino-terminal borders of the hPR interaction domain of dTAF₁₁₀ gave identical results (Fig. 5C).

In addition, a deletion construct lacking both amino- and carboxyl-terminal sequences (TAF₃₇₃–₇₈₇) was tested for its interaction with MH₆-hPR0 and various hPR deletion constructs. As shown in Fig. 6, TAF₃₇₃–₇₈₇ bound efficiently to MH₆-hPR0 (lane 4) and all of the hPR deletion constructs shown to be capable of binding TAF₁–₉₂₁, including MH₆-C (lane 10). Therefore, carboxyl-terminal sequences spanning amino acids 373–787 of dTAF₁₁₀ are sufficient for specific interaction with amino acids 538–639 of hPR.

Sequences Comprising the dTAF₁₁₀ Interaction Domain of hPR Mediate Transactivation in Vivo and in Vitro—Our conclusion that the DNA binding domain of hPR interacts with a TAF prompted us to investigate whether this part of hPR might be sufficient for transcriptional stimulation. To analyze transcriptional stimulation in vivo, an SV40 early promoter-driven expression vector encoding amino acids 556–639 of hPR (hPR(C)) was cotransfected with a CAT reporter construct containing two PREs in front of a TATA box (PRE₂TATA-CAT) into Cos7 cells. Interestingly, expression of hPR(C) stimulated CAT activity to about 10% of the activity observed with MH₆-hPR0 in the presence of progesterone (Fig. 7A). Transactivation by hPR(C) did not require progesterone treatment (Fig. 7A), as expected, but proved to depend on the presence of PREs in the reporter, as no stimulation was observed with a reporter lacking PREs (TATA-CAT, data not shown).

For analysis of the transcriptional activation potential of the DBD of the hPR in vitro, we used a cell-free transcription system, which uses rat liver nuclear extract as a source for general transcription factors (29, 34, 35). Two different test genes containing a TATA box or two PREs and a TATA box in front of a G-free cassette (33) were transcribed simultaneously in all reactions. To determine fold activations, correctly initiated transcripts from PRE₂TATA were normalized to the amount of transcripts obtained from the template containing the TATA box only. As the DNA binding activity in vitro of the E. coli-expressed His-tagged amino acids 556–664 of hPR (MH₆-hPR(DBD)) purified by Ni-NTA chromatography was higher than that of the Sf₂₁-expressed amino acids 538–639 (MH₆-C), we decided to use MH₆-hPR(DBD) in our in vitro transcription experiments. Amino acids 556–664 of hPR are homologous to the minimal deletion mutant of the human estrogen receptor, which is sufficient for stable binding to DNA in vitro (40). As shown in Fig. 7B, addition of recombinant MH₆-hPR0 activated transcription approximately 15-fold, whereas addition of an E. coli mock extract had no effect. Saturating amounts of MH₆-hPR(DBD) activated transcription to about 13% of the activity observed with MH₆-hPR0, which agrees well with the value obtained in the transfection experiments. Together, these
data prove that the DBD of hPR contains significant transactivation potential.

**DISCUSSION**

To detect the interactions between hPR and dTAF\(_{110}\) in vitro and to define the domains involved, we used partially purified hPR proteins bound to a Ni-NTA matrix and in vitro synthesized dTAF\(_{110}\). In this assay, EtBr caused a clear inhibition (Fig. 2), indicating that DNA is somehow involved. However, since dTAF\(_{110}\) alone does not stably bind to DNA (Fig. 1A, lane 5), we exclude the possibility that the association of dTAF\(_{110}\) with hPR loaded beads is simply due to capturing of DNA molecules containing bound dTAF\(_{110}\) through non-specific interactions with the DNA binding domain of hPR. Furthermore, we are convinced that the binding of dTAF\(_{110}\) to the Ni-NTA matrix is due to an interaction with the hPR and not mediated by a natural poly-histidine containing protein, for example TFIIA (41, 42), either present in the reticulocyte lysate or copurified with the PR proteins from infected SF21 cells for the following reasons. First, we were able to detect hPR-dependent dTAF\(_{110}\) binding also in a different assay, in which the hPR was bound to biotinylated DNA fragments immobilized to streptavidin beads (Fig. 1A). Second, the PR dependence of dTAF\(_{110}\) binding to Ni-NTA-agarose beads (Figs. 2A, 4, 5, and 6) rules out the possibility that dTAF\(_{110}\) is directly bound by a natural poly-histidine containing protein present in the reticulocyte lysate used to synthesize the labeled dTAF\(_{110}\) proteins. Third, the negative results obtained with two different hPR mutants (MH6-AB\(_{110}\) core, MH6-B\(_{110}\) core, Fig. 4B), purified from infected SF21 cells by our standard procedure, strongly argue against copurified poly-histidine containing Spodoptera proteins as being responsible for dTAF\(_{110}\) binding. Furthermore, in a chromatographic analysis of a MH\(_{110}\) preparation on a Superose 12 gel filtration column, only one peak of binding activity was observed, which perfectly coincided with the single MH\(_{110}\) polypeptide peak at 13 kDa (data not shown). However, since the dTAF\(_{110}\) proteins used in this study were assayed without further purification, we cannot exclude a model in which the hPR-dTAF\(_{110}\) interaction is mediated or stabilized by an additional protein(s) present in the translation lysate. Highly purified recombinant dTAF\(_{110}\) will be required to investigate this issue.

Prior to this study, two activators have been shown to interact with dTAF\(_{110}\), namely Sp1 and CREB (11, 12, 43). Although the interaction between CREB and dTAF\(_{110}\) has not been mapped to a particular region of dTAF\(_{110}\) (11), sequence similarities of CREB and Sp1 activation domains suggest that these activators may target the same domain of dTAF\(_{110}\) (43). As indicated in Fig. 5A, work of Tjian's group (12) has shown that the amino-terminal 308 amino acids of dTAF\(_{110}\) suffice for interaction with Sp1. In contrast, our experiments establish that the interaction with hPR does not involve the amino-terminal region but depends on a more carboxyl-terminal nonoverlapping domain of dTAF\(_{110}\). Together with the results of the Sp1-dTAF\(_{110}\) interaction, this study identifies dTAF\(_{110}\) as the first TAF containing at least two distinct

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**Fig. 5.** Mapping of the dTAF\(_{110}\) domain required for interaction with MH\(_{110}\)-hPR0 and MH\(_{110}\)-C. A, domain structure of wild type TAF\(_{110}\) (TAF1-921(wt)) and various deletion constructs. The structural motifs of dTAF\(_{110}\) (hatched, S/T-rich; filled, Q-rich; stippled, charged) are shown as described by Hoey et al. (12). Restriction sites used for creating carboxyl-terminal deletion constructs are indicated at the top. The right side of the panel, the potential of the different constructs for interaction with MH\(_{110}\)-hPR0 and MH\(_{110}\)-C is summarized. The regions of dTAF\(_{110}\) shown to be sufficient for interaction with Sp1A and Sp1B (12) and hPR and dTAF\(_{30}\) (48) are indicated at the bottom. B and C, interaction assays were performed with Ni-NTA-agarose beads lacking (−) or containing (+) 0.1 nmol of MH\(_{110}\)-hPR0 (B) or 0.6 nmol of MH\(_{110}\)-C (C) and 4 μl of reticulocyte lysate containing the radiolabeled dTAF\(_{110}\) construct indicated at the top of each lane.
interaction surfaces mediating contacts with activators. Due to the multiplicity of activators and the limited number of TAFs, we expect that multiple interaction domains within TAF polypeptides will turn out as a more general feature of these important molecules.

Work of the groups of Tjian and Roeder (44–48) has indicated that dTAFII110 makes additional contacts with several other TAFs of the TFIID complex. While most TAF-TAF interactions have not been mapped to particular domains of dTAFII110, Yokimori et al. (48) demonstrated that dTAFII110 binds to sequences in the carboxyl-terminal part of dTAFII110 (aa 572–921). In contrast to hPR, dTAFII110 binding was absolutely dependent on the very carboxyl-terminal end of dTAFII110. Therefore, the sequence requirements for binding of hPR and dTAFII110 to dTAFII110 are clearly different.

Perhaps the most surprising result of this study is that an hPR construct (MH6-C) containing only 18 amino acids of AF-1 (aa 538–555) and the DBD (aa 556–639) is sufficient for interaction with dTAFII110. Since two overlapping mutants (MH6-CABcore, MH6-BCcore) containing these 18 amino acids of AF-1 are not sufficient for binding to dTAFII110, the interaction motif is at least partly contained within the DBD of hPR. Another example for an interaction between a DBD and a TAF was recently described by Chiang and Roeder (18), who demonstrated that the DBD of Sp1 binds to a 55-kDa subunit of human TFIID in vitro. Our results thus support the concept that TAFs can interact with multiple distinct domains of transactivators (18).

By cotransfection and in vitro transcription experiments, we were able to demonstrate that the DBD of hPR, which has not been tested for transactivation potential in the studies defining AF-1 and AF-2 of hPR (24, 25, 38), is able to mediate transcriptional activation through PREs located in front of a minimal promoter, albeit with reduced efficiency compared to wild type hPR (Fig. 7). The presence of a previously unidentified activation function within the hPR domain mediating the interaction with dTAFII110 in vitro clearly supports our notion that this interaction reflects an important step in the mechanism of transactivation by hPR.

Interestingly, mutational analysis of the rat and human glucocorticoid receptor DBDs pointed to a role of a basic region immediately following the second zinc finger (49, 50), activation by the DBD of the estrogen receptor may be mediated by a short acidic tract at the carboxyl terminus of the DBD (51). Thus, the presence of an activation function located in the DBD might be a more general feature of steroid receptors.

Analogous to the studies that identified interactions of Sp1 and CREB with dTAFII110 (11, 12), we have investigated interactions between a mammalian activator and a Drosophila TAF, i.e. between proteins from evolutionarily widely separated organisms. Since the cDNA encoding the human homologue of dTAFII110 is not available yet, we have not been able to prove that it also interacts with hPR. However, the part of dTAFII110 sufficient for binding to hPR includes regions with extensive sequence similarity to the human homologue hTAFII110.3 Our results thus raise the possibility that this part of TAFII110 contains a conserved motif mediating interactions with the highly conserved DBDs of members of the steroid receptor superfamily.

Ing et al. (52) have reported that hPR and a truncated chicken PR synthesized in reticulocyte lysate and E. coli, respectively, interact specifically with the basal transcription factor TFIIIB. In contrast, using baculovirus-expressed hPR that is transcriptionally active (Fig. 7B), we have been unable to detect association of hPR and TFIIIB (Fig. 1B). It remains to be seen whether the different sources of the receptors or experimental differences may account for the discrepancy between the two sets of data. Interestingly, the region required for interaction with TFIIIB has been mapped to a 168-amino acid fragment of chicken PR, which includes the highly conserved DBD (52). Since we have shown that the corresponding region of hPR mediates interaction with dTAFII110, it is feasible that the DBDs of PRs are not only involved in the formation of receptor dimers (21, 25), in the recognition of the PREs (21), and in interactions with a TAF, but also in protein-protein interactions with TFIIIB (52). Since the DBD of hPR accounts only for a small part of the overall transactivation potential of

3 R. Tjian and N. Tanese, personal communication.
hPR (Fig. 7), it is likely that hPR contacts additional, yet unidentified components of the initiation complex, possibly through AF-1 and AF-2. Multiple interactions with members of the transcription machinery have been proposed for a growing number of transcriptional activators, including VP16 and the glucocorticoid receptor (13, 53). Such a multiplicity of protein-protein contacts may enable the hPR to affect subsequent rate-limiting steps in the process of preinitiation complex formation and contribute to the more synergistic activation observed with multiple PRs bound to closely adjacent PEs.

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