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The N-terminal region (A/B) of rat thyroid hormone receptors α1, β1, but not β2 contains a strong thyroid hormone-dependent transactivation function

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ABSTRACT **In this study we have investigated the role of the N-terminal region of thyroid hormone receptors (TRs) in thyroid hormone (TH)-dependent transactivation of a thymidine kinase promoter containing TH response elements composed either of a direct repeat or an inverted palindrome. Comparison of rat TRβ1 with TRβ2 provides an excellent model since they share identical sequences except for their N termini. Our results show that TRβ2 is an inefficient TH-dependent transcriptional activator. The degree of transactivation corresponds to that observed for the mutant TRΔNβ1/2, which contains only those sequences common to TRβ1 and TRβ2. Thus, TH-dependent activation appears to be associated with two separate domains. The more important region, however, is embedded in the N-terminal domain. Furthermore, the transactivating property of TRα1 was also localized to the N-terminal domain between amino acids 19 and 30. Using a coimmunoprecipitation assay, we show that the differential interaction of the N terminus of TRβ1 and TRβ2 with transcription factor IIB correlates with the TRβ1 activation function. Hence, our results underscore the importance of the N-terminal region of TRs in TH-dependent transactivation and suggest that a transactivating signal is transmitted to the general transcriptional machinery via a direct interaction of the receptor N-terminal region with transcription factor IIB.**

Thyroid hormone receptors (TRs) are members of the steroid/thyroid hormone (TH) superfamily of nuclear receptors functioning as ligand-dependent transcriptional factors when bound specifically to DNA sequences called TH response elements (TREs, refs. 1–3). Several alternatively spliced forms of two rat (r)TR isotypes encoded by two different genes α and β have been identified.

The TRα2-variant (4, 5), which does not bind TH, is functionally different from TRα1 or TRβ1 and TRβ2, all of which bind TH. rTRβ1 and rTRβ2 comprise 456 and 514 amino acids, respectively (6, 7). Their sequences are identical from the 13 amino acid N terminal to their DNA-binding domains, corresponding to amino acid 89 and 147, respectively, to their C termini (7). Thus, only the N-terminal regions, which show less than 15% sequence similarity, distinguish them from each other. The rTRα1 is 410 amino acids in length and is about 80% similar to rTRβ1 in the DNA-binding and ligand-binding domains (6); however, there is no significant similarity in the N-terminal regions. It is also well established that *in vitro*, TR functions as a repressor of basal promoter activity in the absence of TH, but in the presence of TH, the repression is relieved and there is concomitant transactivation (1, 3, 8–13).

The N-terminal and C-terminal regions of some steroid hormone and retinoic acid receptors possess a transactivating function (14, 15), and the receptors can interact directly with transcription factor IIB (TFIIB; refs. 3 and 16), a member of the general transcriptional machinery (17). It was reasonable to assume that the dissimilar N termini of rTRβ1 and rTRβ2 might transactivate a target gene differently, although Thompson and Evans (18) could not demonstrate a transactivating function for the N terminus of human (h)TRβ1.

This report describes the investigation of the TH-dependent transactivating properties of rTRβ1 and rTRβ2 by using TREs of different structures and provides some information about the N-terminal region of rTRα1. Our results suggest that the mechanism of differential TH-dependent activation mediated by rTRβ1 and rTRβ2 might be the result of different interactions between N-terminal portions of these receptors and hTFIIB.

MATERIALS AND METHODS

Plasmid Construction. The constructions of the myelin basic protein (MBP)-TRE-33 (pseudoinverted palindrome)-thymidine kinase (TK)-chloramphenicol acetyltransferase (CAT) reporter plasmid and the malic enzyme (ME)-TRE (pseudodirect repeat)-TK-CAT (TK1AM) have been described (10, 11). An idealized inverted perfect palindrome (INV PAL +3; ref. 19), (cTGACCTGACTGTCAGGTCAg) TRE-TK-CAT reporter plasmid, and a perfect direct repeat (DR +4; ref. 12), (cTCAGGTCACAGGAGGTCAGAGg) TRE-TK-CAT reporter plasmid, were constructed by inserting each corresponding double-stranded oligonucleotide into *Sph* I and *Bam*HI restriction sites of the BLCAT2 vector (20). The construction of Rous sarcoma virus (RSV)-rTRβ1 and RSV-rTRα1 expression plasmids used in transient transfection assays and SP72-rTRβ1 expression plasmid for *in vitro* transcription/translation has been described (21). To construct rTRβ2 expression plasmids for transient transfection assays or *in vitro* transcription/translation, the rTRβ2 cDNA was modified by replacing the 5′ noncoding region with the Kozak sequence (CCACC; ref. 22), and inserted into either the *Hin*dIII/*Hpa* I site of RSVΔneo (23) or the *Hin*dIII/*Sma* I site of SP72 (Promega). The N-terminal deletion mutants expression plasmids RSV-rTRΔNβ1/2, RSV-rTRΔNα1, RSV-rTRΔN19α1, or RSV-rTRΔN30α1 were prepared by replacing the *Hin*dIII–*Xba* I fragment of RSV-rTRβ1 or the *Hin*dIII–*Age* I, the *Hin*dIII–*Nsp* I, or the *Hin*dIII–*Bst*EII fragments of RSV-rTRα1 with double-stranded oligonucleotides containing a *Hin*dIII restriction site, the Kozak sequence, the initiation codon followed by the nucleotide sequence encoding seven common amino acids starting from amino acid 89 of rTRβ1

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Abbreviations: TR, thyroid hormone receptor; TH, thyroid hormone; TRE, TH response element; ME, malic enzyme; MBP, myelin basic protein; INV PAL +3, inverted perfect palindrome; DR +4, perfect direct repeat; r, rat; h, human; TK, thymidine kinase; CAT, chloramphenicol acetyltransferase; RSV, Rous sarcoma virus.

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and 147 of rTRβ2, including an *Xba* I site (RSV-rTRΔNβ1/2) or encoding 11, 11, or 1 amino acid starting from amino acid 51, 30, or 19 of rTRα1, including *Age* I site (RSV-rTRΔNα1), *Nsp* I site (RSV-rTRΔN30α1), or *Bst*EII site (RSV-rTRΔN19α1), respectively. The chimeric receptor RSV-rTRβ2α1 expression plasmid was obtained by a two-step PCR. The internal primers were 5′-CACAAAAAAGGGTATATCCCCAGCTGTGTCGTGTGTGGGGACAAGGCC-3′ (p1) and 5′-GGCCTTGTCCCCACACACGACACAGCTGGGGATATACCCTTTTTTGTG-3′ (p2). These primers contain sequences of rTRβ2 from amino acid 145 to 152 and rTRα1 from amino acid 53 to 60 (underlined) to replace the DNA-binding domain and C-terminal region of rTRβ1/2 with the DNA-binding domain and C-terminal region of rTRα1. The 5′ external primer 5′-aagcttCCACCATGTGTATGGATGTACGC-3′ (p3) contains the *Hin*dIII site, Kozak sequence, and first six amino acids of rTRβ2. The 3′ external primer 5′-gttaacGCCTGAGGCTTAGAC-3′ (p4) contains the sequence of a *Hpa* I site, a stop codon, and 12 nt of the noncoding region from rTRα1. Two separate primary PCR amplifications were done by pairing p1/p4 and p2/p3. The gel-purified primary products were annealed and served as a template for the secondary PCR amplification using the external primers p3 and p4. The full-length amplified rTRβ2α1 was digested with *Hin*dIII and *Hpa* I and inserted into the *Hin*dIII/*Hpa* I site of RSVΔneo plasmid. The rTRβ1 and rTRβ2 expression plasmids encoding proteins containing hc-myc epitope in their C termini were prepared by PCR by using the primers 5′-ggaccTGCGGATGATTGGAGC-3′ and 5′-gttaacTCACAAGTCCTCTTCAGAAATGAGCTTTTGCTCGTCCTCAAAGACTTCCAAGAAGAG-3′ and RSV-rTRβ1 as a template. The underlined sequence corresponds to the hc-myc epitope sequences (24). The PCR-generated *Ava* II–*Hpa* I fragment was gel-purified and inserted into the *Ava* II/*Hpa* I site of the digested RSV-rTRβ1 and RSV-rTRβ2 plasmids to give RSV-rTRβ1-myc and RSV-rTRβ2-myc expression plasmids. The SP72-rTRβ1_{1-165} and SP72-rTRβ2_{1-223} expression plasmids for coimmunoprecipitation assays were constructed by digesting SP72-rTRβ1 and SP72-rTRβ2 with *Nsi* I, restriction site located in the DNA-binding domain (amino acids 165 and 223, respectively), and *Kpn* I, restriction site located in the 3′ polylinker region of SP72-rTRβ1 and SP72-rTRβ2. The double-stranded oligonucleotide, 5′-tCTGAGTTAACggtac-3′ (upper strand), was inserted in the *Nsi* I/*Kpn* I site of the gel-purified SP72-rTRβ1_{1-165} and SP-rTRβ2_{1-223} fragment to introduce a stop codon (underlined). The sequence of each DNA construct was determined (25) to ensure that constructs had been correctly assembled.

Cell Culture and Transfection Assay. NIH 3T3 cells, a mouse fibroblast cell line, and NG108-15, a neuronal/glial cell line, were cultured and transfected as described (10, 26). At least three independent experiments in duplicate were performed.

Preparation of Nuclear Extract and Western Blot Assay. Nuclear extracts were prepared from NIH 3T3 cells (27) simultaneously transfected with 12.5 μg of RSV-rTRβ1-myc, 12.5 μg of RSV-rTRβ2-myc, and 25 μg of pUC 19 and cultured without TH for 20 h. The proteins were resolved by SDS/10% PAGE, transferred to a nitrocellulose membrane, and probed with anti-human c-myc monoclonal antibody (9E10; ref. 24). The antigen–antibody complexes were detected by the ECL detection system (Amersham) according to the manufacturer's instructions.

Coimmunoprecipitation Experiments. Approximately 3 fmol (0.5 μl) of freshly *in vitro* translated (TNT coupled reticulocyte lysate system, Promega), [^{35}S]methionine-labeled rTRβ1 or rTRβ2 or 0.5 μl (≈1 fmol) of [^{35}S]methionine-labeled rTRβ1_{1-165} or rTRβ2_{1-223} were incubated for 20 min on ice with ≈3 pmol of bacterially expressed hTFIIB (Promega) in 1 ml of modified NENTM buffer (16) containing 20 mM Tris·HCl (pH 8.0), 100 mM NaCl, 1 mM EDTA, 0.5% Nonidet P-40, 0.5% powdered milk, 1 mM dithiothreitol, and 0.01% bovine serum albumin. One half of the reaction mixture was then incubated with 1 μg of anti-hTFIIB monoclonal antibody (Promega) for 60 min on ice and the other half was incubated with 1 μg of anti-β-galactosidase monoclonal antibody (Promega) as a control for nonspecific binding. Then, 50 μl of protein G-agarose beads (Life Technologies, Grand Island, NY), preincubated with the modified NENTM buffer containing 0.1% bovine serum albumin, was added, and the incubation continued for 60 min on ice with gentle, occasional shaking. The beads were washed five times with 1 ml of the modified NENTM buffer in a small column at 4°C. The bound complexes were eluted with 50 μl of prewarmed 2× SDS/PAGE sample buffer (28), resolved by SDS/4–20% gradient PAGE, and visualized by fluorography (Enlightning, NEN). The signals were quantitated by using a phosphorimager [FUJIX (Tokyo), BAS2000].

RESULTS

Effect of rTRβ1 and rTRβ2 on Repression and TH-Dependent Activation of ME and MBP TREs. rTRβ1 and rTRβ2, divergent only in their N-terminal regions, provide an excellent model to study the role of these regions in TH-dependent transactivation. Hence, we wished to compare the

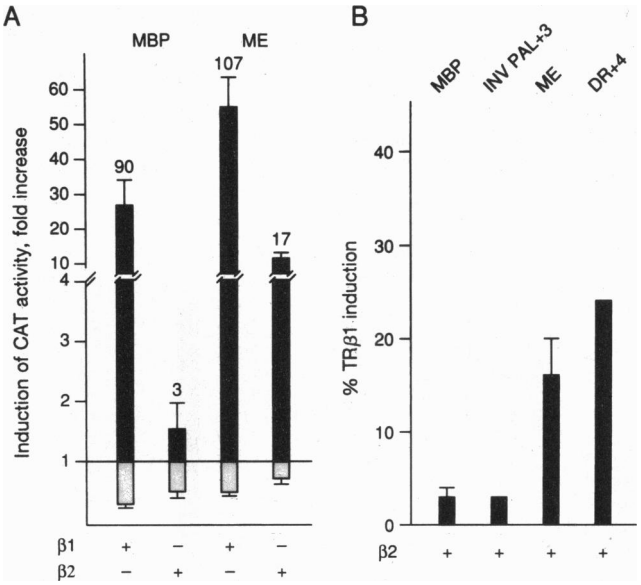

FIG. 1. Effects of rTRβ1 and rTRβ2 on expression of TK-CAT reporters containing MBP, ME, INV PAL +3, or DR +4 TREs. (*A*) Level of expression of MBP-TRE-TK-CAT and ME-TRE-TK-CAT mediated by rTRβ1 and rTRβ2 in the presence (■) and absence (□) of TH. NIH 3T3 cells were cotransfected with 20 μg of either MBP-TRE-TK-CAT (MBP-TRE) or ME-TRE-TK-CAT (ME-TRE) and 12.5 μg of either RSV-rTRβ1 or RSV-rTRβ2 expression plasmids. The cells were plated in 4-well dishes and cultured in the absence or presence of TH. CAT activities were normalized to β-galactosidase activity derived from CMV-β-galactosidase expression plasmid included in every transfection. Results are expressed as the fold increase of CAT activity obtained relative to the activity in the absence of the receptor expression plasmid, which was arbitrarily set at 1. The numbers above the columns show the mean of the ratio of CAT activity in the presence and absence of TH. Values are mean ± SE. (*B*) Comparison of TH-dependent transactivation of different TREs elicited by rTRβ2 with that mediated by rTRβ1. Transient transfection assays were performed as described in *A* by using the reporter genes containing the TREs shown above the bars. The results are expressed as a percentage of the fold of induction (± TH) of the CAT activity obtained with the corresponding reporter in the presence of rTRβ1. Values are mean ± SE.

magnitude of TH-dependent transactivation mediated by rTRβ1 and rTRβ2 by using transient transfection assays and the reporter constructs MBP-TRE-TK-CAT, containing the TRE found in the MBP promoter that we have characterized as an imperfect inverted palindrome (11), and ME-TRE-TK-CAT, containing the TRE found in the ME promoter that we have characterized as an imperfect direct repeat (10). In NIH 3T3 cells, MBP-TRE and ME-TRE conferred increases in the TH inducibility of the TK promoter in the presence of rTRβ1 of 90- and 107-fold, respectively, while increases of only 3- and 17-fold, respectively, were elicited by rTRβ2 (Fig. 1*A*). These results suggested that the differential activation mediated by rTRβ1 and rTRβ2 was most likely due to differences in the amino acid composition in the N-terminal regions. In the absence of TH, rTRβ1 and rTRβ2 both functioned as repressors of MBP-TRE (≈55% inhibition) and ME-TRE (≈40% inhibition) function (Fig. 1*A*).

Transactivation of INV PAL +3 and DR +4 TREs by rTRβ1 and rTRβ2. Next, we investigated the effect of rTRβ1 and rTRβ2 on other TREs, such as an idealized inverted perfect palindrome TRE (INV PAL +3) and a perfect direct repeat TRE spaced by 4 nt (DR +4) by using INV PAL+3-TK-CAT and DR+4-TK-CAT reporter constructs. The results showed that rTRβ2 was a poor activator of all TREs tested, although it proved to activate the prototype TRE, DR +4, or ME-TRE somewhat more efficiently than inverted palindrome type TRE or MBP-TRE. Fig. 1*B* shows that in NIH 3T3 cells, rTRβ2 activation of MBP, INV PAL +3, ME, and DR +4 corresponds to only 3, 3, 16, and 24 percent, respectively, of that obtained with rTRβ1.

Expression of RSV-rTRβ1 and RSV-rTRβ2 in NIH 3T3 Cells and the Dose-Dependency of Transactivation. To determine whether translational efficiencies of rTRβ1 and rTRβ2 in the transfected cells are comparable, we performed an immunoblot assay using nuclear extract from NIH 3T3 cells transfected simultaneously with equal amounts of the RSV-rTRβ1 and RSV-rTRβ2 expression plasmids tagged with the c-myc epitope at their C termini (Fig. 2*A*). The integrated intensities of bands corresponding to rTRβ2-myc (59 kDa) and rTRβ1-myc (54 kDa) were similar. No band at the corresponding position was detected when the cells were transfected with pUC 19 plasmid as a control. Hence, the differential effect of the rTRβ1 and rTRβ2 on TH-dependent transactivation of the TREs cannot be explained by differences in the translational efficiencies of the two expression plasmids. This was further supported by dose–response experiments. As depicted in Fig. 2*B*, the liganded rTRβ1 transactivated the reporter gene construct in a dose-dependent manner, while rTRβ2-mediated transactivation remained low at any rTRβ2 concentrations employed. Similar results were obtained by using NG108-15 neuronal/glial cells (data not shown), suggesting that the difference in TH-dependent transcriptional activation efficiencies mediated by rTRβ1 and rTRβ2 was not cell-type specific.

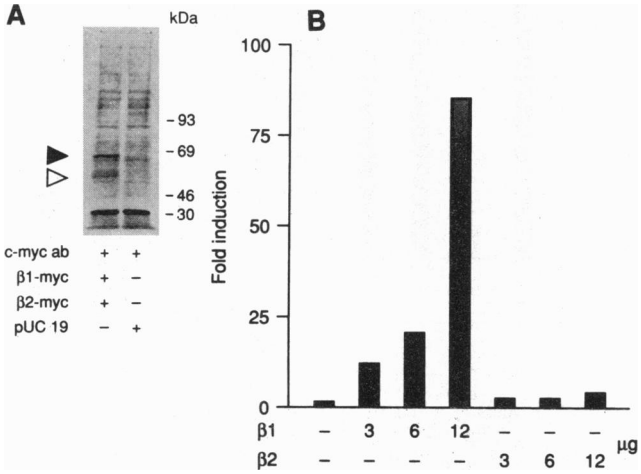

FIG. 2. Expression of RSV-rTRβ1 and RSV-rTRβ2 in transfected NIH 3T3 cells and effects of increasing concentrations of RSV-rTRβ1 and RSV-rTRβ2 on TH responses of MBP-TRE-TK-CAT. (*A*) Immunoblot analysis of nuclear extracts from NIH 3T3 cells transfected with pUC 19, RSV-rTRβ1-myc, or RSV-rTRβ2-myc. Nuclear extracts were prepared as described in *Materials and Methods.* Equal amounts of the extracts were fractionated on SDS/10% polyacrylamide gels and immunoblotted with the anti-c-myc monoclonal antibody (c-myc ab) as described in *Materials and Methods.* The open and filled arrowheads indicate the position of rTRβ1-myc protein and rTRβ2-myc protein, respectively. The positions of molecular weight markers are indicated. (*B*) RSV-rTRβ1 and RSV-rTRβ2 dose-dependent transactivation of MBP-TRE-TK-CAT. Twenty μg of MBP-TRE-TK-CAT reporter was cotransfected with increasing amounts of RSV-rTRβ1 or RSV-rTRβ2 expression plasmids as indicated. The cells were treated and the results were normalized to β-galactosidase activity as described in the legend to Fig. 1. Results are expressed as the fold increase in CAT activity in the presence of TH relative to the activity in the absence of TH.

A Role for the N-Terminal Domain of rTRs on TH-Dependent Transactivation of MBP-TRE. The results noted above suggest that the dissimilar N-terminal regions of otherwise identical rTRβ1 and rTRβ2 play an important role in transactivation. To investigate this further we used an N-terminal deletion mutant rTRΔNβ1/2 which contains only the amino acid sequence shared by both receptors. The rTRΔNβ1/2 mutant was tested by using the MBP-TRE-TK-CAT reporter construct. The results showed (Fig. 3*A*) that this mutant lost about 90% of its TH-dependent transcriptional activity with respect to rTRβ1, a value similar to that obtained with the intact rTRβ2 (Fig. 1*B*) and rTRΔNα1, in which the N-terminal region of the α receptor was deleted (Fig. 3*B*). The rTRα1-mediated TH response on MBP-TRE-TK-CAT reporter was comparable to that obtained with rTRβ1, as we previously reported (11), while rTRΔNα1 lost about 80% of activation function, which was restored by the addition of 34 (rTRΔN19α1) but not 23 (rTRΔN30α1) amino acids N-terminal to rTRα1 DNA-binding domain (Fig. 3*B*). We also investigated the effect of the N-terminal domain of rTRβ2 on rTRα1 function. The chimeric receptor rTRβ2α1 used in transfection assays contained the N-terminal region of rTRβ2, and the remaining sequence was provided by rTRα1. The

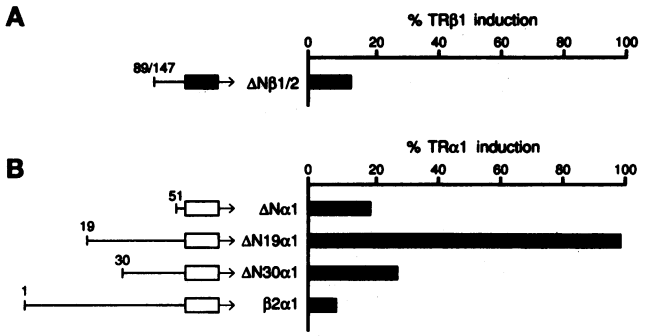

FIG. 3. Effect of the N-terminal regions of rTRs on TH-dependent transactivation of MBP-TRE-TK-CAT. NIH 3T3 cells were transfected with MBP-TRE-TK-CAT and one of the following: RSV-rTRΔNβ1/2 (ΔNβ1/2) (*A*), RSV-rTRΔNα1 (ΔNα1), RSV-rTRΔN19α1 (ΔN19α1), RSV-rTRΔN30α1 (ΔN30α1), or RSV-rTRβ2α1 (β2α1) (*B*) or with RSV-rTRβ1 or RSV-rTRα1 expression plasmids. Diagrams of the N-terminal deletion mutants and the chimeric receptor are shown. Solid lines depict N-terminal regions and boxes are DNA-binding domains (■, β1/β2; □, α1). Amino acid positions from the N termini are indicated. The results are expressed as a percentage of the induction elicited by rTRβ1 (*A*) or rTRα1 (*B*) in the presence and absence of TH. The cells were cultured as described in the legend to Fig. 1 and *Materials and Methods.*

rTRβ2α1 chimeric receptor was an inefficient activator of MBP-TRE-TK-CAT (less than 10% of activation by rTRα1 or rTRβ1; Fig. 3*B*). Interestingly, both rTRΔNβ1/2 and rTRΔNα1 functioned as repressors of MBP-TRE-TK-CAT in the absence of TH (≈45% inhibition; data not shown). Hence, our results demonstrate that most of the hormone-dependent transactivation function is embedded in the N-terminal domain of rTRβ1 and rTRα1, and it is impaired in the N-terminal region of rTRβ2.

Interaction of rTRβ1 and rTRβ2 with hTFIIB Analyzed by Coimmunoprecipitation Assay. Although a large body of evidence indicates that TRs are transcriptional activators in the presence of TH through interacting with TREs (1–3), the mechanism(s) as to how this event affects the RNA polymerase II-mediated rate of transcription is unclear. A protein–protein interaction of the receptors, either via coactivators or directly with the general transcriptional factors assembled in a preinitiation complex, is now considered likely.

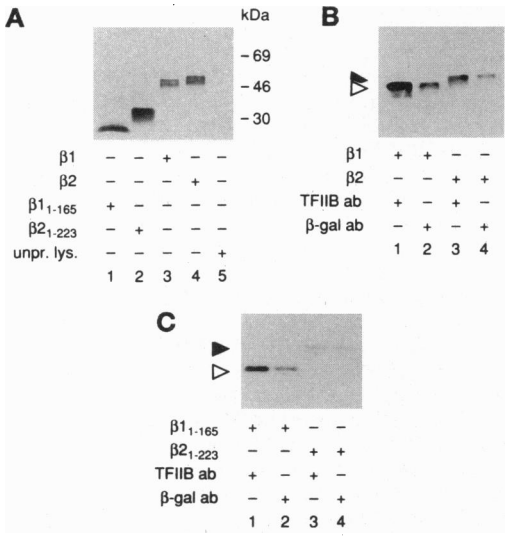

FIG. 4. Interaction of rTRβ1, rTRβ2, rTRβ1$_{1-165}$, and rTRβ2$_{1-223}$ mutants with hTFIIB detected by a coimmunoprecipitation assay. (*A*) Quantitation of *in vitro* translated, [^{35}S]methionine-labeled receptors by SDS/PAGE analysis. Samples of trichloroacetic acid precipitates containing 40,000 dpm of *in vitro* translated, [^{35}S]methionine-labeled rTRβ1$_{1-165}$ (β1$_{1-165}$; lane 1), rTRβ2$_{1-223}$ (β2$_{1-223}$; lane 2), rTRβ1 (β1; lane 3), or rTRβ2 (β2; lane 4) were loaded onto an SDS/4–20% polyacrylamide gel. Lane 5 contained translation product from the control plasmid without insert. The products were analyzed by SDS/4–20% gradient PAGE and autoradiography. The positions of molecular weight markers are indicated. (*B*) Coimmunoprecipitation of hTFIIB with either rTRβ1 or rTRβ2 by anti-hTFIIB monoclonal antibody. Bacterially expressed hTFIIB (≈1.5 pmol) and ≈1.5 fmol of each *in vitro* translated, [^{35}S]methionine-labeled rTRβ1 (β1) (lanes 1 and 2) or rTRβ2 (β2) (lanes 3 and 4) were incubated as described in *Materials and Methods* and then coimmunoprecipitated in modified NENTM buffer with 1 μg of either anti-hTFIIB monoclonal antibody (TFIIB ab; lanes 1 and 3) or anti-β-galactosidase monoclonal antibody (β-gal ab; lanes 2 and 4). Following the washing, as described in *Materials and Methods*, the bound receptors were analyzed by SDS/4–20% gradient PAGE and fluorography. The open arrowhead indicates the position of rTRβ1 protein, while the filled arrowhead marks the position of rTRβ2 protein. (*C*) Coimmunoprecipitation of hTFIIB with the N-terminal mutants either rTRβ1$_{1-165}$ or rTRβ2$_{1-223}$ by anti-hTFIIB antibody. Bacterially expressed hTFIIB (≈1.5 pmol) and ≈0.5 fmol of each *in vitro* translated, [^{35}S]methionine-labeled rTRβ1$_{1-165}$ (β1$_{1-165}$; lanes 1 and 2) or rTRβ2$_{1-223}$ (β2$_{1-223}$; lanes 3 and 4) were preincubated and then coimmunoprecipitated with 1 μg of either anti-hTFIIB monoclonal antibody (TFIIB ab; lanes 1 and 3) or anti-β-galactosidase monoclonal antibody (β-gal ab; lanes 2 and 4) as described in *Materials and Methods*. The washing steps and analysis were performed as in *B*. The open arrowhead indicates the position of rTRβ1$_{1-165}$ protein and the filled arrowhead shows the position of rTRβ2$_{1-223}$ protein.

Since the hTRβ1 N terminus has been shown to interact with hTFIIB (29) and the only difference between rTRβ1 and rTRβ2 is the length and amino acid composition of their N-terminal regions, it was reasonable to assume that these two receptors may interact differently with TFIIB through their N termini, resulting in different TH-dependent transactivation. The interaction of rTRβ1, rTRβ2, and the corresponding mutants rTRβ1$_{1-165}$ and rTRβ2$_{1-223}$ with hTFIIB was studied *in vitro* by using a coimmunoprecipitation assay employing recombinant hTFIIB and a monoclonal hTFIIB antibody. Plasmids expressing rTRβ1$_{1-165}$, rTRβ2$_{1-223}$, rTRβ1, and rTRβ2 were efficiently translated and labeled with [^{35}S]methionine as shown in Fig. 4*A*, lanes 1–4. Equal amounts of rTRβ1 and rTRβ2 (Fig. 4*B*) were preincubated with hTFIIB prior to the addition of the hTFIIB monoclonal antibody (lanes 1 and 3) or a β-galactosidase monoclonal antibody (lanes 2 and 4). Antigen–antibody complexes were precipitated by adding protein G-agarose. The results revealed that while either receptor can interact directly with hTFIIB, the interaction with rTRβ1 (Fig. 4*B*, lane 1) was substantially more efficient than that with rTRβ2 (Fig. 4*B*, lane 3). The ≈2.5-fold difference in specific interactions between rTRβ1 and rTRβ2 with hTFIIB was confirmed by quantifying each signal corrected for the nonspecific signal obtained in the presence of β-galactosidase antibody by using the phosphorimager (Fig. 4*B*, lanes 2 and 4, respectively). Since only the N termini of these receptors are dissimilar, the weak signal of rTRβ2 is surely due to an inefficient interaction of its N terminus with hTFIIB. Thus, equal amounts of *in vitro* translated mutants rTRβ1$_{1-165}$ and rTRβ2$_{1-223}$ were tested for their interaction with hTFIIB as described for rTRβ1 and rTRβ2. The hTFIIB antibody in the presence of hTFIIB precipitated the labeled rTRβ1$_{1-165}$ (Fig. 4*C*, lane 1) efficiently, but the labeled rTRβ2$_{1-223}$ (Fig. 4*C*, lane 3) gave a signal similar to that of the control (Fig. 4*C*, lane 4), suggesting that the weak signal obtained with rTRβ2 (Fig. 4*B*) is most likely due to the C-terminal region. Thus, the different degree of interaction of the N-terminal portion of rTRβ1 and rTRβ2 with hTFIIB offers an explanation for rTRβ isotypes mediating quantitatively different TH-dependent responses.

DISCUSSION

The mechanisms by which transcriptional factors interact with the general transcriptional machinery and then enhance target-gene expression are poorly understood. TFIIB, a member of the general transcriptional machinery, plays a key role in the formation of the preinitiation complex (17) and has been recognized as a target for some trans-acting factors such as COUP-TF, and the estrogen, progesterone, TH, and retinoic acid receptors (3, 16, 29), herpes simplex virus type 1 VP16 protein (30), and fushi tarazu gene product (31).

In this study, we investigated the role of the N-terminal regions of rTRs on TH-dependent transactivation of the TREs found in the ME promoter (pseudodirect repeat), in the MBP promoter (pseudoinverted palindrome), and in constructs containing a perfect direct repeat and a perfect inverted palindrome. We have shown using a coimmunoprecipitation assay that rTRβ1 can directly and efficiently interact with the general transcriptional factor TFIIB; whereas, the alternatively spliced form, rTRβ2, which has a different N terminus, interacts with hTFIIB less efficiently. Furthermore, using N-terminal rTRβ1 and rTRβ2 mutants, our results revealed that the ability of rTRβ1 to interact with hTFIIB is conferred by the N-terminal region, since the N-terminal rTRβ2 mutant failed to interact with hTFIIB. Thus, a weak interaction of the intact rTRβ2 polypeptide is most likely due to interaction of the C terminus with hTFIIB. This is in agreement with the observation of Baniahmad *et al.* (29), who reported that the C-terminal region of hTRβ1, in the absence of TH, contains a repressor activity mediated by direct interaction with hTFIIB

in the absence of TH. Although these investigators showed that the N terminus of hTRβ1 could also interact with hTFIIB, their study focused largely on the C terminus. Their data indicated that hTRβ1 lacking the N-terminal 168 amino acids functioned both as a silencer in the absence of hormone and as an activator when TH was added. In our experiments, in the absence of TH, either rTRβ1 or rTRβ2 functioned as a repressor of a heterologous promoter activity in the NIH 3T3 cell line used in transient transfection assays. However, TH-dependent transactivations of the native TREs, MBP (pseudo-inverted) and ME (pseudodirect repeat), or the idealized inverted palindrome and direct repeat in the context of a heterologous promoter mediated by rTRβ1 and rTRβ2 were quantitatively different. rTRβ2 functioned as a poor activator. Under identical experimental conditions, with a level of expression in transfected cells similar to that of rTRβ1, rTRβ2 gave only ≈3% of the activity obtained with rTRβ1 on an inverted palindrome TRE and ≈20% on a direct repeat TRE.

Kurokawa *et al.* (32) proposed that TR homodimers most likely convey TH-dependent responses to those genes containing TREs arranged as inverted palindromes while those containing direct repeats are activated by TR heterodimers. This might explain why the direct-repeat-containing TREs were activated by rTRβ2 more efficiently than those containing an inverted palindrome, assuming that a direct interaction of the active TR dimeric species with TFIIB is the mechanism whereby TH–TR transfers information to RNA polymerase II. In the case of heterodimers, some interactions with TFIIB could be provided by a TR heterodimerization partner.

rTRα1-mediated, TH-dependent activation in transfection assays is similar to that of rTRβ1 (11) and resides in the N-terminal region since the rTRα 1 N-terminal deletion mutant (rTRΔNα1) gave only ≈20% activation when compared with intact rTRα1. Full activation was seen with rTRΔN19α1 but not with the rTRΔN30α1 mutant, indicating a significant role of the region between amino acids 19 and 30 in transactivation. Recently, it has been reported (H. H. Samuels, personal communication) that the N terminus of chicken TRα1 interacts with TFIIB and is important for the receptor function. Interestingly, comparison of rTRα1 sequence between amino acids 19 and 30 with the N-terminal region of chicken TRα1 showed the conservation of 8 amino acids (Arg..AspGlyLysArgLysArgLys) within otherwise dissimilar N termini. Although we have not studied interaction of this region with TFIIB, our functional data agree with those recently reported (H. H. Samuels, personal communication) and indicate that the N-terminal region of rTRα1 carries transactivation properties which map to the region of conserved amino acids between chicken and rat TRα1. Taken together, our results suggest that the ligand-dependent activating properties of TRs require the presence of both the transactivation domain in the N terminus and the intact ligand-binding domain in the C terminus, while the repression functions reside in the ligand-binding domain and function in the absence of TH and the N-terminal region. Our ongoing studies are directed toward the elucidation of a subregion in the rTRβ1 N-terminal region responsible for direct interaction with hTFIIB, since this interaction appears to correlate with TR-activation functions.

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