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Heterodimerization of thyroid hormone (TH) receptor with H-2RIIBP (RXRβ) enhances DNA binding and TH-dependent transcriptional activation

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ABSTRACT **Steroid/TH receptors mediate transcriptional induction of promoters containing hormone response elements (HREs) through an unclear mechanism that involves receptor binding to both hormone and a HRE. Here we demonstrate that both HRE binding and the transcriptional inducing activities of one member of this family, TH receptor, were markedly enhanced by heterodimerization with H-2RIIBP, a non-TH-binding member of the steroid hormone receptor superfamily. H-2RIIBP, the mouse homologue of human retinoic acid-related receptor, was shown to form stable heterodimers with the TH receptor either in solution or when bound to a TH response element. The results presented indicate that it might be necessary for the TH receptor or other members of this superfamily to have specific partners for heterodimer formation to elicit maximal hormone-specific gene regulation from particular HREs.**

Steroid/TH-dependent transcriptional activation is elicited by the binding of a steroid/TH receptor–hormone complex to specific DNA regulatory sequences found near or within genes whose transcriptional control is responsive to the hormone. Although many reports have demonstrated that nuclear factors form heterodimers with known transcription factors to modify specific DNA binding and transcription (1–8), much less is known about the requirement of nuclear factors for steroid/TH-dependent transcription (9–11). Recent reports have demonstrated that several uncharacterized nuclear proteins present in a variety of tissues and cell lines enhance binding of thyroid hormone receptor (THR) (10, 12–16), retinoic acid receptor (17), and vitamin D receptor (18) to particular hormone response elements (HREs). Although the THR has been shown to bind to several HREs as a homodimer (14, 19, 20), analogous to steroid hormone receptors binding to HREs (21–25), it is still unclear whether the THR can act alone or with another protein(s) to regulate expression from TH-controllable genes.

We investigated the possibility that another protein, namely, the H-2 region II binding protein (H-2RIIBP) (26), could alter the DNA binding or transcriptional inducing activity of rat THRα (rTHRα). H-2RIIBP was believed to interact with rTHRα through a TH response element (TRE) since it was cloned by virtue of its ability to bind a DNA sequence within the promoter of the major histocompatibility complex class I gene that contained the consensus THR binding half-site motif AGGTCA (26, 27). H-2RIIBP, a ubiquitously expressed nuclear protein that is the mouse homologue of the human retinoic acid-related receptor (28–30), was also subsequently shown to regulate the expression of this gene (31). Further, the DNA binding and dimerization domains of H-2RIIBP are similar to the THR and the retinoic acid receptor (26, 32, 33), thereby classifying it in this subgroup of the superfamily and making it an ideal candidate for interacting with the THR.

We report here that H-2RIIBP enhanced rTHRα binding to the rat malic enzyme TRE (ME-TRE) (34, 35) and rTHRα–TH-dependent transcriptional activation. The mechanism appears to be at least partially the result of heterodimer formation since H-2RIIBP–rTHRα heterodimers were detected in solution and bound to the ME-TRE.

MATERIALS AND METHODS

Preparation of Nuclear Extracts and DNA Binding. Nuclear extracts containing high levels of either H-2RIIBP (H2) or rTHRα (R) were prepared from H-2RIIBP (27) or rTHRα (T. Mitsuhashi and V. M. Nikoden, unpublished data) recombinant baculovirus-infected Sf9 cells. The level of rTHRα and H-2RIIBP in Sf9 nuclear extracts was determined by Coomassie blue staining of specific bands resolved by SDS/PAGE compared to known quantities of bovine serum albumin (BSA). The estimated concentration of rTHRα was 3300 fmol/μl in nuclear extract containing 8 μg of total protein per μl and the concentration of H-2RIIBP was 15,700 fmol/μl in nuclear extract containing 2.9 μg of total protein per μl. ME-TRE was prepared from pTK1AM3 (34) as a 72-base-pair *Hin*dIII–*Eco*RI restriction fragment.

DNA binding assays were performed in 1× BB [20 mM Hepes, pH 7.9/2 mM $MgCl_2$/10% (vol/vol) glycerol/1 mM dithiothreitol/0.1% Nonidet P-40/80 mM KCl/0.1 mM EDTA/2 mM sodium pyrophosphate/0.4 mM sodium orthovanadate/10 mM sodium fluoride/aprotinin 2 μg/ml/10 μM leupeptin/0.2 mM phenylmethylsulfonyl fluoride) and 5 μg of poly[d(A-T)] (Pharmacia) in a total volume of 20 μl. After a 4-h incubation on ice, mixtures were subjected to electrophoresis either on a 4–20% linear polyacrylamide gradient gel [30% (wt/vol) acrylamide/0.8% *N,N'*-methylenebisacrylamide] for at least 24 h at 150 V and 4°C or 5% gels for 3–6 h at 150 V. Electrophoresis buffer was 0.5× TBE (45 mM Tris borate/45 mM boric acid/2 mM EDTA). Molecular mass standards were from Pharmacia (BSA, 68 kDa; lactate dehydrogenase, 140 kDa; catalase, 232 kDa; ferritin, 440 kDa; thyroglobulin, 669 kDa) and were detected as described (36). For gradient gels, DNA binding reaction mixtures contained 2 μg of nuclear protein corresponding to 825 fmol of rTHRα and/or 0.03 μg of nuclear protein containing 165 fmol of H-2RIIBP. A DNA binding assay mixture with 16 μg of

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Abbreviations: TH, thyroid hormone; HRE, hormone response element; THR, TH receptor; r, rat; TRE, TH response element; ME-TRE, malic enzyme TRE; BSA, bovine serum albumin; DSS, dissuccinimidyl suberate; TK, thymidine kinase; CAT, chloramphenicol acetyltransferase.

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nuclear protein from wild-type baculovirus-infected Sf9 cells was used as a control. Complexes were labeled by the inclusion of ≈10 fmol of ^{32}P-labeled ME-TRE (5000 cpm) or 0.038 μCi of [^{125}I]TH (3, 5, 3′-triiodothyronine; NEN; specific activity, 2200 Ci/mmol; 1 Ci = 37 GBq).

Identification of ME-TRE-Bound Complexes with Antibodies to rTHRα or H-2RIIBP. Anti-H-2RIIBP monoclonal antibody MOK 13-17, specific for H-2RIIBP, was prepared as described (27). Anti-rTHRα antiserum was prepared by injecting 95% pure denatured rTHRα isolated from recombinant baculovirus-infected Sf9 cells into mice and inducing ascites fluid production as described (37, 38). Ascites fluid was confirmed to contain antibody specific to rTHRα (see Fig. 4). The effect of antisera on protein–DNA complex formation was performed by the addition of either MOK 13-17 (3 μl) or anti-rTHRα antiserum (3 μl) to DNA binding reaction mixtures as described above, with the exception that only 0.5 μg of nuclear protein corresponding to ≈206 fmol of rTHRα and/or 0.00018 μg of nuclear protein containing ≈1 fmol of H-2RIIBP were utilized. Antisera was added 45 min after commencement of DNA binding and continued for 2 h before protein–DNA complexes were separated by PAGE in 5% gels.

Chemical Cross-Linking of rTHRα Homodimer and rTHRα/H-2RIIBP Heterodimer. *In vitro*-translated ^{35}S-labeled rTHRα was prepared as described (35). Labeled rTHRα, 20,000 cpm, was combined with Sf9 nuclear extract containing rTHRα, H-2RIIBP, or nuclear protein from wild-type baculovirus-infected cells in a total of 50 μl of 1× BB. After incubation for 4 h at 0–4°C, the cross-linking agent dissuccinimidyl suberate (DSS) (Pierce, dissolved in dimethyl sulfoxide) was added to a final concentration of 0.25 mM. After an additional 30-min incubation at 4°C, reactions were terminated by the addition of 1 M NH_4Cl and SDS-solubilization buffer containing 5% (vol/vol) 2-mercaptoethanol, heated at 100°C for 5 min, and subjected to SDS/PAGE. Cross-linking of unlabeled rTHRα to H-2RIIBP was performed essentially as described above except products were transferred to a nitrocellulose filter after SDS/PAGE, blocked with 1% BSA in phosphate-buffered saline (PBS), and incubated overnight with rTHRα antiserum in PBS, and antibody-bound products were detected with ^{125}I-labeled protein A as described (37).

Transfections. Transfections and construction of the ME-TRE–thymidine kinase (TK)–chloramphenicol acetyltransferase (CAT) reporter plasmid (pTK1AM3) has been described (34). Plasmid pTK1AM3 was electroporated into 4.4 × 10^6 NIH 3T3 cells with pRSVH-2RIIBP (27, 31) and/or pRSVRTRα expression plasmids (34). After transfections cells were equally divided into four 60-mm dishes, two containing 0.1 μM TH in medium and two containing vehicle, and cultured for 72 h before assaying CAT activity as described (34). Samples were corrected for transfection efficiency by utilizing a β-galactosidase reporter construct included in every transfection. All transfections were performed in duplicate at least three times.

RESULTS

To study whether H-2RIIBP could form a heterodimer with THR and/or enhance binding of the THR to TREs and to determine whether THR or H-2RIIBP could bind a TRE as a homodimer, we used the ME-TRE from the rat malic enzyme promoter positions (−280 to −263). The ME-TRE has been shown (34, 35) to contain two half-sites, both of which were essential for THRα binding and function. Mobility-gel-shift assays using pore gradient gels (36) allowed us to estimate the molecular mass of protein–DNA complexes containing ^{32}P-labeled ME-TRE or [^{125}I]TH.

A nuclear extract (2 μg of protein) from Sf9 cells containing ≈825 fmol of rTHRα (46 kDa) formed an ≈140-kDa complex with the ME-TRE (48 kDa) (Fig. 1, lane 3). However the ME-TRE did not form a complex with any protein in wild-type baculovirus-infected nuclear extract (16 μg) alone (Fig. 1, lane 12). These results suggest that rTHRα was bound to the ME-TRE as a homodimer. Additionally, rTHRα in a nuclear extract from Sf9 cells formed a complex with the palindromic rat growth hormone TRE that had nearly the same mobility as the rTHRα–ME-TRE complex (data not shown). THR has been shown (15, 19) to bind to the palindromic rat growth hormone TRE as a homodimer.

Interestingly, nuclear extract from Sf9 cells containing ≈165 fmol of H-2RIIBP (0.03 μg of nuclear protein) or 60,000 fmol of H-2RIIBP (12 μg of nuclear protein) did not bind the ME-TRE under these conditions (Fig. 1, lane 9, and data not shown, respectively). However, addition of 0.03 μg of nuclear extract containing 165 fmol of H-2RIIBP (52 kDa) to 825 fmol of rTHRα (2.0 μg of nuclear protein) in the DNA binding reaction mixture resulted in a slower migrating complex than was observed with the ME-TRE bound to rTHRα alone (Fig. 1, lanes 6 and 3, respectively). This slower migrating complex had a molecular mass of ≈145 kDa, as predicted for a heterodimer of rTHRα–H-2RIIBP bound to the ME-TRE. Addition of 0.03 μg of wild-type baculovirus-infected Sf9 cell nuclear protein to 2.0 μg of rTHRα-containing nuclear protein did not affect rTHRα binding to the ME-TRE (data not shown).

Addition of H-2RIIBP to the DNA binding reaction mixture containing rTHRα also increased the apparent binding affinity of rTHRα for the ME-TRE since there was a 6-fold increase in the amount of ME-TRE bound in the presence of rTHRα plus H-2RIIBP compared to rTHRα alone (Fig. 1, lanes 6 and 3, respectively). Further, this 6-fold increase in ME-TRE binding was solely due to the formation of the slower migrating complex, as no complex corresponding to the rTHRα homodimer bound to the ME-TRE was observed

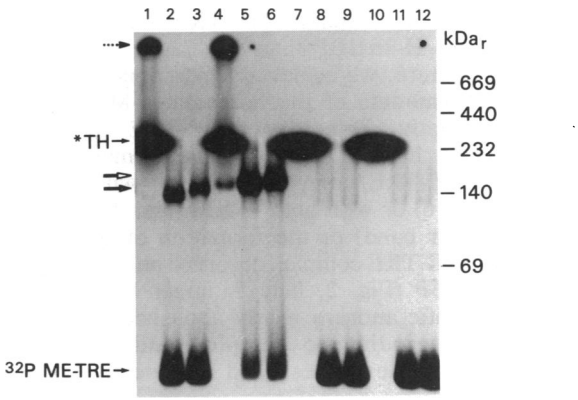

FIG. 1. Binding of rTHRα to the ME-TRE in the presence or absence of H-2RIIBP and/or TH. Autoradiograph of a 4–20% PAGE gradient gel. Lanes: 1–3, nuclear extracts containing rTHRα alone (≈825 fmol/2 μg of nuclear protein); 4–6, both rTHRα (≈825 fmol/2 μg of nuclear protein) and H-2RIIBP (≈165 fmol/0.03 μg of nuclear protein); 7–9, H-2RIIBP alone (≈165 fmol/0.03 μg of nuclear protein); 10–12, wild-type viral extract (16 μg of nuclear protein). Extracts were incubated in the presence of 10 ng of unlabeled ME-TRE and 0.038 μCi of [^{125}I]TH (lanes 1, 4, 7, and 10; 1 Ci = 37 GBq) or 5000 cpm (≈0.5 ng) of ^{32}P-labeled ME-TRE (lanes 2, 3, 5, 6, 8, 9, 11, and 12). Incubations were in the presence of 5 μM TH (lanes 2, 5, 8, and 11) or absence of TH (lanes 3, 6, 9, and 12). Solid or open arrows indicate rTHRα homodimer or rTHRα–H-2RIIBP heterodimer bound to ME-TRE, respectively. *TH designates free [^{125}I]TH, which migrates anomously on these gels presumably from interaction with the gel matrix. Dotted arrow indicates high molecular mass aggregated rTHRα-containing complex bound to [^{125}I]TH. Amount of complexes was quantitated by laser densitometry.

(Fig. 1, lanes 6 and 3, respectively). Although only the slower migrating complex was observed when DNA binding reaction mixtures contained 4-fold less H-2RIIBP than rTHRα, as seen in Fig. 1, both homodimer and heterodimer ME-TRE complexes could be detected in approximately equal amounts when using 206-fold less H-2RIIBP than rTHRα (data not shown and Fig. 2). Therefore, although H-2RIIBP alone did not bind to the ME-TRE in the gel-shift assay, it enhanced rTHRα binding to the ME-TRE, presumably by heterodimer formation.

Addition of TH to the binding reaction increased the formation of both the hetero- and homodimer–^{32}P-labeled ME-TRE complexes ≈2-fold and slightly accelerated the mobility of these complexes (Fig. 1, lane 5 compared with lane 6 and lane 2 compared with lane 3, respectively). It is likely that TH binding to rTHRα induced a conformational change that resulted in the altered mobility of both complexes. Moreover, when [^{125}I]TH was included in the binding reaction mixture containing unlabeled ME-TRE, labeled complexes formed that comigrated with the analogous complexes formed in the presence of unlabeled TH and ^{32}P-labeled ME-TRE (Fig. 1, lanes 1, 2, and 4, 5, respectively). There was 6-fold more [^{125}I]TH bound to the putative heterodimer–ME-TRE complex than to the homodimer–ME-TRE complex (Fig. 1, lanes 4 and 1, respectively). This was precisely the same ratio determined for the amount of ^{32}P-labeled ME-TRE bound in the presence of unlabeled TH (Fig. 1, lanes 5 and 2, respectively). Therefore, the rTHRα homodimer–ME-TRE complex and the putative heterodimer–ME-TRE complex bound virtually the same amount of TH per mol of ME-TRE bound. Similar results were obtained using various concentrations of rTHRα, H-2RIIBP, and ME-TRE (data not shown).

To further demonstrate that the putative heterodimer–ME-TRE complex contained both rTHRα and H-2RIIBP, we examined whether antisera specific for either rTHRα or H-2RIIBP included in the DNA binding reaction mixture altered the formation of this complex. By utilizing 5% polyacrylamide gels to separate ME-TRE-bound complexes and 206-fold less H-2RIIBP than rTHRα in the DNA binding reaction mixture, we achieved separation of approximately equivalent amounts of the homodimer–ME-TRE complex and the putative heterodimer–ME-TRE complex. Preimmune serum had no effect on the formation of the homodimer–ME-TRE complex observed with rTHRα alone (Fig. 2, lane 6) or with both rTHRα and H-2RIIBP (Fig. 2, lane 5, lower band) or the formation of the putative heterodimer–ME-TRE complex observed only with both rTHRα and H-2RIIBP (Fig. 2, lane 5, upper band). Addition of rTHRα-specific antisera nearly abolished formation of the putative heterodimeric complex and the rTHRα homodimeric complex (lanes 2 and 3, respectively). However,

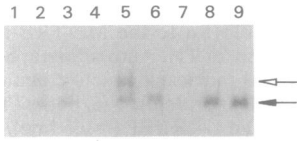

FIG. 2. Identification of rTHRα and H-2RIIBP bound to ME-TRE as a heterodimer with antisera specific for H-2RIIBP or rTHRα. Approximately 206 fmol of rTHRα (0.5 μg of nuclear protein) and/or 1 fmol of H-2RIIBP (0.00018 μg of nuclear protein) were utilized and electrophoresed on a 5% polyacrylamide gel. One of the following sera at 3 μl was added to a ME-TRE binding reaction mixture containing H-2RIIBP alone (lanes 1, 4, and 7), both H-2RIIBP and rTHRα (lanes 2, 5, and 8), or rTHRα alone (lanes 3, 6, and 9): rTHRα antiserum (lanes 1–3), preimmune serum (lanes 4–6), or H-2RIIBP-specific antiserum (lanes 7–9). Solid and open arrows refer to rTHRα homodimer and rTHRα–H-2RIIBP heterodimer bound to the ME-TRE, respectively.

H-2RIIBP-specific antisera added to the binding reaction mixture inhibited the formation of the heterodimer–ME-TRE complex but not the rTHRα homodimer–ME-TRE complex (lanes 8 and 9, respectively). These data, combined with those presented in Fig. 1, indicate that the slower migrating ME-TRE-bound complex formed in the presence of both H-2RIIBP and rTHRα is a heterodimer of H-2RIIBP and rTHRα bound to the ME-TRE.

We investigated whether rTHRα homodimers or rTHRα–H-2RIIBP heterodimers could form in solution in the absence of DNA by using the cross-linking reagent DSS. The rTHRα-containing complexes were detected with ^{35}S-labeled rTHRα or with rTHRα-specific antisera. *In vitro*-translated ^{35}S-labeled rTHRα was cross-linked with excess unlabeled Sf9-cell-derived H-2RIIBP or rTHRα. SDS/PAGE of the putative cross-linked complexes and autoradiography revealed the presence of a ^{35}S-labeled-rTHRα-containing cross-linked complex (95–98 kDa), seen only in the presence of both the cross-linking agent DSS and H-2RIIBP (Fig. 3*A*, lane 1). Furthermore, by using Western blot analysis and the rTHRα-specific antibody for detection, a similar complex was detected when nuclear extracts containing rTHRα and H-2RIIBP from recombinant baculovirus-infected Sf9 cells were mixed prior to cross-linking (Fig. 3*B*, lane 1). No cross-linked ^{35}S-labeled rTHRα complex was observed in the absence of H-2RIIBP (Fig. 3*A*, lanes 3 and 4) or after addition of nuclear extract from wild-type baculovirus-infected Sf9 cells (Fig. 3*A*, lanes 5 and 6). Similarly, no DSS-dependent cross-linked rTHRα-containing complex was detected by Western blot analysis with the rTHRα antibody in rTHRα-containing nuclear extract alone (Fig. 3*B*, lanes 3 and 4) or in that extract mixed with extract from wild-type baculovirus-infected cells (Fig. 3*B*, lanes 5 and 6). Despite the presence of the easily detectable heterodimer in either analysis, no DSS-specific cross-linked rTHRα homodimer was detected.

Since H-2RIIBP formed heterodimers with rTHRα in solution and when bound to the ME-TRE, we anticipated that

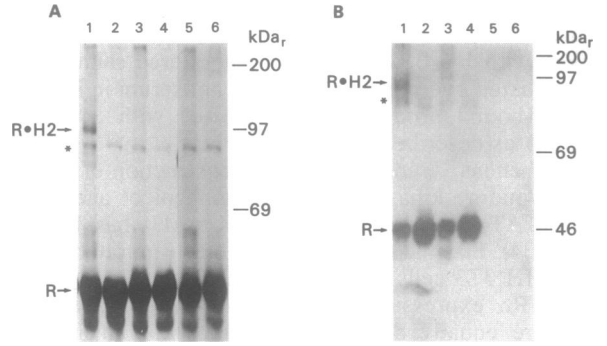

FIG. 3. Chemical cross-linking of rTHRα to H-2RIIBP. (*A*) Cross-linking of [^{35}S]methionine-labeled rTHRα to H-2RIIBP. Autoradiograph of SDS/PAGE-separated products resulting from incubation of [^{35}S]methionine-labeled rTHRα synthesized in an *in vitro* translation system (Promega) with 300 fmol of unlabeled H-2RIIBP (lanes 1 and 2), 300 fmol of rTHRα (lanes 3 and 4), or 8 μg of nuclear extract from wild-type baculovirus-infected Sf9 cells (lanes 5 and 6) for 4 h at 4°C in 1× BB before the addition of the cross-linking reagent DSS to a final concentration of 0.25 mM (lanes 1, 3, and 5). After an additional 30-min incubation, samples were quenched by the addition of 1 μl of 1 M NH_4Cl, analyzed by SDS/PAGE in a 7% gel, and autoradiographed. (*B*) Identification of cross-linked products with rTHRα-specific antiserum. rTHRα (33 μmol, lanes 1–4) and H-2RIIBP (5 μmol, lanes 1, 2, 5, and 6) were cross-linked with DSS as described above (lanes 1, 3, and 5), subjected to SDS/PAGE in a 10% gel, transferred to a nitrocellulose filter, and probed for rTHRα-containing species with rTHRα antisera and ^{125}I-labeled protein A. Molecular mass standards are as follows: myosin, 200 kDa; phosphorylase *b*, 97 kDa; BSA, 69 kDa; ovalbumin, 46 kDa. *, Non-DSS-dependent complex of unknown composition.

expression of H-2RIIBP in cells expressing rTHRα should affect the rTHRα–TH–ME-TRE-dependent transcriptional activation of a reporter gene (34). Transfection of 12.20 μg of only the H-2RIIBP expression plasmid (RSVH-2RIIBP) with the ME-TRE–TK–CAT reporter plasmid had no appreciable effect on CAT expression in the presence or absence of TH (Fig. 4*A*). As expected, transfection of 12.20 μg of the rTHRα expression (RSVRTHRα) plasmid in the presence of TH resulted in a 24-fold increase in CAT expression when compared to the amount of CAT expressed in the absence of TH as reported (34). Nevertheless, when RSVRTHRα expression plasmid (12.20 μg) was cotransfected with gradually increasing amount of RSVH-2RIIBP, higher TH-stimulated CAT expression was observed (Fig. 4*A*). Equivalent amounts of RSVH-2RIIBP and RSVRTHRα expression plasmid cotransfected in the presence of TH resulted in an ≈80-fold increase in TH-stimulated CAT expression compared to the level of CAT expression in the absence of TH. Thus, addition of RSVH-2RIIBP expression plasmid resulted in a 3-fold increase in TH-stimulated CAT expression above that observed with RSVRTHRα alone (Fig. 4*A*). Similar enhancement was observed when utilizing either the palindromic TRE derived from the rat growth hormone gene or by replacing the entire ME-TRE–TK portion of the reporter plasmid with the full-length ME promoter containing the TRE but lacking the TK promoter (data not shown).

Further, we sought to determine whether the concentration of rTHRα affected the observed RSVH-2RIIBP dose-dependent stimulation of rTHRα–TH-responsive CAT expression. In this experiment an increasing amount of the RSVRTHRα expression plasmid was added to 12.20 μg of RSVH2RIIBP. Surprisingly, H-2RIIBP-enhanced rTHRα-mediated transcription was dependent on a minimal concentration of cotransfected RSVRTHRα (Fig. 4*B*). Cotransfection of 12.20 μg of RSVH-2RIIBP with 0.03 μg or 0.13 μg of RSVRTHRα, corresponding to a 5- or 17-fold increase in TH-dependent transcriptional activation in the presence of RSVRTHRα alone, had very little effect on CAT expression (Fig. 4*B*). However, inclusion of higher amounts of RSVRTHRα (0.60–12.20 μg) resulted in a H-2RIIBP-dependent increase in TH-mediated transcription of 3- to 4-fold (Fig. 4*B*).

We hypothesize that when a limiting amount of RSVRTHRα was transfected into NIH 3T3 cells, it complexed with endogenous H-2RIIBP-like protein, and thus, an excess of H-2RIIBP had little effect on CAT expression. However, this could be reversed by adding more RSVRTHRα to that amount of RSVH-2RIIBP. We therefore suggest the existence of a limited amount of endogenous H-2RIIBP-like factor in NIH 3T3 cells, which would be necessary for maximal TH- and THR-mediated induction of gene expression.

DISCUSSION

Our results demonstrate that H-2RIIBP formed a heterodimer with THR and that this heterodimer formation resulted in an apparent increase of affinity of THR for a TRE. These results are similar to those reported by others where protein(s) from crude or partially purified nuclear extracts derived from GH3 cells (13, 39, 40), JEG-3 cells (13, 14), 235-1 cells (13), or liver (15, 16) were shown to enhance the binding of THR to a TRE despite the ability of these extracts to bind a TRE weakly (13) or not at all (15). The observation that some of these protein(s), generally referred to as THR auxiliary proteins, were also shown to alter the mobility of a THR-bound TRE complex in polyacrylamide gels suggests that they may also be binding the TRE (15, 16). Similarly, H-2RIIBP altered the mobility of the rTHRα–ME-TRE complex by forming a heterodimer with rTHRα on the TRE. Whether any of the enhancing activities in these cells could be ascribed to H-2RIIBP present in these extracts must wait for further purification and characterization of those enhancing activities. H-2RIIBP is present in appreciable levels in a variety of tissues and cell lines (26), but it is unlikely that all the enhancing activity is due to H-2RIIBP since the estimated molecular mass of the suspected protein(s) is in the range of 42–63 kDa rather than 44 kDa, the molecular mass of H-2RIIBP (26).

However, the data presented here suggest that it was the formation of heterodimers of rTHRα and H-2RIIBP in solution that modulated the TRE binding activity of rTHRα, since H-2RIIBP bound the ME-TRE only in the presence of rTHRα in our DNA binding assay. Further, rTHRα formed stable heterodimers with H-2RIIBP even in the absence of the ME-TRE. Nevertheless, it is still unclear how H-2RIIBP increased the apparent binding affinity of rTHRα for the TRE. H-2RIIBP could just enable more rTHRα to bind a TRE by simply increasing the amount of species capable of binding this TRE through heterodimer formation (an increase in binding capacity) and/or the heterodimer actually could have a higher affinity for the TRE. Either scenario raises interesting questions regarding the specificity of heterodimer binding, including the role of TH in these processes.

The coexpression of the H-2RIIBP with the rTHRα expression plasmid in NIH 3T3 cells also led to a 3- to 4-fold increase in the amount of TH–rTHRα–ME-TRE–TK-dependent expression of the reporter gene over that observed with rTHRα and TH alone. Thus by considering these results and the binding studies, we speculate that the H-2RIIBP-enhanced transcription was the result of H-2RIIBP recruiting rTHRα to bind more efficiently. However, it is also possible that H-2RIIBP may have the ability to interact with the transcriptional machinery and, thereby, directly contribute to increased transcriptional activation.

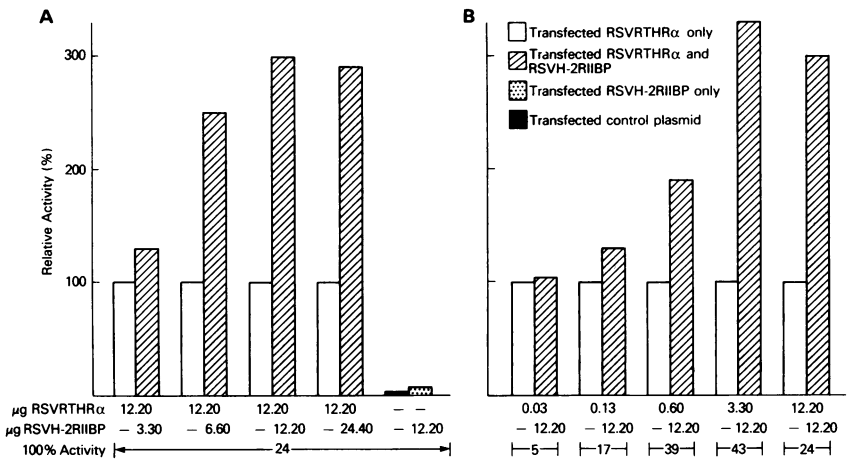

FIG. 4. Effect of rTHRα and H-2RIIBP on rTHRα–TH–ME-TRE-dependent expression of the CAT reporter gene. (*A*) Enhancement of TH-dependent transcriptional activity by H-2RIIBP. Reporter plasmid pTK1AM3 (ME-TRE–TK–CAT) was electroporated into NIH 3T3 cells with H-2RIIBP (pRSVH-2RIIBP), rTHRα (RSVRTHRα), both, or control plasmid alone (RSVΔNEO). Activity at 100% refers to a 24-fold increase in CAT expression obtained with 12.20 μg of transfected RSVRTHRα in the presence and absence of TH. (*B*) Dependence of H-2RIIBP-mediated enhancement of rTHRα–TH-responsive transcription on the concentration of RSVRTHRα. Activity at 100% refers to the increase in CAT expression obtained with transfected RSVRTHRα alone in the presence and absence of TH.

The possibility that rTHRα interacts with a H-2RIIBP-like protein or H-2RIIBP itself to elicit maximal TH-dependent regulation of gene expression *in vivo* is suggested by our experiments in NIH 3T3 cells. We demonstrated that a certain concentration of rTHRα expression plasmid, corresponding to that which we hypothesize is required to titrate endogenous H-2RIIBP-like protein(s), was necessary to observe a H-2RIIBP-dependent increase in transcription. The existence of a natural heterodimer partner is supported further by studies that demonstrated that the ability of THR to heterodimerize and the transcriptional activity of THR were not mutually exclusive. Several mutations constructed in the C terminus of THR abolished the ability of THR to form a heterodimer with an enhancing protein(s) present in 235-1 cells and to activate transcription (41). The requirement for THR to interact with a natural heterodimer partner might also explain why THR can only mediate TH-induced gene expression from some HREs, regardless of the observed high binding affinity that the receptor has for some non-TH-responsive HREs *in vitro* (42–46).

H-2RIIBP and rTHRα join a growing list of transcriptional regulatory proteins (4, 8, 47), including Jun/Fos (1, 2, 5, 7) and Myc/Max (3), that form heterodimers with altered binding and transcriptional activation through respective cis-acting DNA elements. Thus, our results of heterodimer-induced modulation of DNA binding and transcriptional activation underscore the diversity of transcriptional regulation in the steroid/TH receptor superfamily and offer insight into the hormone-specific regulation of gene expression.

Note Added in Proof. Since this manuscript was submitted for review, four papers have been published on the same subject (48–51). In general, our results are concordant with these.

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