Gene Expression, Vol. 6, pp. 169–184, 1996
Printed in the USA. All rights reserved.

1052-2166/96 $10.00 + .00
Copyright © 1996 Cognizant Comm. Corp.

Cross-Talk Between Thyroid Hormone and Specific Retinoid X Receptor Subtypes in Yeast Selectively Regulates Cognate Ligand Actions

P. G. WALFISH,*[1] Y.-F. YANG,* T. YOGANATHAN,* L. A. CHANG,* AND T. R. BUTT†

**Gene Expression Laboratory, Samuel Lunenfeld Research Institute, Division of Endocrinology and Metabolism, Head and Neck Oncology Program, Mount Sinai Hospital and Department of Medicine, University of Toronto Medical School, 600 University Avenue, Room 781, Toronto, Ontario M5G 1X5, Canada*

†Gene Transcription Technology, P. O. Box 1752, Southeastern, PA 19399-1752 and Department of Biochemistry and Biophysics, University of Pennsylvania, School of Medicine, Philadelphia, PA 19104-6059

Thyroid (T3) hormone β_1 (TR) and 9-*cis* retinoic acid (9c-RA) retinoid X receptors (RXR) can form heterodimer complexes that bind to hormone response elements (HREs) in target genes to either activate or repress transcription. However, the action of each cognate ligand and the accessory cellular factors that can differentially regulate the transcriptional responses of a heterodimer–DNA complex are not well understood. Studies in most mammalian cell lines have demonstrated that 9c-RA cannot bind or transactivate TR/RXR–T3 response element (TRE) complexes. In contrast, when identical heterodimer complexes were coexpressed in the yeast (*Saccharomyces cerevisiae*) with single copy typical TREs [i.e., DR+4 (direct repeat), F2 (everted repeat), or PAL (inverted repeat) DNA response elements] we observed that i) unliganded TRβ_1 homodimers had constitutive action on F2 and PAL but not DR4 TREs; ii) TRβ_1 homodimer responsivity to T3 ligand was relatively weak (less than twofold) and was only demonstrable on F2 but not PAL or DR4-TREs, whereas TRβ_1 heterodimers responded to T3 when RXRγ but not RXRα was the heterodimeric partner; iii) RXR responsivity to 9c-RA (three- to sixfold) could be demonstrated only on palindromic TREs that could be enhanced by TRβ_1 on all TREs; iv) T3 + 9c-RA ligands increased (additively or synergistically) transactivation when RXRγ but not α heterodimerized with TRβ_1 on both typical as well as atypical (DR1, DR3, DR5, and F2M) TREs. Substitutions for wild-type TRβ_1 of C-terminus mutants deficient in dimerization with RXRs abrogated the anticipated single and dual cognate ligand-induced effects on TRβ_1/RXRγ transactivation of DR4 TREs, whereas mutants with preserved dimerization function but impaired T3 transactivation regions could maintain an enhanced 9c-RA response but were devoid of the anticipated T3 and dual (T3 + 9c-RA) cognate ligand-induced effects. Thus, the ligand-inducible respones of TR and RXR homodimers expressed in yeast are relatively weak but can be further enhanced by TRβ_1 cross-talk with specific RXR subtypes in the presence of both cognate ligands.

Thyroid hormone (T3) receptors Retinoid X nuclear receptors Transcriptional regulation
Subtype specificity allosterism Ligand-dependent synergy

Received September 6, 1996; revision accepted October 21, 1996.
[1]Address correspondence to P. G. Walfish, Mount Sinai Hospital, Endocrine Unit, Room 781, 600 University Avenue, Toronto, Ontario, M5G 1X5, Canada.

THYROID hormone (T3) receptor (TR) and retinoid X receptor (RXR) are members of the nuclear receptor superfamily of transcriptional proteins that regulate complex genetic networks and therefore control diverse biological aspects of growth, development, and homeostasis [(14,15, 43) and the references therein]. TR and RXR are activated by their cognate ligands L-triiodothyronine (T3) and 9-*cis* retinoic acid (9c-RA) (20,37), respectively. Additionally, multiple subtypes derived from different genes have been detected for TR α and β (28) and RXR α, β, and γ (35). Although these receptor subtypes are differentially expressed in mammalian tissues (28,35), their precise functional roles are not known. These nuclear receptors modulate gene expression by binding as monomers, homodimers, or heterodimers to AGGTCA hexameric core motifs with variations in sequence, spacing, and orientation called hormone response elements (HREs), which are located in the regulatory region of target genes (14,15,43). Typical thyroid response elements (TREs) have been identified in the rat growth hormone gene as an inverted repeat with 0 bp core spacing (PAL), the chicken lysozyme gene promoter as an everted repeat with 6 bp spacing (F2), and direct repeats (DRs) spaced by 4 bp in such natural genes as malic enzyme and myosin heavy chain (15). Compared to the steroid (class I subgroup) nuclear receptors, which bind to HREs only as homodimers, TRs and other members of the class II subgroup [i.e., retinoic acid receptor (RAR) and vitamin D (VDR) receptors] bind with high affinity to specific HREs as heterodimers with RXRs (43). Consequently, RXR may serve as an important pleiotropic coregulator of TR, RAR, and VDR receptor signaling pathways through their formation of heterodimers with these receptors to enhance DNA binding [(14,15,43) and the references therein]. RXR can also can function as an active receptor that is responsive to its cognate ligand 9c-RA as either a homodimer or when it heterodimerizes with specific orphan receptors (29,43).

Traditional assays for measuring the transactivational properties of nuclear receptors have utilized mammalian cell systems that contain variable amounts of endogenous TR and RXR subtypes as well as cognate ligands. Therefore, the elucidation of precise contributions of each heterodimer partner to ligand-dependent transcription in mammalian cells has been difficult to unravel. Current knowledge of transcriptional regulation suggests that TR and RXR must specifically interact with a number of other transcription factors and the basal transcriptional apparatus (59) to determine whether transrepression or transactivation will occur in the presence or absence of their cognate ligands. The identification of the functional roles of such interacting proteins with TRs and RXRs would provide novel and important insights into the molecular mechanisms of signal transduction. Previous reports supporting successful reconstitution of many mammalian nuclear receptors function in yeast cells such as steroid (40–42,45,55), TR (17,33,39,49,52,63), RAR (1,18,19,40), and VDR (23,25,44) strongly suggest that the basic mechanisms of RNA polymerase II transcription between yeast and mammalian cells are very similar. The yeast, *S. cerevisiae*, is a primitive eukaryote devoid of nuclear receptor homologues and the ligands commonly present in mammalian cells. Thus, yeast could provide a simple and unique cellular context to study protein-protein and protein-DNA interactions regulating the action of class II nuclear receptors and their cognate ligands [(5) and the references therein].

In the present report, we have attempted to reconstruct in yeast the transactivational responses of TRβ_1 and two different RXR subtypes (α and γ) to their cognate ligands using single copy DNA response elements of different configurations. Novel paradigms of transcriptional regulation of TR/RXR-DNA complexes have emerged from these studies that differ from those observed in mammalian cells. We show that yeast facilitates the detection of TRβ_1 constitutive function on TRE palindromes but mediates only weak ligand-dependent responses. These functions contrast with the property of TRβ_1 to act as a transcriptional silencer in mammalian cells and modulate a potent ligand-inducible response (12,13,36). We also demonstrate that, contrary to those studies in mammalian cells, RXR can function as an active ligand-responsive receptor when coexpressed in yeast with TRβ receptors that have intact dimerization subdomains. Consequently, our studies of TR and RXR receptor function in yeast have permitted the detection of dual ligand-dependent effects on heterodimers that can be modulated by different RXRs subtypes and DNA configurations.

MATERIALS AND METHODS

Yeast Strains and Media

The *S. cerevisiae* strains BJ2168 (*MATa, ura*3, *leu*2, *trp*1) used for triple transformation and

YPH499 (*MATa, ura3, lys2, ade2, trp1, his3, leu2*) used for quadruple transformation were grown in YPD or selective medium (0.67% yeast nitrogen base and 2% glucose), and utilized for the coexpression of receptor proteins and for β-galactosidase assays (β-GAL). For YPH499, the selective medium was supplemented with adenine (40 mg/l) and lysine (40 mg/l).

Yeast Plasmids for Nuclear Receptors and Reporter Genes

Rat TRβ$_1$ was cloned downstream of a CUP1 promoter into a 2 μ multiple copy (20–30 copies/cell) yeast expression (Yep) vectors containing TRP1 or LEU2 selectable markers (49). The mouse RXRα was under the control of a PGK promoter of Yep10 vector containing a TRP1 marker (18). The human TRβ$_1$ was cloned downstream of a GPD promoter into a p2HG vector containing HIS3 selectable marker (17). The chicken RXRγ was inserted downstream of a PGD promoter of a pG1 vector containing TRP1 marker (17). Human RXRα and mouse RXRγ were cloned downstream of CUP1 promoter of Yep vector (1). For reporter plasmids, double-stranded oligonucleotides containing one copy (two half-sites) DNA response elements of F2, F2M, PAL, or PAL × 3 copies elements were inserted into *Xho*I sites upstream from a proximal cytochrome C (CYC1) promoter of yeast plasmid pC2 that was linked to the *E. coli lacZ* gene expressing β-GAL. Reporter plasmids containing one copy DR1, DR3, DR4, and DR5 HREs were constructed by methods previously described (17). D300A and Δ286–305 were constructed by substituting the TRβ$_1$ wild-type Sfi I-Pst I restriction fragment with the corresponding fragment excised from respective mutant cDNAs (48). Construction of TRβ$_1$ T419 and T455 mutants was by methods previously described (39,49).

Analyses of Nuclear Receptor Transactivational Function Using Yeast

Yeast cells were transformed as described previously (49). Transformants were isolated and maintained using the appropriate minimal medium. Cells were treated with appropriate ligand at a final concentration of 1 μM and grown overnight, harvested, washed, resuspended in Z buffer (0.1 M sodium phosphate buffer containing 0.01 M KCl, 1 mM $MgSO_4$, 0.05 M β-mercaptoethanol) and lysed with glass beads (425–600 μ) before centrifugation. The supernatant was collected and the protein concentration was determined by Lowry's method (38) using BSA as a standard. Twenty micrograms of protein was used for β-GAL assay and the activity expressed in Miller units (46). All the data presented represent results obtained from at least three independent experiments.

Electrophoretic Mobility Shift Assays (EMSA)

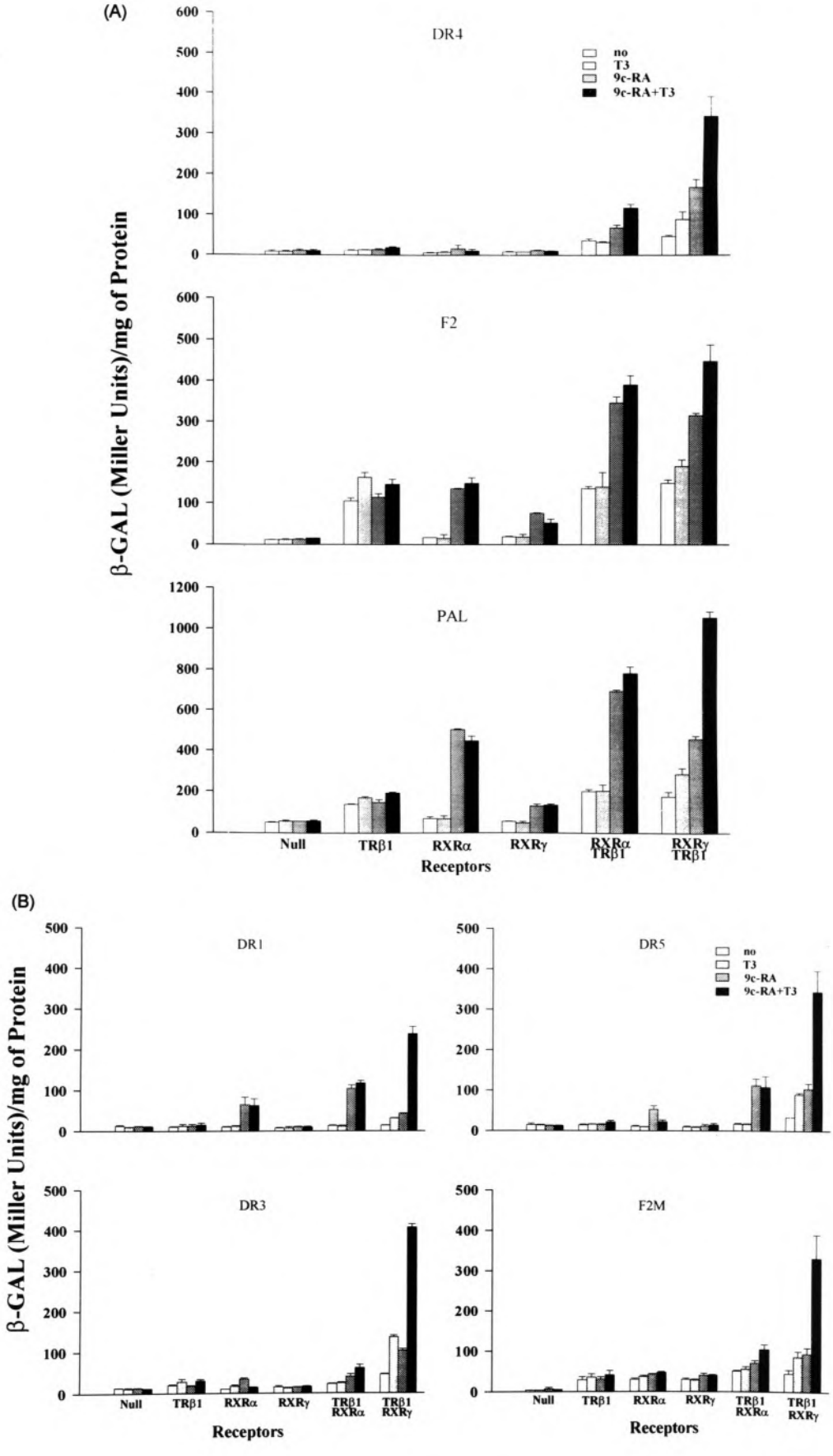
Procedures used to obtain yeast extracts for EMSA were similar to those of β-GAL assays except that yeast culture was treated with 100 μM $CuSO_4$ 2–3 h before harvesting, and yeast cells were resuspended in medium salt buffer [20 mM Tris (pH 7.4), 0.15 M KCl, 20% (v/v) glycerol] for disruption by glass beads. EMSA analyses were performed by a modification of a previous method (47). Briefly, 10 μg of protein was preincubated with binding buffer [20 mM HEPES (pH 7.8), 50 mM KCl, 5% (v/v) glycerol, 1 mM β-mercaptoethanol, 2 μg of poly(dI-dC)·poly(dI-dC)] for 10 min at room temperature before end-labeled ^{32}P-probes (30,000 cpm) (Du Pont-NEM) were added, and the incubation was continued for 15 min. The DNA–receptor complexes were resolved by electrophoresis on a 5% polyacrylamide gel with 0.5 × Tris-borate, EDTA buffer.

Oligonucleotides

Sequences for the sense strand of oligonucleotides for F2, F2M, and PAL for transcriptional studies are as shown in Fig. 1A. The precise DNA sequences utilized for EMSA analyses for DR1, DR3, DR4, and DR5 were identical to those described by other workers (58,62), namely: F2, AT TGACCCCagctgAGGTCAAG; F2M, attGACC GCTctgAGGTCAgg; PAL, tcAGGTCATGACC TgaTCAGGTCATGACCTG DR1, agcttcttcAG GTCAcAGGTCAGagag; DR3, agcttcttcAGGTC AacgAGGTCAGagag; DR4, agcttcttcAGGTCA caggAGGTCAGagag; DR5, agcttcttcAGGTCA ccgaaAGGTCAgagag.

Western Blot Analyses

Procedures used to obtain yeast extracts for Western blot were similar to EMSA. The yeast extract containing receptor or null extract (10 μg total protein) was added to 2× sodium dodecyl sulfate (SDS)/polyacrylamide gel electrophoresis buffer, electrophoresed via 10% SDS polyacrylamide gel, and transferred to nitrocellulose membranes (BioRad Laboratories, Richmond, CA). Membranes were blocked in 5% Carnation milk in


(A)
DR4
F2
PAL
no
T3
9c-RA
9c-RA+T3
β-GAL (Miller Units)/mg of Protein
Null
TRβ1
RXRα
RXRγ
RXRα TRβ1
RXRγ TRβ1
Receptors
(B)
DR1
DR5
DR3
F2M
β-GAL (Miller Units)/mg of Protein
Null
TRβ1
RXRα
RXRγ
TRβ1 RXRα
TRβ1 RXRγ
Receptors

Tris-buffered saline (TBS). Primary TRβ or RXRs antibodies (Santa Cruz Biotechnology Inc., Santa Cruz, CA) were incubated with membranes overnight at room temperature in 1% milk/TBS + 0.1% Tween-20. Goat anti-rabbit secondary antibodies conjugated to horseradish peroxidase (BioRad) were incubated with membranes for 1 h at room temperature in 1% milk/TBS + 0.1% Tween-20. Washes and development of color were as described in the Amersham protocol.

RESULTS

Differences in the Functional Properties of TR and RXR Homodimers

To test the hypothesis that the transactivation properties of either TR and RXR homodimers are dependent upon DNA response element configurations, we first coexpressed TRβ_1, RXRα, and RXRγ with a β-GAL yeast reporter plasmid containing a single copy of one of three differently configured TREs (i.e., DR4, F2, or PAL). The null (control) receptor had small differences on basal transcription that were correlated with TRE configuration (i.e., PAL > F2 > DR4). Because the null receptor was also unresponsive to T3 or 9c-RA on all TREs tested, the absence of endogenous TRs and RXR was again confirmed (Fig. 1A, column 1). Unliganded TRβ_1 but not RXRα or γ homodimers increased basal transcription when coexpressed with a single copy of F2 or PAL-TREs (Fig. 1A, columns 2 to 4, middle and lower panel). In contrast, no significant constitutive or ligand-induced response could be detected on a DR4 TRE when either TRβ_1, RXRα, or RXRγ was present (Fig. 1A, columns 2 to 4, upper panel). Added T3 ligand induced a significant but a weak transcriptional enhancement on a TRβ_1 homodimer when transformed with an F2 but not PAL or DR4 TREs. However, a significant (twofold) increase in transactivation induced by T3 above basal was detected when a multicopy PAL $\times$ 3 reporter was utilized (data not shown). 9c-RA increased the responses of RXRα and RXRγ homodimers above basal by approximately four- to sixfold and two- to threefold, respectively, on F2 and PAL but not a DR4 TRE (Fig. 1A, columns 3 and 6). Thus, the expression of these nuclear receptors in the presence of single copy TREs in the context of yeast detected TRβ_1 homodimer constitutive function and very weak ligand-inducible increases in transactivation.

Differences in TRβ_1/RXR Heterodimer Subtype Responses to Cognate Ligand

Next, we examined the transactivational properties of TR/RXR heterodimers in the absence and presence of cognate ligands. TRβ_1 was coexpressed with RXRα or RXRγ in the presence of single copy DR4, F2, or PAL reporter genes. Compared to their effects as homodimers, coexpression of TRβ_1 with RXRα or RXRγ in the absence of cognate ligands (Fig. 1A, open bars, column 5 and 6) resulted in further transactivational increases only on a DR4 TRE. As a heterodimer partner with TRβ_1, RXRγ facilitated a weak increase in the transactivational response to T3 and to 9c-RA on F2 and DR4 TRE, but not PAL TRE. In contrast, when RXRα was the heterodimeric partner with TRβ_1 no T3-inducible response was observed for TRβ_1. Regardless of RXR subtype, a significant enhancement of 9c-RA responses was retained by heterodimers on all TREs. In contrast to the functional responses of TR/RXR heterodimers cotransfected in mammalian cell wherein 9c-RA is devoid of binding or transactivational effects (12,13,26,36), we observed that identical TR/RXR-TRE complexes in yeast are paradoxically more responsive to 9c-RA than T3 (Fig. 1A, striped bars, columns 5 and 6).

RXR Subtype and Yeast Cell Context Facilitate Dual Ligand-Dependent Synergy

The transactivational properties of TR/RXR heterodimers were also evaluated when both cognate ligands were simultaneously coadded (Fig.

FACING PAGE

FIG. 1. Transactivational responses in yeast of TRβ_1 and RXR receptors when coexpressed with consensus and natural T3 response elements. (A) Ligand-dependent transactivation of TREs by TR and RXRs expressed as homo- or heterodimers. Yeast triple transformants expressing TRβ_1, RXRs, alone or in combination, with either DR4, F2, or PAL *lacZ* reporter were grown in the presence of no ligand (open bar), 1 μM T3 (stripped bar), 1 μM 9c-RA (hatched bar), or 1 μM T3 + 9c-RA (solid bar). β-Galactosidase (β-GAL) assay was performed with yeast extract and the activity was expressed as Miller units/mg protein. The mean and SEs of three different experiments are shown. "Null" indicates the basal reporter activity using expression plasmids devoid of receptor. (B) Ligand-dependent transactivation of atypical TREs by TR and RXRs expressed as homo- or heterodimers. Reporter plasmids containing either DR1, DR3, F2M, or DR5 elements were cotransfected with TRβ_1 and/or RXRs in the presence of no ligand, T3, 9c-RA, or T3 + 9c-RA and similarly analyzed as in (A).

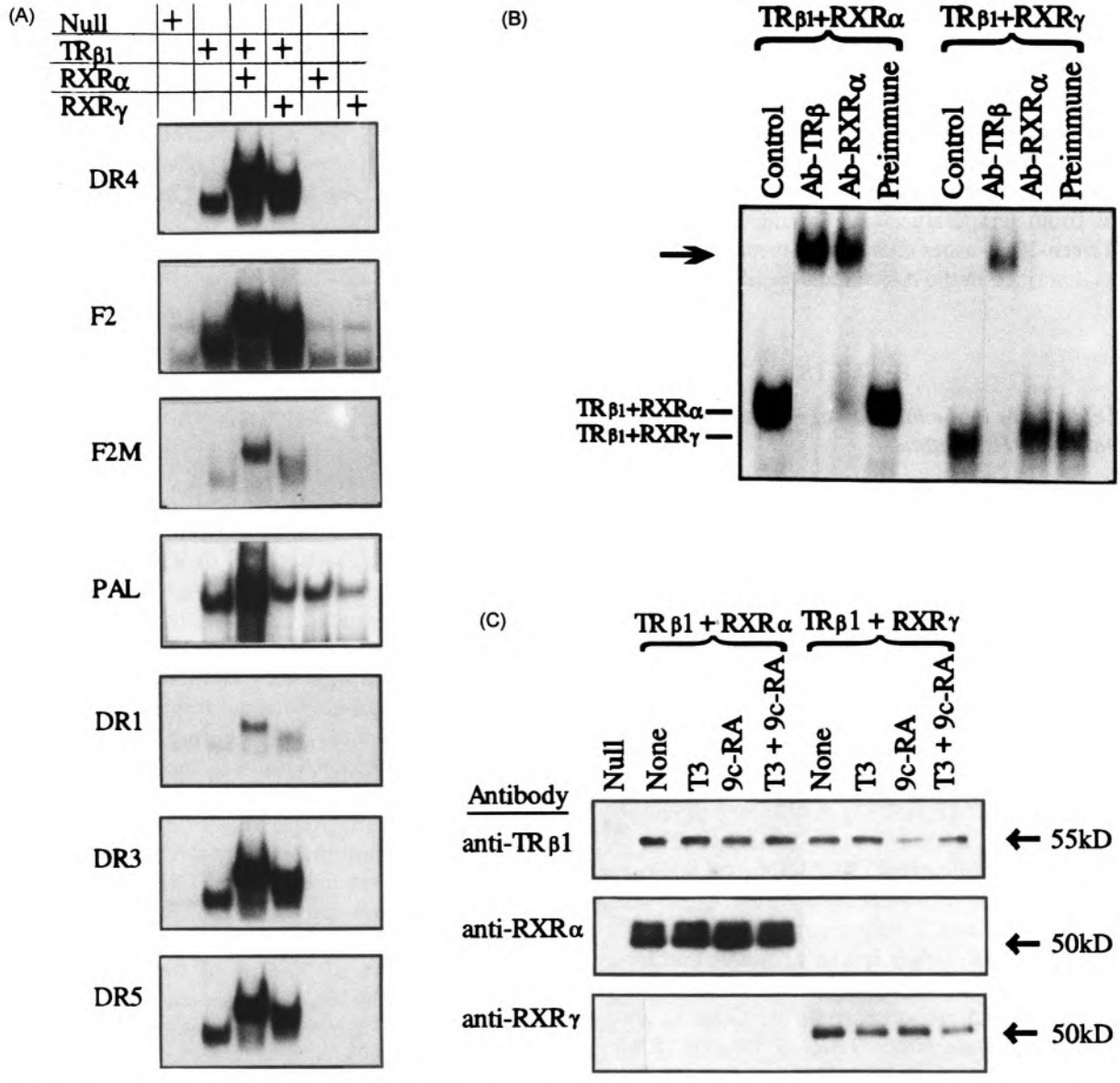


FIG. 2. Electrophoretic mobility shift assays (EMSA) to determine DNA binding properties of recombinant TR and RXRs produced in yeast cells. (A) EMSA of radiolabeled TRE probes with TRβ_1 and RXRs. EMSA were performed using a labeled F2, F2M, or PAL (sequences given in Fig. 1B), DR1, DR3, DR4, and DR5 (sequences given in the Materials and Methods section) in the presence of TRβ_1 or RXR(α or γ), alone or in combination as indicated. "Null" represents the basal DNA binding activity of vector devoid of a nuclear receptor. (B) Binding specificity of DNA–receptor complex. EMSA was performed with a labeled DR4 probe and yeast extracts containing TRβ_1/RXRs heterodimers. The panel shows specific and supershifted complexes of TRβ_1/RXRα heterodimer with polyclonal antibodies of TRβ (Ab-TRβ) or RXRα (Ab-RXRα) as indicated. The supershifted complex is indicated by an arrow. "Control" indicates no antibody was added to the binding reaction, and "preimmune" represents serum collected prior to immunization. (C) Western blot analysis of recombinant receptors. Yeast extracts prepared in the presence or absence of cognate ligands containing homo- or heterodimeric receptors were blotted where indicated with anti-TR or RXRs antibody as described in the Materials and Methods section.

1A, solid bars, columns 5 and 6). Compared to the 9c-RA alone (hatched bars), coexpression of TRβ_1 with RXRα in the presence of coadded T3 + 9c-RA resulted in a small transactivational increase on a DR4 but not F2 and PAL TREs. However, when T3 and 9c-RA were coadded to TRβ_1/RXRγ heterodimers, additive or synergistic (i.e., effects that are greater than those attainable solely by either cognate ligand) transactivational responses were observed that could be modulated by TRE configuration. Although additive or synergistic responses to coadded Triac (10 μM) and 9c-RA (10 μM) or a racemic mixture of retinoids on a chicken RXR and human TRβ_1 heterodimer coexpressed in yeast with a multicopy PAL-TRE reporter have been previously noted (17), we have

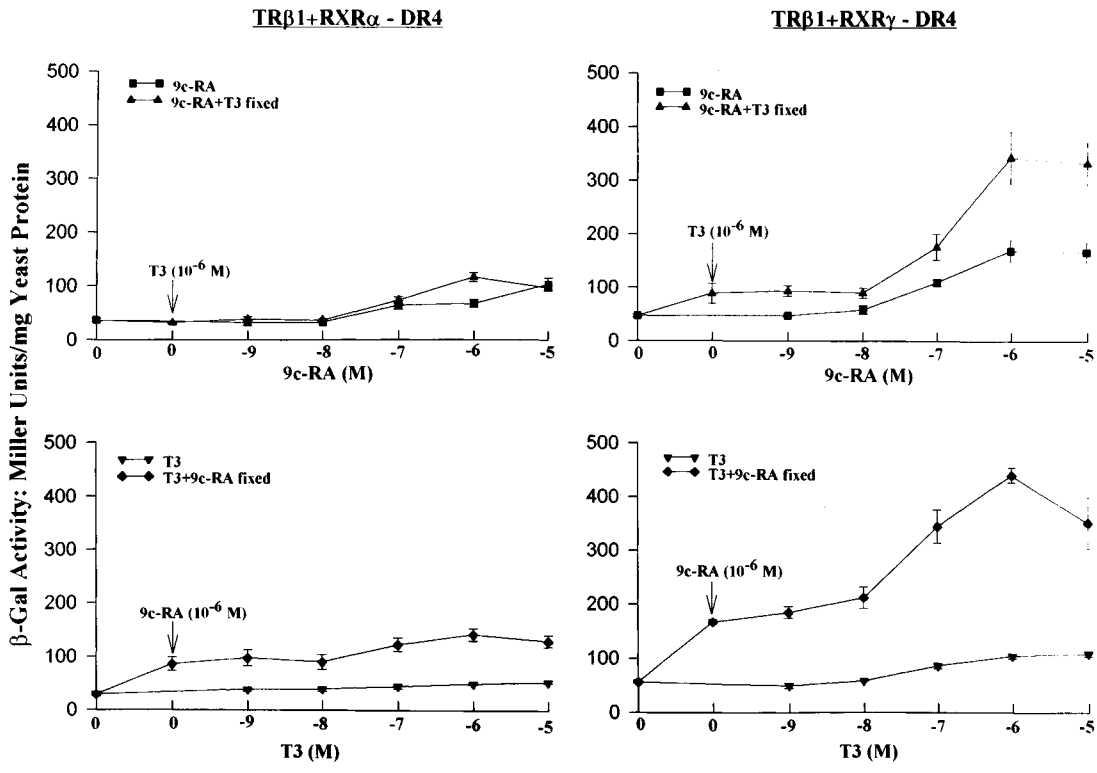


FIG. 3. Allosteric regulation of dual cognate transcriptional enhancement by RXR subtype-specific heterodimers. Ligand-dependent transactivation of TRβ_1/RXR(α or γ) heterodimers on a DR4 TRE reporter plasmid. TRβ_1 coexpressed with RXRα or γ were analyzed for transcriptional responses in the presence of varying concentrations of T3 and/or 9c-RA. The left panels for each figure refer to TRβ_1/RXRα and the right panels to TRβ_1/RXRγ heterodimer responses. The upper panels refer to the transactivation responses to serial concentrations of 9c-RA in the absence (■) or presence (▲) of a fixed saturating concentration (10^{-6} M) of T3, whereas the lower panels show the transactivation responses to serial concentrations of T3 in the absence (▼) or presence (◆) of a fixed saturating concentration (10^{-6} M) of 9c-RA.

demonstrated that the regulation of cognate ligand by the RXR α subtype is different from that of RXRγ. To our knowledge, these are the first studies to demonstrate that different heterodimer subtypes can selectively regulate the action of each cognate ligand.

TRβ_1/RXRγ Heterodimers Permit Transcriptional Synergy on Atypical TREs

Because the above-mentioned studies utilized TREs established to have high DNA binding and transactivational specificity for TR (15), we also assessed the functions of different TR/RXR heterodimer subtypes in the presence of other DNA response element configurations (DNA sequences depicted in the Materials and Methods section). As shown in Fig. 1B, DR1 and DR5 elements (upper panels), considered to be retinoid receptor response elements, as well as a DR3 and the F2M palindrome (lower panels), considered to be VDR elements, had the anticipated weak T3 and 9c-RA-induced effects on TR and RXR homodimers. Unexpectedly, 9c-RA exerted a two- to threefold increase in transactivation on either RXRα or γ/TRβ_1 heterodimers whereas T3 coadded to 9c-RA produced a synergistic response (i.e., fivefold greater transcriptional activation than either ligand alone) when RXRγ, but not RXRα, was the heterodimeric partner with TRβ (see Fig. 1B). Thus, despite the weak unliganded and single cognate ligand TR/RXR responses inducible on these HREs (i.e., three- to fivefold less than on a DR4 TRE) (Fig. 1A), a potent 9c-RA and dual (9c-RA + T3) ligand-dependent synergistic transactivational response could be clearly documented when RXRγ, but not RXRα, was the heterodimer partner.

Correlations of DNA Binding and Transactivation

Using electrophoretic mobility shift assays (EMSA), the DNA binding characteristics of yeast-synthesized TR and RXR receptors were studied. Compared to extracts containing a null

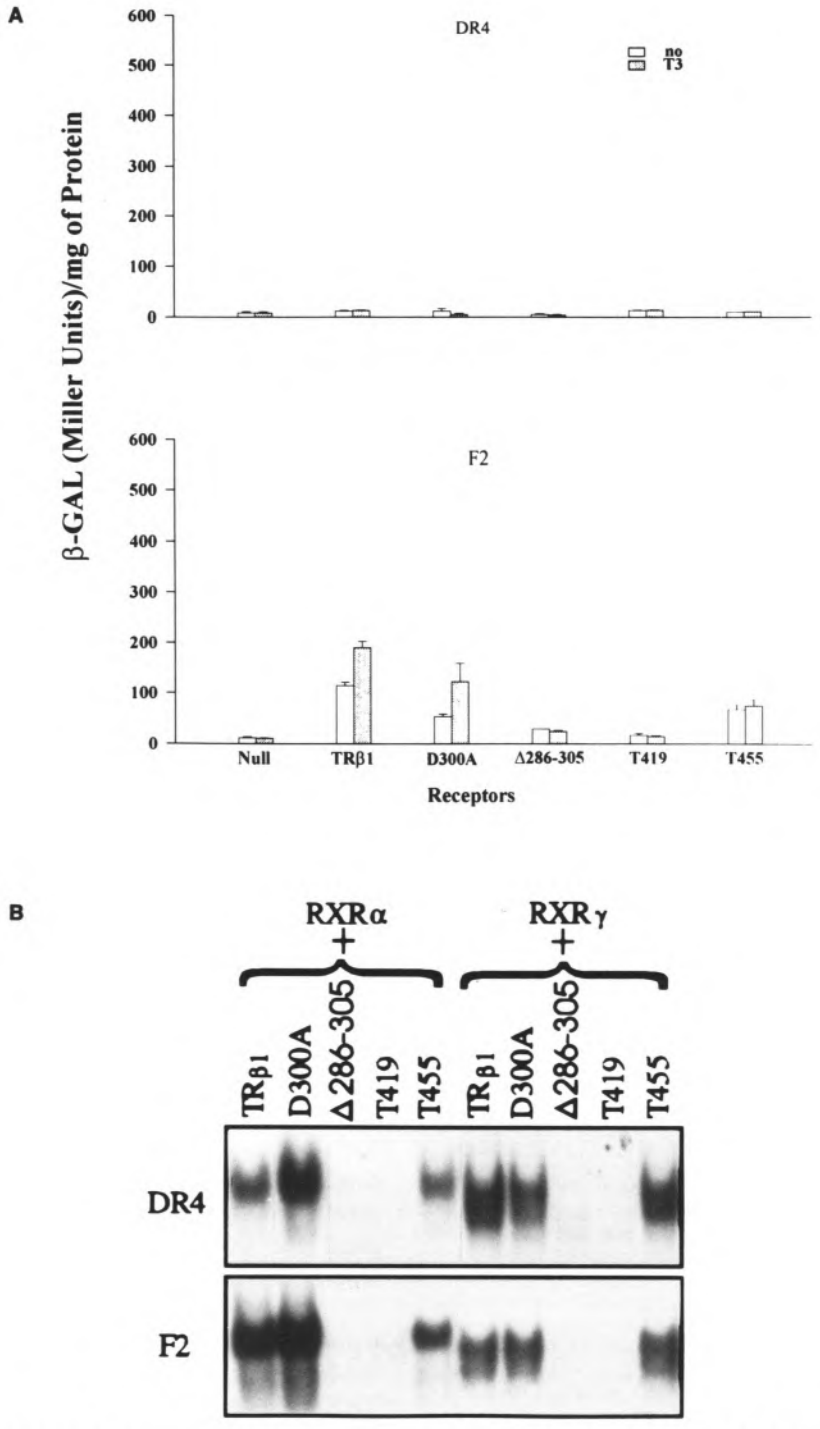


FIG. 4. Structural functional characteristics of C-terminus mutants of TRβ_1 with various LBD C-terminus mutants. (A) Transactivation properties of TRβ_1 and C-terminus mutants compared to wild-type when coexpressed with a F2 reporter plasmid in the absence of ligand (open bar) or presence of T3 (striped bar). "Null" indicates the basal reporter activity using expression plasmids devoid of receptors. (B) DNA binding properties of the four C-terminus mutants of TRβ_1 mutants when coexpressed with RXRs. EMSA was performed using a labeled DR4 or F2 probe with LBD mutants coexpressed with RXRs as indicated.

reporter (Fig. 2A, lane 1), the presence of wild-type TRβ_1 resulted in the formation of a retarded complex in the presence of differently configured typical and atypical HREs (Fig. 2A, lane 2). Retarded bands indicative of heterodimer complex were also demonstrated on all TREs tested when TRβ_1 was mixed with yeast-synthesized RXR (Fig. 2A, lanes 4 and 5). Yeast-synthesized TRβ_1 and RXR homodimer also formed detectable complexes with atypical TREs and formed slower migrating bands with TREs indicative of TR/RXR heterodimers when TR and RXR were present (Fig. 2A, lanes 3 and 4). Not surprisingly, a precise correlation between the DNA binding results by EMSA for these TR/RXR–HRE complexes with the observed single or dual cognate ligand transactivational effects could not be uniformly elicited. Similar discordances in nuclear receptor functions have been reported when using yeast (19,25) or mammalian cell (12,36,61) systems. Taken together, variations in posttranscriptional as well as conformational effects induced by cognate ligand likely regulate transactivation by mechanims which cannot be detected by EMSA.

The specificity of these complexes was confirmed by the elimination of TR/RXR complexes when excess unlabeled DR4 TRE was coadded and the failure of a 100-fold excess of nonspecific DNA to disrupt the complex formation (data not shown). Anti-TRβ_1 or anti-RXRα antiserum produced the appropriate supershifted complex reactions (see Fig. 2B). The addition of ligand did not alter the binding affinity of TR and RXR receptors to DR4 and F2 (data not shown). Western blot analyses confirmed that these receptors expressed in yeast had approximately equal protein concentrations and that the addition of ligand did not significantly alter the level of protein expression (Fig. 2C).

Allosteric Regulation of Cognate Ligand Action by Subtype-Specific Heterodimers

The previously observed dual cognate ligand-dependent enhancement on TR/RXRγ subtype-specific heterodimers raises the possibility that the actions of cognate ligands can be allosterically controlled. To evaluate whether such mechanisms could be operative, the transactivational effects exerted by varying concentrations of each cognate ligand on different TR/RXR heterodimer subtypes were analyzed. Incubation of these transformants with variable concentrations of 9c-RA either singly or with a coadded fixed saturating concentration (10^{-6} M) of T3 demonstrated that the action of 9c-RA induced a signficant enhancement of transactivation only in the presence of the RXRγ, but not RXRα, heterodimer (Fig. 3). Similarly, stimulation with variable concentrations of T3 compared to when a fixed saturating concentration (10^{-6} M) of 9c-RA was coadded achieved enhanced transactivation only on the RXRγ, but not α, heterodimer. Compared to single ligand effects, coadded 9c-RA and T3 at 10^{-6} M augmented by sixfold the absolute transcriptional responses of TRβ_1/RXRγ compared to TRβ_1/RXRα heterodimers. Identical results were obtained when a DR5 TRE β-GAL reporter was utilized (data not shown). Thus, these studies have demonstrated that the optimal action of both cognate ligands is regulated by subtle changes in TR/RXR subtype conformations that will determine the contact of the liganded C-terminus of these receptors with the basal transcriptional appartus of yeast.

Properties of TRβ_1 LBD Mutants Expressed in Yeast

To validate that the differential effects of cognate ligands on these TR/RXR heterodimers were modulated by specific TRβ_1 C-terminus domain, we selected four rat TRβ_1 C-terminus mutants having the following structural modifications: i) a mutation with an aspartic acid to alanine change at aa position 300 (D300A), which retains dimerization with RXR but has a partial reduction in transactivation by T3 in mammalian cells (48) and has a predicted α helix (H) location within the H3–H5 loop of the LBD (65); ii) a mutant with a wide deletion of a highly conserved 20-aa sequence in the DD1 region (Δ286–305), which is essential for dimerization with RXRs (32,48) as well as for T3 transactivation (48) located in the H3–H5 loop (65); iii) a mutant with a 42-aa C-terminus truncation (T419) to include the loss of the ninth heptad (aa 421–428), which disrupts the other dimerization region (DD2) and deletes the C-terminal T3 transactivation region (2) and which would be predicted to eliminate H11 and H12 to expose the LBD cavity and disrupt a dimerization interface (65); and iv) a mutant with a 6-aa C-terminus truncation (T455), which deletes the α-amphipathic-helix region essential for T3 transactivation function (4,60) and prevents T3 ligand contact with H12 (65), while leaving intact the DD1 and DD2 regions necessary for dimerization with RXR.

The transactivational function of these TRβ_1 mutants coexpressed in yeast with either a DR4 or F2 TRE reporter is illustrated in Fig. 4A. Com-

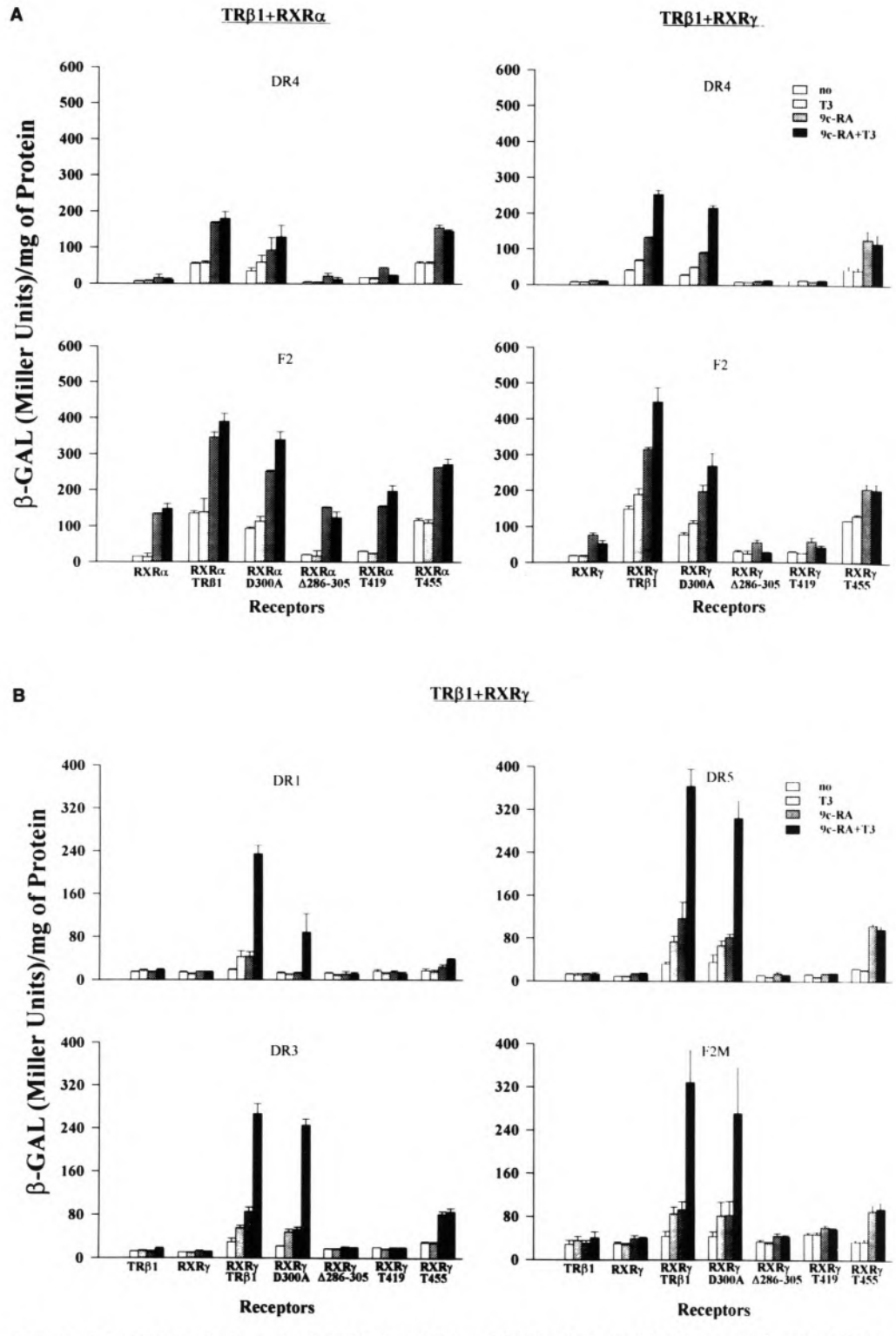


FIG. 5. Substitution of *cis* terminal TRβ_1 for wild-type inhibits single and dual cognate ligand action on heterodimers. (A) The transactivation properties of wild-type TRβ_1 compared to TRβ_1 LBD C-terminus mutants RXR heterodimers RXRs in the presence of DR4 and F2 TREs. LBD TRβ_1 mutants coexpressed with RXRα or RXRγ were analyzed in the presence of no ligand, T3, 9c-RA, or T3 + 9c-RA on β-GAL reporter plasmids. (B) Transactivation properties of wild-type compared to mutant TRβ_1/RXRγ heterodimer in the presence of β-GAL reporter plasmids containing either DR1, DR5, DR3, or F2M HREs in the presence of no ligand, T3, 9c-RA, or T3 + 9c-RA. (C) Effects of C-terminus TRβ_1 mutant D300A compared to T455 on dual cognate-dependent transcriptional enhancement when coexpressed with a β-GAL DR4 reporter. The transactivational responses for 9c-RA alone (■) and in the presence of fixed concentration (10^{-6} M) of T3 (▲) are shown for the D300A/RXRγ heterodimer (upper panels) and for T455/RXRγ heterodimers (lower panels).

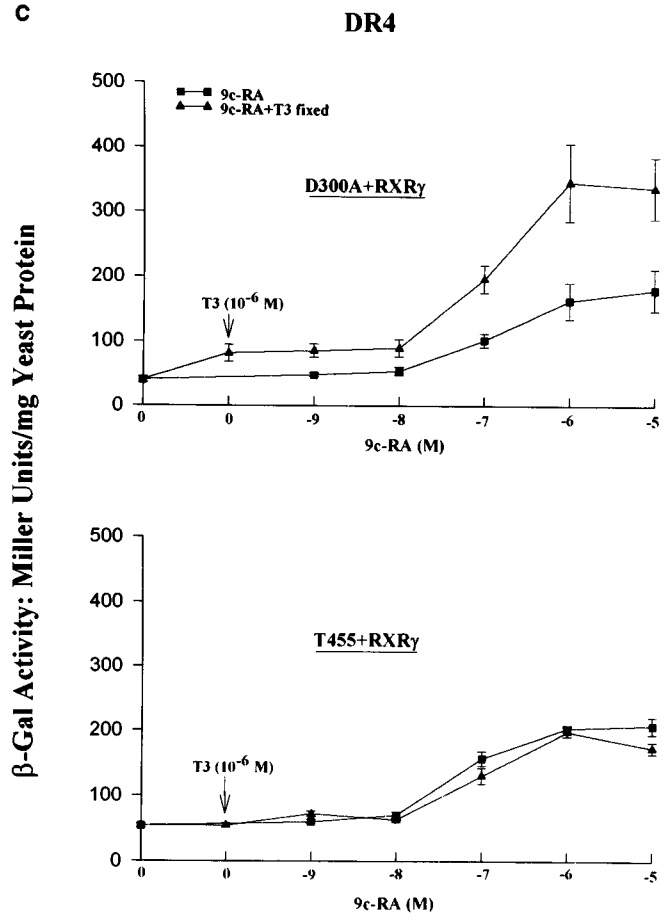


FIG. 5C.

pared to wild-type TRβ_1, Δ286–305 and T419 mutants were virtually devoid of all constitutive and ligand-inducible transactivational function, whereas the D300A TRβ_1 mutant retained detectable but reduced constitutive and T3-induced transactivational function (Fig. 4A). In comparison, the T455 TRβ_1 mutant had reduced constitutive function (Fig. 4A, column 6) and was completely unresponsive to T3 even at concentrations as high as 10^{-4} M (data not shown). To analyze the DNA binding properties of these TRβ_1 C-terminus mutants EMSA studies were performed using yeast extracts. Wild-type TRβ_1 as well as D300A and T455 TRβ_1 mutants formed heterodimers with RXRα and RXRγ, whereas the TRβ_1 Δ286–305 and T419 TRβ_1 mutants failed to dimerize with RXRs (Fig. 4B). Because Western blot analyses confirmed that equal amounts of recombinant proteins were produced in yeast (data not shown), the differences in the transactivation function of these TR mutants therefore cannot be explained by variations in protein expression.

Thus, the transcriptional and DNA binding properties of these mutants when reconstituted in yeast are in agreement to their observed function in JEG-3 mammalian cells (48) and in accord with a report demonstrating that the TRβ_1 DD1 region facilitates homodimerization (32). The near complete loss of T3-inducible transactivation for the T419 and T455 TRβ_1 mutants agrees with previous reports on the functional role for the ninth heptad (2) and the LBD carboxy-terminus F subdomain containing an amphipathic α-helix with hydrophobic and negatively charged faces when transfected in mammalian cells (4,60).

Substitution of TRβ_1 LBD Mutants for Wild-Type Selectively Inhibits Transactivation

We next studied the effects on transactivational function of these TRβ_1 LBD mutants when substituted for wild-type TRβ_1 as a heterodimer partner with either RXRα or RXRγ. Coexpression of the Δ286–305 or T419 TRβ_1 mutants with RXRα or

γ in yeast completely abrogated the anticipated increased transactivation observed for the 9c-RA-induced effect on an RXR homodimer (Fig. 5A, column 4 and 5). These findings were in accord with their failure to form heterodimers (Fig. 4B). Compared to wild-type TRβ_1, the D300A TRβ_1 mutant/RXR heterodimer maintained T3 and 9c-RA responsivity (Fig. 5A, column 3), and the T455 TRβ_1 mutant/RXR sustained an increased transactivational response to 9c-RA when RXRγ was the heterodimer partner (Fig. 5, hatched lines, colum 6,) but could not support either a T3 or T3 + 9c-RA-dependent increase in transactivation (Fig. 5A, column 6). Similar results were obtained when identical studies were performed in the presence of β-GAL reporters containing either DR1, DR5, DR3, or F2M DNA response elements (Fig. 5B). These studies in yeast have established that TRβ_1 dimerization with RXR is essential for an 9c-RA-induced increase in heterodimer function and that T3 transactivational subdomains of TRβ_1 must also be intact to facilitate dual cognate ligand-dependent additive or synergistic transactivational effects on specific heterodimer subtypes.

The T455 and D300A TRβ_1 mutants that could dimerize with RXRs were selected for further studies to compare their effects on the previously observed allosteric control of wild-type TRβ_1/RXRγ heterodimer responsivity to cognate ligands (Fig. 3). When these mutants were substituted for wild-type TRβ_1 as a heterodimer partner with RXRγ, the D300A TRβ_1/RXRγ heterodimer responses to 9c-RA and T3 were not significantly altered (Fig. 5C, upper panel) compared to wild-type TRβ_1/RXRγ (Fig. 3). Although the T455 TRβ_1/RXRγ heterodimer could also maintain 9c-RA-induced transactivation identical to wild-type and D300A TRβ_1/RXRγ heterodimers, it was unresponsive to T3 and could not support anticipated increases in dual ligand (T3 + 9c-RA) transactivation (Fig. 5C, lower panel).

DISCUSSION

Understanding of mammalian nuclear receptor function in yeast is a long-term goal of our laboratory. The current report focuses on the functional properties in yeast of the full-length TRβ_1/RXR receptor heterodimer subtypes and the effect of single and dual (T3 and 9c-RA) ligands on nuclear receptor function on single copy TREs. Owing to the simple and unique cellular context of yeast, new insights into TRβ_1/RXR homodimer function have been achieved and novel molecular paradigms mediated by cross-talk of TRβ_1 on different RXR subtypes demonstrated. We have shown that the unliganded TRβ_1 homodimer did not function as a transcriptional silencer in yeast and that the T3 ligand-inducible transactivational function on TR homodimers and RXR heterodimers could be regulated by TRE configuration and RXR subtype. Moreover, when RXR was a heterodimeric partner with TRβ_1 in yeast it was not silent but was responsive to 9c-RA regardless of RXR subtype and TRE configuration. We also demonstrate that the further additive or synergistic increase in transactivation induced by the coaddition of T3 + 9c-RA in yeast can be regulated by RXR subtype and TREs configuration. A simple interpretation of these studies is that the unique cellular context of yeast is devoid of mammalian-specific transcriptional coregulatory proteins mediating not only constitutive silencing but also those cofactors necessary for a potent ligand-dependent transactivation. Alternatively, the functional behavior of these nuclear receptors in yeast could reflect evolutionary differences from mammalian cells in basal transcriptional machinery.

Additionally, these observations suggest that functional regulation of heterodimers by dual cognate ligand interaction may be a more common theme than previously recognized. Such potential molecular mechanisms could serve to generate diversity in mammalian cell function. A critical question in defining the function of heterodimer receptors is to what extent dual ligands are operative in the control of transcriptional function. Do RXRs merely act as agents that increase the binding of TRβ_1 (and other class II nuclear receptors) to respective HREs or do they play a more active role? Several theories have been proposed which suggest that RXRs can act either as silent (13,43) or active (13,29) partners in promoting 9-c-RA responsivity in cells. To date, the study of nuclear receptors has focused primarily on a limited number of mammalian cell lines (i.e., CV1 cells) (12,13,36), which may not contain the same repertoire of RXRs and regulatory cofactors as other cells. In contrast to the downregulatory effects of coadded 9c-RA to T3 in mammalian cells (12,13,36), addition of 9c-RA to TRβ_1/RXR heterodimers expressed in yeast induces a further increase in transactivation. Thus, the context of yeast facilitates a heterodimer subtype-specific dual ligand-dependent optimization of conformational interactions with one or more coactivators (64) and the transcriptional initiation complex (24). It is also possible that interactions with other basal transcription factors such TBP (9) and

TFIIB (3,51) with TRβ_1 and RXR could be different among mammalian cells. Consequently, it is of interest that T3 + 9-c-RA ligand-dependent synergism has been observed in JEG-3 cells in the presence of a rat GH promoter (53) and *Drosophila* SL3 cells for RXRs and VDR in the presence of an osteopontin promoter (6). In contrast, the studies of VDR action in COS-7 cells and rat osteoblast-like osteosarcoma cell line ROS 17/2.8 (which contain endogenous RXRα and β but not γ) had responses to 1,25 $(OH)_2$ D3, which could not be further enhanced by 9c-RA (11). These studies therefore support the notion that differences in cell-specific accessory factors and promoters of target genes could play an important role in transcriptional regulation of class II nuclear receptor homodimer and heterodimer function.

The observation that RXR can function in vivo as a bonafide receptor in yeast is in accord with the observation that it can act as an active hormone receptor in pituitary cells (10) and when it heterodimerizes with specific orphan receptors (29). It has been postulated that the ratio of RXR to TR must be high for RXR to function as a 9c-RA-responsive homodimer in CV-1 mammalian cells (15,43). However, we observed that when equal amounts of TR and RXR receptors were coexpressed in yeast, RXRs were clearly responsive to 9c-RA. Thus, yeast facilitates the active responsiveness of RXR to 9c-RA when it heterodimerizes with TRβ_1 (27) and might permit a direct contact between 9c-RA and the AF2 region of RXR with specific transcriptional machinery proteins such as TBP (56) or other interacting RXR proteins (5,64). Substitution of wild-type TRβ_1 with a variety of LBD mutations as a heterodimeric partner with RXRs validated the important role of TRβ_1 cross-talk in mediating 9c-RA and selective 9c-RA + T3 enhancement of transactivation in yeast. Mutants that could maintain efficient dimerization with RXRs also functioned as potent enhancers of the 9c-RA-induced signaling in yeast. Thus, we have demonstrated that RXR functions as an active heterodimer partner with TRβ_1 in yeast and DD1 and DD2 dimerization subdomains of TRβ_1 play an essential role in the action of 9c-RA.

Mammalian cells are not entirely suited to study pure heterodimeric function because they contain a repertoire of endogenous receptors and their ligands. Although yeast by no means replaces a mammalian system, our studies demonstrate that it can be used as a model to dissect interactions between various heterodimeric partners and reconstruct the components of eukaryotic transcription regulation. For example, VDR behave differently in yeast than mammalian cells [see (5) and references therein]. Our studies have not determined whether the observed functional differences occurring in yeast can be attributed to differences from mammalian cells in basal transcription apparatus or cell-specific transcriptional cofactors. Very recently, ligand-dependent interactions for TR and RAR nuclear receptors with specific mammalian corepressor proteins N-CoR (22), SMRT (8), and TRAC 2 (54) have been identified, to silence basal transcription by binding to class II nuclear receptors, but are released in the presence of hormone (8,22). In addition, specific mammalian coactivators that function as interacting proteins to include a mouse TIFI (30,34), the human homolog of the adaptor SUGI; TRIPI (31,34) and other T4 interacting proteins (TRIPs) (31); RXR interacting proteins (57); and the estrogen receptor ER associated protein, ER-API60 (16) also termed RIP160; a RIP140 (7); SRC-1 (50), GRIP (21), and ARA_{70} (66) have been discovered and that function as ligand-dependent cofactors. Interestly, a GEN BANK search with the National Institutes of Health BLAST program detected no significant homology of these mammalian coactivators and corepressor to yeast proteins. Although this finding does not preclude the presence in yeast of other functional adaptors, we nevertheless have discovered using yeast that TRβ_1 has both enhanced constitutive and impaired ligand-dependent properties. These observations could result from the absence of specific mammalian nuclear receptor corepressor and ligand-dependent coactivator class II mammalian nuclear receptor interacting proteins in yeast. Such cell-specific differences provide another possible source of diversity in the functionality of the yeast transcriptional machinery. It will therefore be of interest to explore the interactive effects of putative mammalian coregulatory proteins on these nuclear receptors in yeast model system.

In conclusion, our studies of TRβ_1/RXR nuclear receptors homodimer and heterodimer function in yeast have permitted the detection of novel molecular paradigms controlling the action of cognate ligands. TR/RXR heterodimer transactivational function has been observed to be a highly dynamic process that could be regulated by differently configured DNA response elements, cell-specific RXR subtypes, and the actions of both cognate ligands. We believe that yeast provides a valuable tool to unravel the diversities and complexities of eukaryotic transcription and elucidate

the regulatory actions of nuclear receptors and their cognate ligands on target genes.

ACKNOWLEDGEMENTS

We thank R. Y. Wang, E. Pisano, I. Frenkel, L. Xia, X. Zhu, and H. Ohashi for technical assistance; E. Lee for computer graphic assistance; C. Walfish for the preparation of the manuscript; A. Levin of Hoffmann-La Roche, Inc. for supplies of 9 *cis*-retinoic acid; R. Lossant and P. Chambon for the mouse RXRα; B. L. Hall, Z. Smit-McBride, and M. L. Privalsky for chicken RXRγ and reporter genes containing various direct repeats; A. O'Donnell and R. J. Koenig for mutants D300A and Δ286-305; L. J. DeGroot and S.-Y. Cheng for TRβ antibody; H. Towle for rat $TR\beta_1$ and α1 cDNAs; P. Brickell for chicken RXRγ cDNA; R. Evans and J. Dyck for RXRα antibody and cDNA; V. Giguere and M. Shago for technical advice; R. L. Wagner, J. D. Baxter, and R. J. Fletterick for their helpful comments and advice. This work was funded by a grants from Medical Research Council of Canada #MT-12604, the W. Garfield Weston Foundation, The Brian Davidson Memorial Fund of the Ontario Grocery Industry Foundation, The Mount Sinai Hospital Department of Medicine Research Fund, and The Saul A. Silverman Family Foundation, Temmy Latner/Dynacare and The Meadowcroft Group as well as a Research Fellowship from the Thyroid Foundation of Canada (T.Y.).

REFERENCES

1. Allegretto, E. A.; McClurg, M. R.; Lazarchik, S. B.; Clemm, D. L.; Kerner, S. A.; Elgort, M. G.; Boehm, M. F.; White, S. K.; Pike, J. W.; Heyman, R. A. Transactivation properties of retinoic acid and retinoid X receptors in mammalian cells and yeast: Correlation with hormone binding and effects of metabolism. J. Biol. Chem. 268:26625-26633; 1993.
2. Au-Fliegner, M.; Helmer, E.; Casanova, J.; Raaka, B. M.; Samuels, H. H. The conserved ninth C-terminal heptad in thyroid hormone and retinoic acid receptors mediates diverse responses by affecting heterodimer but not homodimer formation. Mol. Cell. Biol. 13:5725-5737; 1993.
3. Baniahmad, A.; Ha, I.; Reinberg, D.; Tsai, S.; Tsai, M. J.; O'Malley, B. W. Interaction of human thyroid receptor β with transcription factor TFIIB may mediate target gene derepression and activation by thyroid hormone. Proc. Natl. Acad. Sci. USA 90: 8832-8836; 1993.
4. Barettino, D.; Ruiz, D. M. V.; Stunnenberg, H. G. Characterization of the ligand-dependent transactivation domain of the thyroid hormone receptor. EMBO J. 13:3039-3049; 1994.
5. Butt, T. R.; Walfish, P. G. Human nuclear heterodimers: Opportunities for detecting targets of transcriptional regulation using yeast. Gene Expr. 5: 255-268; 1996.
6. Carlberg, C.; Bendik, I.; Wyss, A.; Meier, E.; Sturzenbecker, L. J.; Grippo, J. F.; Hunziker, W. Two nuclear signalling pathways for vitamin D. Nature 361:657-660; 1993.
7. Cavailles, V.; Cauviuos, S.; L'Horset, F.; Lopez, G.; Hoare, S.; Kushner, P. J.; Parker, M. G. Nuclear factor RIP140 modulates transcriptional activation for the estrogen receptor. EMBO J. 14:3741-3751; 1995.
8. Chen, J. D.; Evans, R. M. A transcriptional co-repressor that interacts with nuclear hormone receptors. Nature 377:454-457; 1995.
9. Cormack, B. P.; Strubin, M.; Stargell, L. A.; Struhl, K. Conserved and non-conserved functions of yeast and human TATA-binding proteins. Genes Dev. 8:1335-1343; 1994.
10. Davis, K. D.; Berrodin, T. J.; Stelmach, J. E.; Winkler, J. D.; Lazar, M. A. Endogenous retinoid X receptor can funtion as hormone receptors in pitutary cells. Mol. Cell. Biol. 14:7105-7110; 1994.
11. Ferrara, J.; McCuaig, K.; Hendy, G.; Uskokovic, M.; White, J. H. High potency transcriptional activation by 16-ene derivators of 1,25 dihydroxy vitamin D_3. J. Biol. Chem. 269:2971-2981; 1994.
12. Force, W. K.; Tillman, J. B.; Sprung, C. N.; Spindler, S. R. Homodimer and heterodimer DNA binding and transcriptional responsiveness to triiodothyronine (T3) and 9-cis-retinoic acid are determined by the number and order of high affinity half-sites in a T3 response element. J. Biol. Chem. 269:8863-8871; 1994.
13. Forman, B. M.; Umesono, K.; Chen, J.; Evans, R. M. Unique response pathways are established by allosteric interactions among nuclear hormone receptors. Cell 81:541-550; 1995.
14. Giguere, V. Retinoic acid receptor and cellular retinoid binding proteins: Complex interplay in retinoid signalling. Endocr. Rev. 15:61-79; 1994.
15. Glass, C. K. Differential recognition of target genes by nuclear receptor monomers, dimers and heterodimers. Endocr. Rev. 15:391-407; 1994.
16. Halachimi, S.; Marden, E.; Martin, G.; MacKay, H.; Abbondanza, C.; Brown, M. Oestrogen receptor-associated proteins: Possible mediators of hormone-induced transcription. Science 264:1455-1458; 1994.
17. Hall, B. L.; Smit-McBride, Z.; Privalsky, M. L. Reconstitution of retinoid X receptor function and

combinatorial regulation of other nuclear hormone receptor in the yeast *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA 90:6929-6933; 1993.
18. Heery, D. M.; Pierrat, B.; Gronemeyer, H.; Chambon, P.; Losson, R. Homo- and hetero-dimers of the retinoid X receptor (RXR) activate transcription in yeast. Nucleic Acids Res. 22:726-731; 1994.
19. Heery, D. M.; Zacharewski, T.; Pierrat, B.; Gronemeyer, H.; Chambon, P.; Losson, R. Efficient transactivation by retinoic acid receptors in yeast require retinoid X receptors. Proc. Natl. Acad. Sci. USA 90:4281-4285; 1993.
20. Heyman, R. A.; Mangelsdorf, D. L.; Dyck, J. A.; Stein, R. B.; Eichele, G.; Evans, R. M.; Thaller, C. 9-cis retinoic acid is a high affinity ligands for the retinoid X receptor. Cell 68:397-406; 1992.
21. Hong, H.; Kohli, K.; Triverdi, A.; Johnson, D. L.; Stallcup, M. R. GRIP1, a novel mouse protein that serves as a transcriptional coactivator in yeast for the hormone binding domains of steroid receptors. Proc. Natl. Acad. Sci. USA 93:4948-4952; 1996.
22. Horlein, A. J.; Naar, A. M.; Heinzel, T.; Torchia, J.; Gloss, B.; Kurokawa, R.; Ryen, A.; Kamei, Y.; Soderstrom, M.; Glass, C. K.; Rosenfeld, M. G. Ligand-independent repression by the thyroid hormone receptor mediated by a nuclear receptor corepressor. Nature 377:397-403; 1995.
23. Jin, C. H.; Pike, J. W. Human vitamin D receptor-dependent transactivation in *Saccharomyces cerevisiae* requires retinoid X receptor. Mol. Endocrinol. 10:196-205; 1996.
24. Katzenellenbogen, J. A.; O'Malley, B. W.; Katzenellenbogen, B. S. Tripartite steroid hormone receptor pharmacology: Interaction with multiple effector sites as a basis for cell- and promoter-specific action of these hormones. Mol. Endocrinol. 10:119-131; 1996.
25. Kephart, D. D.; Walfish, P. G.; DeLuca, H.; Butt, T. R. RXR isotype identity directs human vitamin D receptor heterodimer transactivation from the 24-hydroxylase vitamin D response elements in yeast. Mol. Endocrinol. 10:408-419; 1996.
26. Kurokawa, R.; Yu, V.; Narr, A.; Kyakumoto, S.; Han, Z.; Silverman, S.; Rosenfeld, M. G.; Glass, C. K. Differential orientations of the DNA-binding domain and carboxy terminal dimerization and interface regulate binding site selection by nuclear receptor hetero-dimers. Genes Dev. 7:1423-1435; 1993.
27. Kurokawa, R.; DiRenzo, J.; Boehm, M.; Sugarman, J.; Gloss, B.; Rosenfeld, M. G.; Heyman, R. A.; Glass, C. K. Regulation of retinoid signalling by receptor polarity and allosteric control of ligand binding. Nature 371:528-531; 1994.
28. Lazar, M. A. Thyroid hormone receptors: Multiple forms, multiple possibilities. Endocr. Rev. 14:184-193; 1993.
29. Leblanc, B. P.; Stunnenberg, H. G. 9-cis retinoic acid signalling: Changing partners causes some excitement. Genes Dev. 9:1811-1816; 1995.
30. Le Douarin, B.; Zechel, C.; Garnier, J. M.; Lutz, Y.; Tora, L.; Pierrat, B.; Heery, D.; Gronemeyer, H.; Chambon, P.; Losson, R. The N-terminal part of TIF1, a putative mediator of the ligand-dependent activation function (AF-2) of nuclear receptors is fused to B-raf in the oncogenic protein T18. EMBO J. 14:2020-2033; 1995.
31. Lee, J. W.; Choi, H. S.; Gyuris, J.; Brent, R.; Moore, D. D. Two classes of proteins dependent on either the presence or absence of thyroid hormone for interaction with the thyroid hormone receptor. Mol. Endocrinol. 9:243-254; 1995.
32. Lee, J. W.; Gulick, T.; Moore, D. D. Thyroid hormone receptor dimerization function maps to a conserved subregion within the ligand binding domain. Mol. Endocrinol. 6:1867-1873; 1992.
33. Lee, J. W.; Moore, D. D.; Heyman, R. A. A chimeric thyroid hormone receptor constitutively bound to DNA requires retinoid X receptor for hormone-dependent transcriptional activation in yeast. Mol. Endocrinol. 8:1245-1252; 1994.
34. Lee, J. W.; Ryan, F.; Swaffield, J. C.; Johnston, S. A.; Moore, D. D. Interaction of thyroid-hormone receptor with a conserved transcriptional mediator. Nature 374:91-94; 1995.
35. Leid, M.; Kastner, P.; Chambon, P. Multiplicity generates diversity in the retinoic acid signalling pathways. Trends Biochem. Sci. 17:427-433; 1992.
36. Leng, X.; Blanco, J.; Tsai, S. Y.; Ozoto, K.; O'Malley, B. W.; Tsai, M. J. Mechanisms for synergistic activation of thyroid hormone receptor and retinoic X receptor on different response elements. J. Biol. Chem. 269:31436-31442; 1994.
37. Levin, A. A.; Sturzenbecker, L. J.; Kazmer, S.; Bosakowski, T.; Huselton, C.; Allenby, G.; Speck, J.; Kratzeisen, C.; Rosenberger, M.; Lovey, A. 9-cis retinoic acid stereoisomer binds and activates the nuclear receptor RXR alpha. Nature 355:359-361; 1992.
38. Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193:265; 1951.
39. Lu, C.; Yang, Y. F.; Ohashi, H.; Walfish, P. G. In vivo expression of rat liver c-erbA β thyroid hormone receptor in yeast *Saccharomyces cerevisiae*. Biochem. Biophys. Res. Commun. 171:138-142; 1990.
40. Mak, P.; Fuernkranz, H. A.; Ge, R.; Karathanasis, S. K. Retinoid X receptor homodimers function as transcriptional activators in yeast. Gene 145:129-133; 1994.
41. Mak, P.; McDonnell, D. P.; Weigel, N. L.; Schrader, W. T.; O'Malley, B. W. Expression of functional chicken oviduct progesterone receptors in yeast (*Saccharomyces cerevisiae*). J. Biol. Chem. 264:21613-21618; 1989.
42. Mak, P.; Young, C. Y. F.; Tindall, D. J. A novel yeast expression system to study androgen action. Recent Prog. Horm. Res. 49: 347-352; 1994.

43. Mangelsdorf, D. J.; Evans, R. M. The RXR heterodimers and orphan receptors. Cell 83:841–850; 1995.
44. McDonnell, D. P.; Pike, W.; Drutz, D. J.; Butt, T. R.; O'Malley, B. W. Reconstitution of the vitamin D-responsive osteocalcin transcription unit in *Saccharomyces cerevisiae*. Mol. Cell. Biol. 9:3517–3523; 1989.
45. Metzger, D.; White, J. W.; Chambon, P. The human oestrogen receptor function in yeast. Nature 334:31–36; 1988.
46. Miller, J. H. Assays for β-galactosidase In: Experiments in molecular genetics. Cold Spring Harbor: Cold Spring Harbor Laboratory; 1972:352.
47. Nelson, C. C.; Hendy, S. C.; Faris, J. S.; Romaniuk, P. J. The effects of P-box substitutions in thyroid hormone receptor on DNA binding specificity. Mol. Endocrinol. 8:829–840; 1994.
48. O'Donnell, A. L.; Koenig, R. J. Mutational analysis identifies a new functional domain of the thyroid hormone receptor. Mol. Endocrinol. 4:715–720; 1990.
49. Ohashi, H.; Yang, Y. F.; Walfish, P. G. Rat liver c-erb A β1 thyroid hormone receptor is a constitutive activator in yeast (*Saccharomyces cerevisiae*): Essential role of domains D, E and F hormone dependent transcription. Biochem. Biophys. Res. Commun. 178:1167–1175; 1991.
50. Onate, S. A.; Tsai, S. Y.; Tsai, M. J.; O'Malley, B. W. Sequence and characterization of a coactivator for the steroid hormone receptor superfamily. Science 270:1354–1357; 1995.
51. Pinto, I.; Ware, D. E.; Hampsey, M. The yeast SUA7 gene encodes a homolog of human transcription factor TFIIB and is required for normal start site selection in vivo. Cell 68:977–988; 1992.
52. Privalsky, M. L.; Sharif, M.; Yamamoto, K. R. The viral erbA oncogene protein, a constitutive repressor in animal cells, is a hormone regulated activator in yeast. Cell 63:1277–1286; 1990.
53. Rosen, E. D.; O'Donnell, A.; Koenig, A. J. Ligand-dependent synergy of thyroid hormone-retinoid X receptors. J. Biol. Chem. 267:22010–22013; 1992.
54. Sande, S.; Privalsky, M. L. Identification of TRACs (T_3 receptor-associating cofactors), a family of cofactors that associate with, and modulate the activity of nuclear hormone receptors. Mol. Endocrinol. 10:813–825; 1996.
55. Schena, M.; Yamamoto, K. R. Mammalian glucocorticoid receptor derivatives enhance transcription in yeast. Science 241:965–967; 1988.
56. Schulman, I. G.; Chakravarti, D.; Juguilon, H.; Romo, A.; Evans, R. M. Interaction between the retinoid X receptor and conserved region of the TATA-binding protein mediate hormone-dependent transactivation. Proc. Natl. Acad. Sci. USA 92:8288–8292; 1995.
57. Seol, W.; Choi, H. S.; Moore, D. D. Isolation of proteins that interact specifically with the retinoid X receptor: Two novel orphan receptors. Mol. Endocrinol. 9:72–85; 1995.
58. Shulemovich, K.; Dimaculangan, D. D.; Katz, D.; Lazar, M. DNA binding by thyroid hormone receptor: Influence of half-site spacing and RXR. Nucleic Acids Res. 23:881–818; 1995.
59. Tjian, R.; Maniatis, T. Transcriptional activation: A complex puzzle with few easy pieces. Cell 77:5–815; 1994.
60. Tone, Y.; Collingwood, T. N.; Adams, M.; Chatterjee, V. K. Functional analysis of a transactivation domain in the thyroid hormone β receptor. J. Biol. Chem. 269:31157–31161; 1994.
61. Tsai, S. Y.; Tsai, M. J.; O'Malley, B. W. Cooperative binding of steroid hormone receptor contributes to transcriptional synergism at target enhancer elements. Cell 57:443–448; 1989.
62. Umesono, K.; Murakami, K. K.; Thompson, C. C.; Evans, R. M. Direct repeats as selective response elements for the thyroid hormone, retinoic acid, and vitamin D3 receptors. Cell 65:1255–1266; 1991.
63. Uppaluri, R.; Towle, H. C. Genetic dissection of thyroid hormone receptor β: Identification of mutations that separate hormone binding and transcription activation. Mol. Cell. Biol. 15:1499–1512; 1995.
64. vom Baur, E.; Zechel, C.; Heery, D.; Heine, M.; Garnier, J. M.; Vivat, V.; LeDourin, B.; Gronemeyer, H.; Chambon, P.; Losson, R. Differential ligand-dependent interactions between the AF2 activating domain of nuclear receptors and the putative transcriptional intermediary factors in mSUG1 and T1F1. EMBO J. 15:110–124; 1996.
65. Wagner, R. L.; Apriletti, J. W.; McGrath, M. E.; West, B. L.; Baxter, J. D.; Fletterick, R. J. A structural role for hormone in the thyroid hormone receptor. Nature 378:690–697; 1995.
66. Yeh, S.; Chang, C. Cloning and characterization of a specific coactivator, ARA70, for the androgen receptor in human prostate cells. Proc. Natl. Acad. Sci. USA 93:5517–5521; 1996.