Heterodimer Formation by Retinoid X Receptor: Regulation by Ligands and by the Receptor's Self-Association Properties†

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Received March 11, 1998; Revised Manuscript Received May 28, 1998

ABSTRACT: The retinoid X receptor (RXR), a nuclear receptor that is activated by 9-*cis*-retinoic acid (9cRA), can regulate transcription as a homodimer or as a heterodimer with numerous other receptors. It was previously shown that, in the absence of ligand, RXR self-associates into homotetramers which are transcriptionally silent, and that ligand-binding induces dissociation of RXR tetramers into active species, dimers and monomers. Here, the implications of tetramer formation by RXR for the ability of the receptor to heterodimerize with the retinoic acid and the vitamin D receptors (RAR and VDR) were studied. In addition, the effects of cognate ligands for RXR and for RAR and VDR on formation of the respective heterodimers were examined. The data indicate that RXR subunits that are sequestered in tetramers were not available for interactions with RAR or VDR and, consequently, that in the absence of a RXR ligand, only a small fraction of this receptor became involved in heterodimers. RXR-selective ligands led to tetramer dissociation, but also inhibited the formation of heterodimers, directing a significant fraction of RXR into homodimers. Ligand binding by either heterodimerization partner significantly stabilized the respective heterodimer. Thus, maximal heterodimerization was observed in the presence of both 9cRA, acting to release active RXR species from tetramers, and the partner's cognate ligand, acting to overcome the inhibitory effect of 9cRA on heterodimer formation. These observations suggest that, by modulating protein−protein interactions within homo- and hetero-oligomers of RXR, cognate ligands control the relative distribution of potential RXR-containing complexes, thereby determining the transcriptional pathways that may be invoked under particular conditions in vivo.

The retinoid X receptor (RXR) is a ligand-inducible transcription factor that belongs to the superfamily of nuclear hormone receptors. Members of this family, which includes receptors for small hydrophobic hormones such as steroids, retinoic acid, vitamin D, thyroid hormone, and metabolites of long-chain fatty acids, associate with specific DNA response elements (REs) in the promoter region of target genes and act either to activate or to repress transcription (*1*−*3*). REs for nuclear receptors are usually comprised of two repeats of the hexameric sequence PuG(G/T)TCA arranged in direct, inverted, or everted repeats, with a variable number of base pairs between the two half-sites (*4*−*6*). As suggested by the repeat structure of their REs, most nuclear receptors bind to cognate DNA as dimers. RXR, which is activated by the 9-cis isomer of retinoic acid (9cRA), can bind to cognate DNA with a high affinity and regulate transcription as a homodimer (*7*−*14*). In contrast, tight binding of some other receptors, e.g., retinoic acid receptor (RAR), vitamin D receptor (VDR), thyroid hormone receptor (TR), and the peroxisome proliferator-activated receptor (PPAR), to cognate DNA usually requires that they heterodimerize with RXR, and their transcriptional activities seem to be exerted mainly via these heterodimers (*15*−*20*). RXR thus plays a central role in regulating a number of signaling pathways.

The molecular mechanisms by which hydrophobic hormones regulate the transcriptional activities of their respective receptors are not completely understood as yet, but available information suggests that ligand binding serves to modulate protein−protein interactions of nuclear receptors with multiple targets. For example, binding of ligands to several receptors, including RAR, TR, and RXR, allows them to interact with coactivator proteins that, presumably, link them with the general transcription machinery (*21*−*28*). Ligand binding by RAR and TR also induces the release of a corepressor that associates with these receptors in the absence of ligands. The corepressor interacts with RXR only weakly and in a ligand-independent fashion, suggesting that the activity of this receptor might be regulated by a different mechanism (*29*−*34*).

In addition to the ability of ligands to modulate the interactions of nuclear receptors with accessory proteins, ligand binding can also influence the self-association properties of these proteins. For example, it was demonstrated that apo-RXR self-associates into homotetramers with a high affinity and that the receptor is predominantly tetrameric at concentrations as low as 70 nM. Little information is currently available on the in vivo concentrations of RXR, but it has been estimated that the nuclear concentration of RAR in HL-60 promyelocytic cells is on the order of 500

† This work was supported by Public Health Service Grant CA68150 from the National Cancer Institute and by Grant 89-34115-4498 from the United States Department of Agriculture.

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S0006-2960(98)00561-3 CCC: $15.00
Published on Web 07/07/1998

nM (*35*; for discussion, see ref *36*). Thus, if the intracellular concentration of RXR is as high as that of RAR, tetramers can be expected to be the major RXR species in cells. Indeed, the presence of RXR tetramers has been demonstrated in mammalian cells as well as in mouse embryos overexpressing this receptor (*37*, *38*). It has been demonstrated that RXR tetramers rapidly dissociate into dimers and monomers upon binding of 9cRA (*36*, *39*, *40*), and it was suggested that the ligand-induced change in the oligomeric state of RXR is the first step in the activation of this receptor (*14*). Interestingly, it was previously observed that ligand binding induces an increase in the population of RXR dimers, and it was proposed that the ligand acts to stabilize RXR homodimers (*18*, *41*). The findings described above suggest that the increase in RXR homodimer population may be an outcome of the ligand-induced dissociation of receptor tetramers. In additional studies, the protein region that mediates tetramer formation by RXR was found to critically contain two phenylalanine residues in helix 11 near the carboxyl terminal of the receptor's ligand binding domain (helix numbering according to ref *42*), and it was demonstrated that protein−protein interactions via this region are abolished upon ligand binding (*14*). Examination of the oligomerization behavior and transcriptional activities of two site-specific mutants of mRXRα has shown that a mutation (F318A) that impairs the ability of RXR to form tetramers results in a receptor that displays substantial transcriptional activity even in the absence of ligand, and that a mutation (R321A) that negates the ability of RXR tetramers to dissociate upon ligand binding abolishes the ligand-dependent transcriptional activity of the receptor (*67*). Taken together, these findings indicate that, in the absence of a cognate ligand, RXR sequesters itself into transcriptionally inactive tetramers and thus functions as an autosilencer. One function of the ligand in activating RXR is thus to induce dissociation of tetramers into dimers which are the receptor's active species. Because, as discussed above, many of the regulatory functions of RXR are exerted via its heterodimers with other nuclear receptors, an important question that arises is how the propensity of RXR to self-associate into tetramers affects its interactions with various partners. Here, the implications of tetramer formation by RXR for the ability of the receptor to heterodimerize with two partner receptors, RAR and VDR, are examined.

Cognate ligands may also function to regulate the interactions of RXR with partner receptors directly. For example, it was reported that 9cRA decreases the stability of heterodimers of RXR with VDR (*41*), with RAR (*18*), and with TR (*43*) and that the VDR ligand 1α,25-dihydroxyvitamin D_3 (D_3) and the TR ligand 3,3′,5-triiodo-L-thyronine (T_3) enhance heterodimerization between RXR and their respective receptors (*41*, *43*−*46*). Transcriptional regulation by RXR-containing heterodimers can potentially respond to the two ligands of the individual subunits. Indeed, a number of studies indicated that, in cells in which RXR is coexpressed with a partner receptor, maximal transcriptional responses require the presence of ligands for both RXR and the partner receptor (*47*). Interestingly, it was reported that RXR-selective ligands do not elicit transcriptional response in cells coexpressing RXR and either RAR or TR. It was consequently suggested that RXR within RXR−RAR and RXR−TR heterodimers is transcriptionally inhibited and serves as a silent partner (*48*−*51*). It was observed further that binding of ligand by the partner subunit can relieve the inhibition and allows RXR to become responsive to its ligand, resulting in a synergistic response to the ligands of the two heterodimerization partners (*47*, *50*−*52*). To explain these observations, it was proposed that the inability of RXR to transactivate when associated with unliganded RAR or TR stems from the receptor's inability to bind ligand under these conditions (*49*, *50*). Inconsistent with this proposition, it was demonstrated that neither the equilibrium binding affinity nor the kinetic parameters of the interactions of RXR with its ligand are affected by formation of RXR−RAR heterodimers, regardless of whether the heterodimers are in solution or bound to cognate DNA (*53*, *54*). The molecular mechanism underlying the inability of RXR ligands to activate this receptor via heterodimers with unliganded TR and RAR is still incompletely understood (for review, see ref *47*).

Detailed knowledge on the effects of cognate ligands on heterodimerization of RXR with partner receptors is thus important for understanding how the transcriptional activities of these heterodimers are regulated. However, most previous studies on this issue were nonquantitative, and even in cases where quantitation was sought (*41*), data analysis did not take into account the self-association properties of RXR or the profound effect of RXR ligands on the oligomeric state of this receptor. Here, the equilibrium dissociation constants characterizing RXR−RAR and RXR−VDR heterodimers were measured, and the effects of cognate ligands on formation of these complexes were examined to gain better insight into the regulatory features of these systems. The findings of this study suggest a molecular mechanism by which ligands may determine the transcriptional pathways that will be invoked under particular conditions in vivo.

EXPERIMENTAL PROCEDURES

Ligands. 9-*cis*-Retinoic acid was a gift from Hoffman La Roche (Nutley, NJ). All-*trans*-retinoic acid was purchased from Kodak. 1α,25-Dihydroxyvitamin D_3 was purchased from Calbiochem. LG1069 and TTNPB were gifts from Ligand Pharmaceuticals (San Diego, CA).

Antibodies were provided by the group of P. Chambon (LGME, Strasbourg, France).

Proteins. Histidine-tagged mouse RXRα and human RARα lacking the N-terminal A/B domain (RXRαΔAB and RARαΔAB) were obtained by overexpression in *Escherichia coli* and isolated as described previously (*36*). Histidine-tagged full-length human VDR was obtained by overexpression in *E. coli* and purified by Ni^{2+}−nitrolotriacetic acid (NTA) agarose beads (Qiagen). Bacteria harboring the VDR expression vector were provided by L. Freedman (Memorial Sloane-Kettering Cancer Research Center, New York). Purified proteins were dialyzed against a buffer containing 10 mM Hepes (pH 8.0), 0.1 mM EDTA, 0.4 mM DTT, 400 mM KCl, and 5% glycerol and stored at −20 °C in 50% glycerol. Protein concentrations were determined with the Bradford assay (Bio-Rad, Hercules, CA) using bovine serum albumin as a standard.

Oligonucleotides. Oligonucleotides containing the following response elements were used: DR-1, a response element containing two direct repeats spaced by a single

nucleotide (5′-TCGAGGGTAGGGGTCAGAGGTCACTCGTCGA-3′), and DR-3, a response element comprised of two direct repeats spaced by three nucleotides (5′-TCGAGGGCAGTTCAAGGAGTTCAGACGTCGA-3′).

The oligonucleotides were synthesized and purified at the Cornell DNA synthesis facility. Single-stranded DNA was annealed (*55*) and double-stranded DNA isolated on Centrex centrifugal filter units. Oligonucleotides were end-labeled with [^{32}P]dCTP by filling in with Klenow fragments, and free nucleotides were removed with the Qiagen nucleotide removal kit.

Electrophoretic Mobility Shift Assays (EMSAs). One microliter of labeled oligonucleotide (50−200 nM, final concentration of 2.5−10 nM) and 1 μL of 2.4 mg/mL dI-dC were mixed with the indicated amounts of receptors in 18 μL of 10 mM Hepes (pH 8.0), 0.1 mM EDTA, 0.4 mM DTT, 100 mM KCl, and 15% glycerol. Ligands were added from a concentrated solution in ethanol or DMSO to a final concentration of 1 μM, and mixtures were incubated for 15 min at room temperature. For the supershift assay, antibodies were then added and the mixture was incubated for an additional 5−10 min at room temperature. Protein−DNA complexes were resolved by electrophoresis on 5% polyacrylamide gels (0.5× TBE, 2−3 h prerun at 100 V, 2 h run at 25 mA/gel); the gel was dried, and protein−DNA complexes were visualized by autoradiography. During electrophoresis, the gel was cooled with circulating water at 12 °C.

Fluorescence anisotropy titrations were carried out using a SPEX (Metachen, NJ) fluorolog-2 spectrofluorometer equipped with Glan-Thompson polarizers. RXRαΔAB was covalently labeled with the fluorescent probe fluorescein as previously described (*36*, *39*). Labeled RXR in a buffer containing 10 mM Hepes (pH 8.0), 0.1 mM EDTA, 0.4 mM DTT, 100 mM KCl, and 5% glycerol was mixed with a partner receptor at the denoted concentrations in the absence or in the presence of a 10-fold (moles of ligand per mole of protein) excess of ligands which were added from a concentrated ethanolic solution. Mixtures were incubated for 15 h at 4 °C, followed by equilibration at room temperature for 1 h. The fluorescence anisotropy ($\lambda_{ex} = 491$ nm, $\lambda_{em} = 516$ nm) was measured four times to obtain a mean. Equilibrium dissociation constants characterizing the heterodimers were obtained by fitting the titration curves to an equation derived from simple binding theory.

RESULTS

Measurements of Equilibrium Dissociation Constants Governing Heterodimer Formation by RXR. Measurements of binding affinities within heterodimers of RXR and a partner receptor are somewhat complicated because of the possible formation of multiple protein complexes. As discussed in the introductory section, RXR self-associates not only into homodimers but also into homotetramers, and tetramer formation proceeds with a high affinity and in a cooperative fashion. Thus, in mixtures containing RXR and a partner receptor, several protein complexes might coexist depending on relative binding affinities and on concentrations of the different receptors present. Reactions 1−4 outline the multiple equilibria that can exist in mixtures containing RXR and a heterodimerization partner (HP).

$$\mathrm{RXR} + \mathrm{RXR} \leftrightarrow (\mathrm{RXR})_2 \quad (1)$$

$$(\mathrm{RXR})_2 + (\mathrm{RXR})_2 \rightleftarrows (\mathrm{RXR})_4 \quad (2)$$

$$\mathrm{HP} + \mathrm{HP} \leftrightarrow (\mathrm{HP})_2 \quad (3)$$

$$\mathrm{RXR} + \mathrm{HP} \leftrightarrow \mathrm{RXR{-}HP} \quad (4)$$

Clearly, investigation of the formation of heterodimers (reaction 4) will be simplified if experimental conditions can be set such that this reaction predominates, i.e., that reactions 1−3 are negligible within the experiment. K_ds for formation of RXR dimers and tetramers were reported to be 60 and 3 nM, respectively, and from these values, it was calculated that at a total RXR concentration of 10 nM, the receptor is >90% monomeric (*39*). Consequently, reactions 1 and 2 can be excluded if the concentration of RXR within an experiment is kept at or below this value. The K_d governing the formation of RAR homodimers has not been reported, but several lines of evidence indicated that RAR homodimers are formed with an affinity significantly lower than those of RXR homodimers (*1*, *2*). K_d for formation of VDR homodimers was reported to be about 600 nM (*41*), and similar to that for RAR, available qualitative information indicates that VDR homodimers do not readily form (*56*). Thus, under conditions where the concentrations of RAR or VDR are kept at or below 50−100 nM, formation of RAR or VDR homodimers (reaction 3) will be minimal. Overall, observed protein−protein interactions in mixtures containing 10 nM RXR and either RAR or VDR at concentrations that are lower than 50 nM will be almost exclusively heterodimerization (reaction 4).

To monitor heterodimer formation, RXR was covalently labeled with the fluorescent probe fluorescein and the labeled protein was titrated with either RAR or VDR. The progress of the titrations was monitored by following changes in the fluorescence anisotropy of labeled RXR. Fluorescence anisotropy is a sensitive measure of the rotational volume of a fluorescent molecule, and has been extensively used to monitor variations in the size of a fluorophore such as those that accompany protein−protein interactions (*57*−*60*). For example, we previously demonstrated that the self-association of RXR into dimers and tetramers can be quantitatively studied by monitoring the concentration dependence of the fluorescence anisotropy of fluorescein-labeled RXR (*36*, *39*, *40*).

Effects of Ligands on Heterodimer Formation by RXR and RAR. Protein−protein interactions between RXR and RAR were studied by using receptors that lack the amino-terminal A/B domain, a region which is not involved in dimer or tetramer formation by either receptor (*14*, *61*). We also previously showed that RXRΔAB is essentially identical to the full-length protein in its ligand binding, DNA binding, and self-association properties (*62*). Histidine-tagged proteins were obtained by overexpression in *E. coli* and purified using Ni affinity chromatography, and RXRΔAB was labeled with fluorescein (see Experimental Procedures). Labeled RXRΔAB, at a concentration at which it is predominantly monomeric (10 nM), was mixed with RARΔAB at various concentrations in the range of 0−20 nM. Mixtures were incubated for 15 h at 4 °C followed by a 1 h equilibration at room temperature prior to measurement of the fluorescence

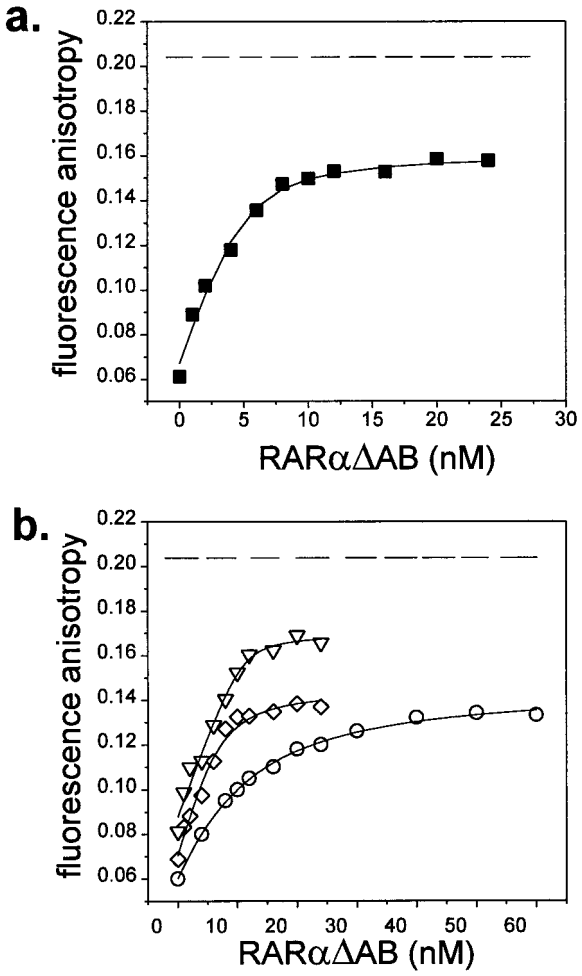

FIGURE 1: Fluorescence anisotropy titration of monomeric RXR with RAR. Fluorescein-labeled mRXRαΔAB at a concentration where it is predominantly tetrameric (10 nM, see the text) was mixed with hRARαΔAB at the denoted final concentrations. Mixtures were incubated, and the fluorescence anisotropy was measured as described in Experimental Procedures. A representative curve obtained in the absence of ligands is shown in panel a. Panel b shows representative titration curves obtained in the presence of a 10-fold (moles of ligand per mole of protein) excess of the following ligands: the RAR ligand tRA (▽), the pan-agonist 9cRA (◇), or the RXR-selective ligand LG1069 (○). Solid lines through data points represent the fits of the titration curves to an equation derived from simple binding theory. Equilibrium dissociation constants obtained in the analyses are given in Table 1. The dashed line indicates the value of the fluorescence anisotropy of tetrameric fluorescein-labeled mRXRαΔAB.

anisotropy. As shown by the representative titration depicted in Figure 1a, the fluorescence anisotropy of labeled RXR increased upon titration with RAR, reflecting heterodimer formation, until a plateau value was reached at saturation. For comparison, the self-association of RXR was monitored under the same conditions by examining the concentration dependence of the fluorescence anisotropy of RXR alone, and the final point of this titration is shown in Figure 1 as a dashed line. The larger value of fluorescence anisotropy that was reached when the self-association of RXR was monitored, as compared with titrations of RXR with RAR, reflects the fact that while RXR self-associates into tetramers, the interactions of RXR with RAR only lead to formation of dimers. The equilibrium dissociation constant (K_d) governing the formation of RXR−RAR heterodimers obtained by analyzing the titration curves is shown in Table 1 alongside the K_ds governing the self-association of RXR. The data indicate that the K_d characterizing RXR−RAR heterodimers is in the sub-nanomolar range and more than 2 orders of magnitudes lower than the K_d for formation of RXR homodimers.

To examine the effects of cognate ligands on the interactions of RXR with RAR, fluorescence anisotropy titrations were carried out in the presence of an RXR-selective ligand (LG1069) or RAR-selective ligands (either tRA or TTNPB). Heterodimerization was also studied in the presence of ligands for both receptors, either a combination of LG1069 and TTNPB or the pan-agonist 9cRA. Representative titration curves obtained in the presence of ligands are shown in Figure 1b. Notably, a considerably higher concentration of RAR was required to reach saturation when the RXR-selective ligand was present alone (○) than under any other condition. These observations suggest that binding energies within RXR−RAR heterodimers are significantly weaker in the presence of the RXR ligand. Analysis of the data (Table 1) indeed indicated that the K_d for the RXR−RAR heterodimer was more than 5-fold higher in the presence of LG1069 as compared to the K_d obtained in the absence of ligands. In contrast with the pronounced effect of the RXR-selective ligand, RAR-selective ligands had little effect on the heterodimerization process on their own. However, in the presence of cognate ligands for both receptors, the RAR ligand negated the inhibitory effect of the RXR ligand, and led to a 2-fold increase in the affinity between the receptors (Table 1). The data reveal that when both partners within RXR−RAR heterodimers are liganded, the complex is 1 order of magnitude stronger than under conditions when only an RXR ligand is present.

Effects of Ligands on Heterodimer Formation by RXR and VDR. Protein−protein interactions leading to formation of RXR−VDR heterodimers were studied by titrating fluorescein-labeled RXRΔAB with full-length VDR (see Experimental Procedures for protein expression and purification protocols). Again, to ensure that self-association of RXR was negligible within the experiments, a concentration of RXR at which the receptor is predominantly monomeric was used. Representative titration curves obtained in the absence of ligands and in the presence of the RXR ligand 9cRA, or the VDR ligand D_3, or both ligands, are shown in Figure 2. Similar to the response of RXR alone (*36*), addition of 9cRA to mixtures containing RXR and VDR led to a decrease in the fluorescence anisotropy, reflecting the fact that ligand binding induced a conformational change in the RXR component within the heterodimer. In contrast, addition of D_3 did not affect the fluorescence anisotropy of the RXR−VDR heterodimer as could be seen by the similar final anisotropy values obtained in the absence and in the presence of this ligand. These observations suggest that ligand binding by VDR did not result in a significant change in the overall rotational volume of this receptor. Fluorescence anisotropy values reached upon saturation of RXR with VDR were similar to those observed for the RXR−RAR dimers and significantly smaller than those of the RXR tetramer, confirming that RXR−VDR interactions lead to formation of dimers but not to formation of higher-order oligomers. K_d values for RXR−VDR heterodimers in the absence and in the presence of ligands are shown in Table 2. Similar to RXR−RAR heterodimers, K_ds characterizing the RXR−

Table 1: Dissociation Equilibrium Constants of RXR−RAR Heterodimers[a]

	K_d (nM)				
complex	no ligand	LG1069	tRA/TTNPB[g]	9cRA	LG1069 and tRA/TTNPB
RXR−RAR	0.43 ± 0.07[b]	2.23 ± 0.65[c]	0.33 ± 0.02[d]	0.21[e]	0.21 ± 0.01[d]
RXR−RXR	130.0[f]			63.0[f]	
$(RXR)_2$−$(RXR)_2$	3.0[f]			high[f]	

[a] Values were obtained from fluorescence anisotropy titrations carried out in the absence or in the presence of the cognate ligands denoted. [b] $n = 5$. [c] $n = 4$. [d] $n = 3$. [e] $n = 2$. [f] Reference *24*. [g] Due to the similarity of the values obtained in the presence of either of the RAR-selective ligands, these data are grouped together as tRA/TTNPB.

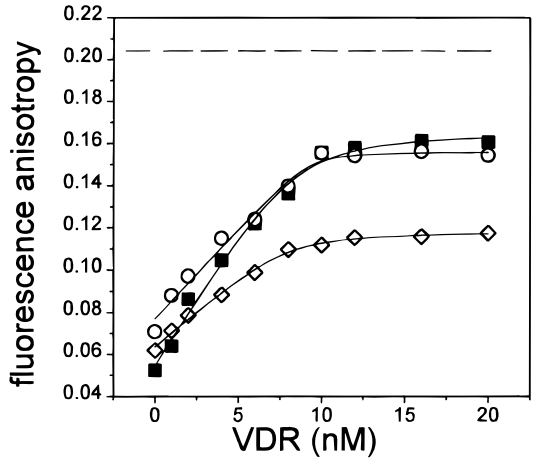

FIGURE 2: Fluorescence anisotropy titration of monomeric RXR with VDR. Fluorescein-labeled mRXRαΔAB at a concentration where it is predominantly tetrameric (10 nM, see the text) was mixed with hVDR at the denoted final concentrations. Mixtures were incubated, and the fluorescence anisotropy was measured as described in Experimental Procedures. Representative titration curves obtained in the absence of ligands (■) and in the presence of a 10-fold (moles of ligand per mole of protein) excess of D_3 (○) or 9cRA (◇) are shown. Solid lines through data points represent the fits of the titration curves to an equation derived from simple binding theory. Equilibrium dissociation constants obtained in the analyses are given in Table 2. The dashed line indicates the value of the fluorescence anisotropy of tetrameric fluorescein-labeled mRXRαΔAB.

Table 2: Dissociation Equilibrium Constants of RXR−VDR Heterodimers[a]

	K_d (nM)			
complex	no ligand	9cRA	D_3	9cRA and D_3
RXR−VDR	0.32 ± 0.02[b]	0.78 ± 0.07[c]	0.14 ± 0.05[d]	0.16[e]

[a] Values were obtained from fluorescence anisotropy titrations carried out in the absence or in the presence of the cognate ligands denoted. [b] $n = 6$. [c] $n = 4$. [d] $n = 3$. [e] $n = 2$.

VDR heterodimer were found to be in the sub-nanomolar range, indicating that protein−protein interactions leading to the formation of the heterodimers were 2 orders of magnitude stronger as compared with those for the formation of the RXR homodimers. 9cRA weakened RXR−VDR interactions by about 2.5-fold. In contrast, addition of D_3 resulted in 2-fold stronger interactions between RXR and VDR. In the presence of both ligands, the K_d was similar to that observed in the presence of D_3 alone, revealing that the VDR cognate ligand can negate the inhibitory effect of 9cRA on formation of the RXR−VDR heterodimers. Thus, similar to the effects of ligand on RXR−RAR interactions (Table 1), the data indicate that when both partners within RXR−VDR heterodimers are liganded, the complex is significantly stronger than under conditions when only the RXR partner is saturated with ligand.

RXR Tetramers Do Not Dissociate in the Presence of either RAR or VDR. Our previous work demonstrated that RXR tetramers are extremely stable and dissociate at a very slow rate in the absence of their cognate ligand (*40*). In contrast, upon binding of ligand, RXR tetramers dissociate with a $t_{1/2}$ of about 80 ms (*40*). As many of the functions of RXR are exerted via its heterodimers with other nuclear receptors, and as this receptor is predominantly tetrameric at physiological concentrations, it is important to consider how RXR tetramerization affects the ability of the receptor to interact with its partners. The data in Tables 1 and 2 show that binding energies within RXR−RAR and of RXR−VDR heterodimers are considerably stronger compared to those of RXR tetramers. Consequently, at equilibrium, it can be expected that in the presence of either partner receptor, RXR tetramers will dissociate and heterodimers will form in their place. However, when the extremely slow rate by which RXR tetramers dissociate is considered, it is possible that the kinetic barrier will retard the attainment of equilibrium, i.e., that tetramers will remain stable thereby hindering formation of heterodimers.

To examine the ability of RXR subunits that are incorporated in tetramers to interact with RAR or with VDR, fluorescein-labeled RXR at a concentration at which it is predominantly tetrameric (400 nM, see ref *39*) was titrated with either RAR or VDR. The fluorescence anisotropy of RXR tetramers is notably higher than that of RXR within heterodimers (see Figures 1 and 2). Consequently, if RXR tetramers dissociate and heterodimers are formed upon titration with a partner receptor, a significant decrease in the fluorescence anisotropy will be observed. Labeled RXRΔAB was mixed with either RARΔAB or VDR at various concentrations in the range of 0−800 nM. Mixtures were incubated for 15−20 h at 4 °C and equilibrated at room temperature prior to measurement of the fluorescence anisotropy. As can be seen in Figure 3, the fluorescence anisotropy of 400 nM labeled RXR remained at a level reflecting RXR tetramers and did not change in response to addition of either RAR or VDR. These data clearly demonstrate that tetrameric RXR did not appreciably dissociate to yield heterodimers within the time frame of the experiment. It seems then that the slow rate of dissociation of RXR tetramers comprises a critical barrier which prevents the interactions of the tetrameric fraction of the receptor with its partners.

DNA-Bound Dimeric RXR Readily Forms RXR−RAR Heterodimers, but DNA-Bound Tetrameric RXR Does Not.

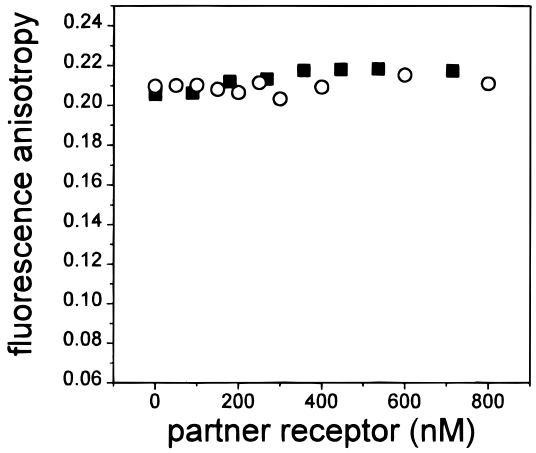

FIGURE 3: Fluorescence anisotropy titrations of tetrameric RXR with RAR or VDR. Fluorescein-labeled mRXRαΔAB at a concentration where it is predominantly tetrameric (400 nM, see the text) was mixed with either hRARαΔAB (■) or hVDR1 (○) at the denoted final concentrations. Mixtures were incubated, and the fluorescence anisotropy was measured as described in Experimental Procedures.

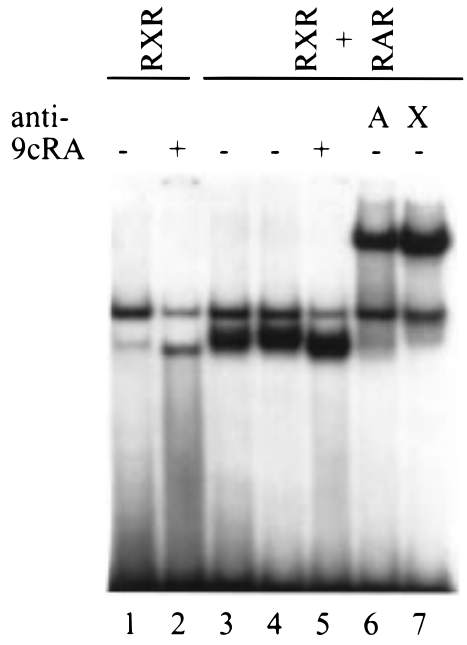

FIGURE 4: Interactions of DNA-bound RXR with RAR. The DNA binding patterns of mRXRαΔAB (100 nM, lanes 1 and 2) or mixtures of mRXRαΔAB and hRARαΔAB (100 nM each, lanes 3−7) in the presence of oligonucletides containing a DR-1 RE were examined with an EMSA. 9cRA (1 μM) was added as indicated. Supershifting experiments were carried out using antibodies directed against RXR (lane 7) or RAR (lane 6, see Experimental Procedures for experimental details).

The interactions of RXR with partner receptors were further examined by electrophoretic mobility shift assays (EMSAs). We previously demonstrated that RXR forms two distinct protein−DNA complexes with oligonucleotides containing RXREs and that these complexes correspond to DNA-bound RXR dimers and DNA-bound RXR tetramers (*62*). EMSA is thus a useful tool for visualizing the various RXR oligomers. The interactions between RXR and RAR were studied by EMSA utilizing oligonucleotides containing DR-1 RE, a sequence that is recognized by RXR homodimers and homotetramers as well as by RXR−RAR heterodimers (*1*, *2*, *62*). As previously noted, two bands were observed in mixtures of RXR and the DR-1 oligonucleotides (Figure 4, lane 1). The slower-moving band, reflecting the presence of tetrameric RXR−DNA complexes, which predominated in the absence of ligand, dissociated to yield DNA-bound RXR dimers in the presence of ligand (Figure 4, lane 2). Addition of RAR (Figure 4, lane 3) did not affect the tetrameric RXR−DNA complex. In contrast, the homodimeric RXR−DNA band disappeared upon addition of RAR, and a more intense band with a slightly slower mobility was observed in its place. This new band corresponded to RXR−RAR heterodimers, a conclusion that was confirmed by the observations that it was efficiently supershifted by antibodies against either RAR or RXR (Figure 4, lanes 6 and 7, respectively). Note that while the RXR antibody efficiently supershifted the RXR−RAR−DNA complex, it did not affect the mobility of the tetrameric RXR−DNA complex (lane 6). These observations are consistent with our previous report that this antibody, which was raised against the D−E regions of *E. coli* mRXRα (4RX-1D12-1; *63*, *64*), is directed toward an epitope which is masked within RXR tetramers, and thus does not efficiently recognize tetramers (*14*). The observation that the intensity of the heterodimeric DNA complex was stronger than that of the RXR homodimer most likely reflects a stronger DNA binding affinity of the heterodimers than of the homodimers.

Addition of 9cRA to mixtures of RXR and RAR (Figure 4, lane 5) resulted in a diminished RXR tetrameric complex and a further enhancement of the band representing DNA-bound RXR−RAR heterodimers. Ligand binding also somewhat increased the mobility of the heterodimeric complex, reflecting the more compactly folded structure of the liganded receptors (compare lanes 4 and 5).

Overall, the observations in Figure 4 indicate that, similar to RXR tetramers in solution, DNA-bound RXR tetramers remain stable and do not dissociate in response to addition of RAR. On the other hand, DNA-bound homodimers are in a dynamic equilibrium and the RXR subunits that are involved in homodimers readily interact with RAR to form heterodimers. As binding affinities within the heterodimers are significantly higher than those characterizing RXR homodimers (Table 1), essentially all of the dimeric RXR is converted into RXR−RAR heterodimers. The observations in Figure 4 also show that RXR subunits that are released from tetramers upon addition of 9cRA become available for interactions with RAR to form heterodimers.

DNA-Bound Dimeric RXR Interacts with VDR in a Ligand-Dependent Fashion, while DNA-Bound Tetrameric RXR Does Not Interact with VDR at All. The DNA binding patterns of mixtures of RXR and VDR were analyzed by EMSAs in the presence of oligonucleotides containing either a DR-1 RE, which binds RXR but does not efficiently associate with either VDR or RXR−VDR heterodimers, or a DR-3 RE, which binds RXR−VDR complexes with a high affinity but does not associate with RXR (*5*).

The results of the EMSA carried out using DR-1-containing oligonucleotides are shown in Figure 5. As noted above, both RXR tetramers and dimers associated with DR-1 RE, with the tetrameric complex predominating in the absence of 9cRA (Figure 5, lane 1). Addition of VDR did not affect the tetrameric RXR−DNA complex (Figure 5, lane 2), indicating that RXR subunits incorporated in this complex

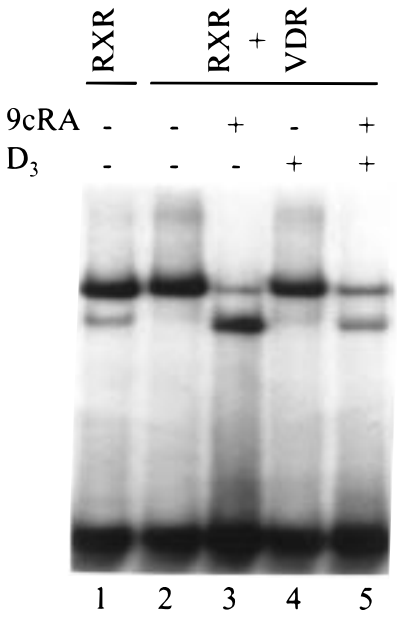

FIGURE 5: Interactions of DNA-bound RXR with VDR. The effects of hVDR and cognate ligands on mRXRαΔAB bound to oligonucletides containing a DR-1 RE were examined with an EMSA. Assays were carried out as described in Experimental Procedures using mRXRαΔAB (100 nM) in the absence (lane 1) or in the presence (lanes 2−5) of hVDR (300 nM). 9cRA (1 μM) or D_3 (1 μM) was added as indicated.

are not available for interactions with VDR. In contrast, the band representing the homodimeric RXR−DNA complex completely disappeared in the presence of VDR (Figure 5, lane 2). These observations suggest that RXR subunits within RXR homodimers readily interacted with VDR to form RXR−VDR heterodimers. As these heterodimers do not bind to a DR-1 RE, they could not be visualized by this assay and the dimeric band "disappeared". Addition of 9cRA to mixtures containing RXR and VDR led to dissociation of RXR tetramers, as indicated by the diminishing of the tetrameric band, and, concomitantly, to enhancement of the band corresponding to DNA-bound RXR homodimers (Figure 5, lane 3). The observation that a large fraction of the available RXR did not interact with VDR but remained in the form of RXR homodimers in the presence of 9cRA is consistent with the conclusion that this ligand inhibits RXR−VDR heterodimerization (Table 1).

The VDR cognate ligand D_3 had little effect on the pattern of the EMSA of RXR and VDR in the absence of 9cRA (Figure 5, lane 4). However, in the presence of both D_3 and 9cRA, the pattern was dramatically altered (Figure 5, lane 5). Under these conditions, not only was the population tetrameric RXR−DNA complex reduced, but the homodimeric RXR band became considerably weaker (compare lanes 3 and 5). It thus seems that addition of D_3 induced the conversion of the RXR homodimer population into RXR−VDR heterodimers, which do not associate with the DR-1 RE. These observations concur with the data in Table 2 that indicated that binding of D_3 to VDR negates the inhibitory effect of 9cRA, and significantly stabilizes the RXR−VDR heterodimers.

To directly follow the formation of RXR−VDR heterodimers, EMSAs were also carried out using oligonucleotides containing a DR-3 RE (Figure 6). VDR did not efficiently associate with these oligonucleotides on its own

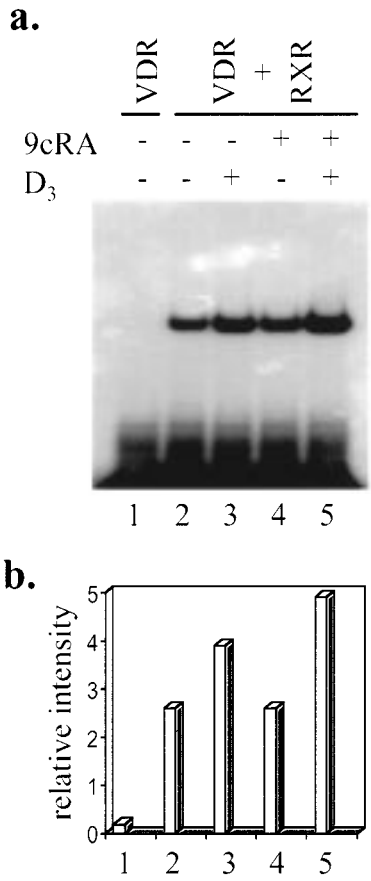

FIGURE 6: Effects of ligands on the DNA binding pattern of RXR−VDR heterodimers. The DNA binding patterns of mixtures of hVDR and mRXRαΔAB in the presence of oligonucleotides containg a DR-3 RE were examined by EMSA. (a) An EMSA was carried out as described in Experimental Procedures using hVDR1 (50 nM) in the absence (lane 1) and presence (lanes 2−5) of mRXRαΔAB (50 nM). 9cRA (1 μM) or D_3 (1 μM) was added as indicated. (b) The relative intensities of the bands obtained by the EMSA in panel a were quantitated using a Bio-Rad Phosphorimager.

(Figure 6a, lane 1), but RXR−VDR heterodimers formed stable complexes with the DR-3 RE (Figure 6a, lane 2). Addition of D_3 enhanced the intensity of the DNA-bound RXR−VDR band (Figure 6a, lanes 3), consistent with the observation that this ligand stabilizes the interaction by about 2-fold (Table 2). Addition of 9cRA had little effect on the intensity of the heterodimeric band (Figure 6, lane 4) and, in some cases, somewhat reduced the RXR−VDR population (data not shown). In the presence of both ligands, the RXR−VDR−DNA complex was significantly enhanced compared with those in all other treatments (Figure 6, lane 5).

Overall, the observations in Figures 5 and 6 show that DNA-bound RXR tetramers are stable in the absence of a RXR cognate ligand and do not dissociate upon addition of VDR. In contrast, DNA-bound RXR dimers readily interact with VDR to yield heterodimers. The data also show that although 9cRA induces dissociation of RXR tetramers and thereby increases the population of available RXR, addition of this ligand does not enhance the formation of heterodimers. This latter observation can be explained by the inhibitory effect of 9cRA on heterodimerization of RXR with VDR (Table 2). This inhibition can be overcome by D_3, and consequently, the combined effects of 9cRA, acting to increase the fraction of available RXR, and of D_3, acting to

enhance the stability of RXR−VDR heterodimers, enable a large fraction of RXR present to interact with VDR.

DISCUSSION

RXR is unique among nuclear receptors in that it can affect transcription both as a homodimer and as a heterodimer with numerous other receptors. It is thus often referred to as "a master regulator", reflecting its central position as a participant in several signaling pathways. Hypothetically, the transcriptional activities of RXR-containing heterodimers may be controlled by the RXR cognate ligand, or by the ligand for the partner receptor, or by both. A possible mechanism of action of cognate ligands is that they control nuclear receptor function by determining which of the possible RXR-containing complexes will predominate, and thus, which signaling pathways will be invoked when RXR and several of its partner receptors are present in a cell simultaneously.

Examination of the effects of ligands on binding energies governing the formation of RXR−RAR heterodimers (Table 1) revealed that the RXR-selective ligand LG1069 significantly decreased the binding affinity characterizing this complex. In contrast, RAR-selective ligands stabilized the heterodimer. Remarkably, the RXR−RAR complex was found to be strongest when both receptors were liganded such that the K_d of the heterodimer was 1 order of magnitude lower in the presence of both ligands compared to when only the RXR-selective ligand was present (Table 1). A similar pattern was observed when the formation of RXR−VDR heterodimers was examined (Table 2). Consistent with a recently reported qualitative study (*68*), the data show that the RXR ligand inhibited while D_3 enhanced the formation of this heterodimer. In the presence of both ligands, K_d was as low as it was with D_3 alone, or some 5-fold lower than it was with 9cRA alone.

In a previous study, the K_d values characterizing RXR−VDR heterodimerization were found to be 282 and 41 nM in the absence and in the presence of D_3, respectively (*41*). These values, which were obtained by using surface plasmon resonance methodology, are several orders of magnitude higher than the sub-nanomolar values reported here, and seem to be too weak to allow heterodimer formation to occur at physiological concentrations. The origin of the discrepancy is not clear to us but might be due to artifacts of the surface plasmon resonance methodology (*65*), or to uncertainties regarding the oligomeric state of the immobilized RXR used in that study. It is interesting to note, however, that despite the discrepancies in K_d values, both the study of Cheskis and Freedman (*41*) and this work point at the conclusion that D_3 functions to stabilize the RXR−VDR heterodimer.

As discussed in the introductory section, it was previously demonstrated that, in the absence of ligand, RXR forms tetramers which are transcriptionally inactive, and that ligand binding, which induces dissociation of RXR tetramers into transcriptionally active dimers and monomers (*14*, *36*, *39*, *40*, *67*). Here, the implications of the self-association of RXR into tetramers for the ability of the receptor to form heterodimers were examined. The equilibrium dissociation constants characterizing the interactions of RXR with RAR and with VDR were found to be in the sub-nanomolar range, indicating that heterodimers form with a much higher affinity

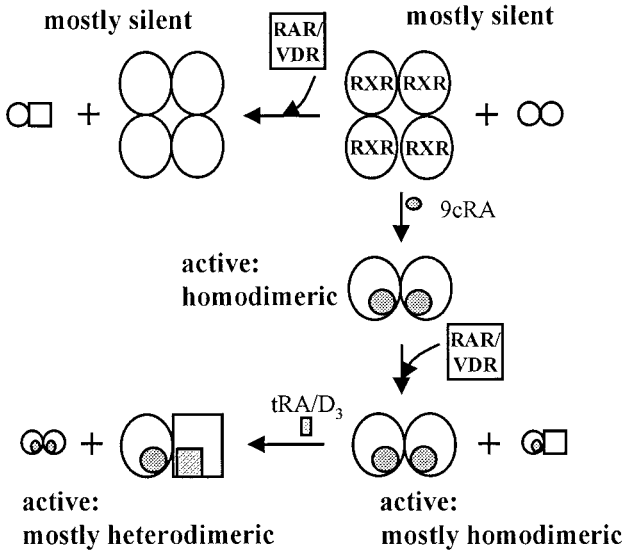

FIGURE 7: Model for the mechanisms by which cognate ligands direct RXR to various signaling pathways. See the text.

compared to the affinity driving the self-association of RXR into either dimers or tetramers (Tables 1 and 2). Consequently, when mixtures of RXR with either RAR or VDR reach equilibrium, heterodimers can be expected to predominate over RXR self-oligomers. Nevertheless, it was found that tetrameric RXR, either in solution (Figure 3) or when bound to DNA (Figures 4 and 5), does not dissociate to form heterodimers upon addition of either RAR or VDR. These findings indicate that the slow rate by which RXR tetramers dissociate (*40*) impedes the attainment of equilibrium, and comprises a critical barrier that does not allow protein subunits that are incorporated in tetramers to interact with partner receptors. In contrast to the behavior of RXR tetramers, it was found that the limited fraction of apo-RXR that exists as a homodimer could readily interact with the partner receptors and was rapidly converted into heterodimers (Figures 4 and 5). In addition, the data indicated that although 9cRA induced dissociation of DNA-bound RXR tetramers, a significant fraction of the released dimer population remained as a RXR homodimer when only the RXR ligand was present. Finally, it is shown that addition of ligands for both RXR and its heterodimerization partners resulted in conversion of a large fraction of the RXR into heterodimers. Taken together, these findings reveal a pattern by which cognate ligands for RXR and for its two partner receptors control the relative distribution of the various oligomers in which RXR can take part. On the basis of these observations and on the basis of previous findings that apo-RXR is predominantly tetrameric at physiological concentrations, a model outlining how ligands direct RXR into homo- and heterodimeric transactivation pathways can be proposed. According to this model (Figure 7), the main fraction of RXR in the absence of ligand is sequestered into tetramers and is thus transcriptionally silent. The small fraction of apo-RXR dimers and monomers which coexists with tetrameric RXR is at a dynamic equilibrium, and can be converted into heterodimers if a partner receptor is present. Addition of 9cRA induces dissociation of RXR tetramers. However, because of the inhibitory effect of this ligand on heterodimer formation, a large fraction of RXR in mixtures containing an RXR-selective ligand and either RAR or VDR will be

present as RXR homodimers. Thus, the RXR ligand will direct a large fraction of the population of RXR into an active homodimeric pathway. Addition of a cognate ligand for the partner receptor, by stabilizing the respective heterodimers, results in diversion of most of the RXR into an active heterodimeric complex.

It has been reported that RXR-selective ligands do not induce transcriptional responses via RAR−RXR and RXR−TR response elements when the partner receptors are unliganded and that RXR regains its transcriptional activity in the context of such heterodimers when the partner receptor becomes liganded (*50*, *51*). To explain these observations, it was proposed that RXR within RXR−RAR and RXR−TR heterodimers is unable to associate with its ligand and that this inhibition, which is imposed by the partner receptors, is relieved when the partner receptor becomes liganded (*50*). However, this suggestion is incompatible with several lines of evidence, and the observations that RXR ligands have a profound effect on the interactions of RXR with RAR (Table 1) further support the conclusion that RXR can interact with its ligand within the RXR−RAR heterodimer (*47*, *53*, *54*). Alternatively, the findings of this work suggest that transcriptional responses to heterodimers may be low in the presence of RXR-selective ligands because these ligands destabilize heterodimeric complexes. These findings also indicate that maximal stabilization of heterodimeric population requires the presence of ligands for heterodimerization partners. This outcome of the combined effect of the RXR ligand, acting to release RXR species that are able to interact with a partner receptor, and the ligand of the partner receptor which stabilizes the heterodimeric complexes provides an explanation for the observed release of inhibition exerted by the partner receptor ligand as well as for the often observed synergism in transcriptional response invoked by the two ligands.

An important question is whether these findings point at a general mechanism by which available RXR subunits partition between their various signaling pathways, or whether they apply only to the formation of RXR−RAR and RXR−VDR heterodimers. Interestingly, it has been reported that heterodimerization of RXR with TR is enhanced by thyroid hormone and inhibited by 9cRA, similar to these findings (*43*). However, conflicting conclusions have also been reported suggesting that both T_3 and 9cRA stabilize RXR−TR heterodimers (*45*). To the best of our knowledge, no systematic examination of the effects of ligands on the interactions of RXR with PPAR has been reported. This system is of particular interest because it has been suggested that, unlike RAR and TR that act as "nonpermissive" partners of RXR, other receptors, most notably PPARs, allow RXR to be transcriptionally active within the context of the heterodimer (*66*).

The findings of this work, taken together with previous observations, show that binding of 9cRA to RXR plays at least three distinct roles in regulating the transcriptional activity of the receptor. This ligand induces dissociation of RXR tetramers, thereby releasing active subunits of the receptor; it inhibits the formation of heterodimers, and thus directs RXR to a homodimeric transactivation pathway, and it allows the receptor to interact with nuclear receptor coactivators that function to link it to the general transcription machinery (*42*, *61*). The first one of these functions seems to be unique for the RXR ligand. The ligand for the partner receptor, in turn, acts to stabilize the heterodimers and thus activates heterodimeric pathways. It is also responsible for regulating the interactions of its respective receptor with accessory corepressors and coactivators (*22*−*28*). The interplay between the two ligands allows for modulation of the relative abundance of the various possible complexes in which RXR is involved. In a final note, it should be pointed out that the abundance of these complexes will be determined not only by ligand-regulated changes in relative binding energies but also by the relative concentrations of the various proteins involved. The full spectrum of factors that modulate the concentrations of nuclear receptors in vivo is not clear at the present time.

ACKNOWLEDGMENT

We thank Hinrich Gronemeyer and Pierre Chambon for providing plasmids and antibodies, Leonard Freedman for providing the expression vector for hVDR, Hoffman La Roche for providing 9cRA, and Ligand Pharmaceuticals for the gift of LG1069 and TTNPB.

REFERENCES

1. Gronemeyer, H., and Laudet, V. (1995) *Protein Profile 2*, 1173−1308.
2. Mangelsdorf, D. J., Tummel, C., Beato, M., Herrlich, P., Schutz, G., Umesono, K., Blumberg, B., Kastner, P., Mark, M., Chambon, P., and Evans, R. M. (1995) *Cell 83*, 835−839.
3. Enmark, E., and Gustafsson, J.-C. (1996) *Mol. Endocrinol. 10*, 1293−1307.
4. Naar, A. M., Boutin, J. M., Lipkin, S. M., Yu, V. C., Holloway, J. M., Glass, C. K., and Rosenfeld, M. G. (1991) *Cell 65*, 1267−1279.
5. Umesono, K., Murakami, K. K., Thompson, C. C., and Evans, R. M. (1991) *Cell 65*, 1255−1266.
6. Mader, S., Leroy, P., Chen, J.-Y., and Chambon, P. (1993) *J. Biol. Chem. 268*, 591−600.
7. Mangelsdorf, D. J., Umesono, K., Kliewer, S. A., Borgmeyer, U., Ong, E. S., and Evans, R. M. (1991) *Cell 66*, 555−561.
8. Rottman, J. N., Widom, R. L., Nadal-Ginard, B., Mahdavi, V., and Karathanasis, S. K. (1991) *Mol. Cell. Biol. 11*, 3814−3820.
9. Zhang, X.-K., Hoffmann, B., Tran, P. B.-V., Graupner, G., and Pfahl, M. (1992) *Nature 355*, 441−446.
10. Davis, K. D., Berrodin, T. J., Stelmach, J. E., Winkler, J. D., and Lazar, M. A. (1994) *Mol. Cell. Biol. 14*, 7105−7110.
11. Leid, M. (1994) *J. Biol. Chem. 269*, 14175−14181.
12. Spanjaard, R. A., Sugawara, A., Ikeda, M., and Chin, W. W. (1995) *J. Biol. Chem. 270*, 17429−17436.
13. Vu-Dac, N., Schoonjans, K., Kosykh, V., Dallongeville, J., Heyman, R. A., Staels, B., and Auwerx, J. (1996) *Mol. Cell. Biol. 16*, 3350−3360.
14. Kersten, S., Reczek, P. R., and Noy, N. (1997) *J. Biol. Chem. 272*, 29759−29768.
15. Yu, V., Delsert, C., Andersen, B., Holloway, J. M., Devary, O. V., Naar, A. M., Kim, S. Y., Boutin, J.-M., Glass, C. K., and Rosenfeld. M. G. (1991) *Cell 67*, 1251−1266.
16. Durand, B., Saunders, M., Leroy, P., Leid, M., and Chambon, P. (1992) *Cell 71*, 73−85.
17. Hallenbeck, P. L., Marks, M. S., Lippoldt, R. E., Ozato, K., and Nikodem, V. M. (1992) *Proc. Natl. Acad. Sci. U.S.A. 89*, 5572−5576.
18. Zhang, X.-K., Lehmann, J., Hoffman, B., Dawson, M. I., Cameron, J., Graupner, G., Hermann, T., Tran, P. B.-V., and Pfahl, M. (1992) *Nature 358*, 587−591.
19. Gearing, K. L., Goettlicher, M., Teboul, M., Widmark, E., and Gustafsson, J.-C. (1993) *Proc. Natl. Acad. Sci. U.S.A. 90*, 1440−1444.

20. Leid, M., Kastner, P., Lyons, R., Nakshatri, H., Saunders, M., Zacharewski, T., Chen, J.-Y., Staub, A., Garnier, J.-M., Mader, S., and Chambon, P. (1992) *Cell 68*, 377−395.
21. Le Douarin, B., Zechel, C., Garnier, J. M., Lutz, Y., Tora, L., Pierrat, P., Heery, D., Gronemeyer, H., Chambon, P., and Losson, R. (1995) *EMBO J. 14*, 2020−2033.
22. Onate, S. A., Tsai, S. Y., Tsai, M.-J., and O'Malley, B. W. (1995) *Science 270*, 1354−1357.
23. Schulman, I. G., Chakravarti, D., Juguilon, H., Romo, A., and Evans, R. M. (1995) *Proc. Natl. Acad. Sci. U.S.A. 92*, 8288−8292.
24. Kamei, Y., Xu, L., Heinzel, T., Torchia, J., Kurokawa, R., Gloss, B., Lin, S.-C., Heyman, R. A., Rose, D. W., Glass, C. K., and Rosenfeld, M. G. (1996) *Cell 85*, 403−414.
25. Le Douarin, B., Nielsen, A. L., Garnier, J.-M., Ichinose, H., Jeanmougin, F., Losson, R., and Chambon, P. (1996) *EMBO J. 15*, 6701−6715.
26. L'Horset, F., Dauvois, S., Heery, D., Cavailles, V., and Parker, M. (1996) *Mol. Cell. Biol. 16*, 6029−6036.
27. Voegel, J. J., Heine, M. J., Zechel, C., Chambon, P., and Gronemeyer, H. (1996) *EMBO J. 15*, 3667−3675.
28. vom Baur, E., Zechel, C., Heery, D., Heine, M. J. S., Garnier, J. M., Vivat, V., Le Douarin, B., Gronemeyer, H., Chambon, P., and Losson, R. (1996) *EMBO J. 15*, 110−124.
29. Chen, J. D., and Evans, R. M. (1995) *Nature 377*, 454−457.
30. Horlein, A. J., Naar, A. M., Heinzel, T., Torchia, J., Gloss, B., Kurokawa, R., Ryan, A., Kamei, Y., Soderstrom, M., Glass, C. K., and Rosenfeld, M. G. (1995) *Nature 377*, 397−403.
31. Kurokawa, R., Soderstrom, M., Horlein, A., Halachmi, S., Brown, M., Rosenfeld, M. G., and Glass, C. K. (1995) *Nature 377*, 451−454.
32. Chen, J. D., Umesono, K., and Evans, R. M. (1996) *Proc. Natl. Acad. Sci. U.S.A. 93*, 7567−7571.
33. Sande, S., and Privalsky, M. L. (1996) *Mol. Endocrinol. 10*, 813−825.
34. Seol, W., Mahon, M. J., Lee, Y.-K., and Moore, D. D. (1996) *Mol. Endocrinol. 10*, 1646−1655.
35. Nervi, C., Grippo, J. F., Sherman, M. I., George, M. D., and Jetten, A. M. (1989) *Proc. Natl. Acad. Sci. U.S.A. 86*, 3888−3892.
36. Kersten, S., Kelleher, D., Chambon, P., Gronemeyer, H., and Noy, N. (1995) *Proc. Natl. Acad. Sci. U.S.A. 92*, 8645−8649.
37. Chen, Z.-P., Shemshedini, L., Durand, B., Noy, N., Chambon, P., and Gronemeyer, H. (1994) *J. Biol. Chem. 269*, 25770−25776.
38. Vivat, V., Zechel, C., Wurtz, J.-M., Bourguet, W., Kagechika, H., Umemiya, H., Shudo, K., Moras, D., Gronemeyer, H., and Chambon, P. (1997) *EMBO J. 16*, 5697−5709.
39. Kersten, S., Pan, L., Chambon, P., Gronemeyer, H., and Noy, N. (1995) *Biochemistry 34*, 13717−13721.
40. Kersten, S., Pan, L., and Noy, N. (1995) *Biochemistry 34*, 14263−14269.
41. Cheskis, B., and Freedman, L. P. (1996) *Biochemistry 35*, 3309−3318.
42. Renaud, J. P., Rochel, N., Ruff, M., Vivat, V., Chambon, P., Gronemeyer, H., and Moras, D. (1995) *Nature 378*, 681−689.
43. Collingwood, T. N., Butler, A., Tone, Y., Cliftin-Bligh, R. J., Parker, M. J., and Chatterjee, K. K. (1997) *J. Biol. Chem. 272*, 13060−13065.
44. Cheskis, B., and Freedman, L. P. (1994) *Mol. Cell. Biol. 14*, 3329−3338.
45. Kakizawa, T., Miyamata, M., Atsuko, K., Yajima, H., Ichikawa, K., and Hashizume, K. (1997) *J. Biol. Chem. 272*, 23799−23804.
46. Zhao, X.-Y., Eccleshal, T. R., Krishnan, A. V., Gross, C., and Feldman, D. (1997) *Mol. Endocrinol. 11*, 366−378.
47. Minucci, S., and Ozato, K. (1996) *Curr. Opin. Genet. Dev. 6*, 567−574.
48. Lehmann, J. M., Jong, L., Fanjul, A., Cameron, J. F., Lu, X. P., Haefner, P., Dawson, M. I., and Pfahl, M. (1992) *Science 258*, 1944−1946.
49. Kurokawa, R., DiRenzo, J., Boehm, M., Sugarman, J., Gloss, B., Rosenfeld, M. G., Heyman, R. A., and Glass, C. K. (1994) *Nature 371*, 528−531.
50. Forman, B. M., Umenoso, K., Chen, J., and Evans, R. M. (1995) *Cell 81*, 542−550.
51. Botling, J., Castro, D. S., Oberg, F., Nilsson, K., and Perlmann, T. (1997) *J. Biol. Chem. 272*, 9443−9449.
52. Durand, B., Saunders, M., Gaudon, C., Roy, B., Losson, R., and Chambon, P. (1994) *EMBO J. 13*, 5370−5382.
53. Apfel, C. M., Kamber, M., Klaus, M., Mohr, P., Keidel, S., and LeMotte, P. K. (1995) *J. Biol. Chem. 270*, 30765−30772.
54. Kersten, S., Dawson, M. I., Lewis, B. A., and Noy, N. (1996) *Biochemistry 35*, 3816−3824.
55. Kadonaga, J. T., and Tijian, R. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 5889−5893.
56. Carlberg, C. (1996) *Endocrine 4*, 91−105.
57. Lakowicz, J. A. (1983) in *Principles of fluorescence spectroscopy*, Plenum Press, New York and London.
58. Fernando, T., and Royer, C. (1992) *Biochemistry 31*, 3429−3441.
59. Noy, N., Slosberg, E., and Scarlata, S. (1992) *Biochemistry 31*, 11118−11124.
60. Kwon, O.-S., and Churchich, J. E. (1994) *J. Biol. Chem. 266*, 266−271.
61. Bourguet, W., Ruff, M., Chambon, P., Gronemeyer, H., and Moras, D. (1995) *Nature 375*, 377−382.
62. Kersten, S., Gronemeyer, H., and Noy, N. (1997) *J. Biol. Chem. 272*, 12771−12777.
63. Bortell, R., Owen, T. A., Shalhoub, V., Heinrichs, A., Aronow, M. A., Rochette-Egly, C., Lutz, Y., Stein, J. L., Lian, J. B., and Stein, G. S. (1993) *Proc. Natl. Acad. Sci. U.S.A. 90*, 2300−2304.
64. Rochette-Egly, C., Lutz, Y., Pfister, V., Heyberger, S., Scheuer, I., Chambon, P., and Gaub, M. P. (1994) *Biochem. Biophys. Res. Commun. 204*, 525−536.
65. Schuck, P. (1997) *Curr. Opin. Biotechnol. 8*, 498−502.
66. Direnzo, J., Soderstrom, M., Kurokawa, R., Ogliastro, M.-H., Ricote, M., Ingrey, S., Horlein, A., Rosenfeld, M., and Glass, C. K. (1997) *Mol. Cell. Biol. 17*, 2166−2176.
67. Kersten, S., Reczek, P. R., and Noy, N. (1998) *J. Mol. Biol.* (submitted for publication).
68. Thompson, P. D., Jurutka, P. W., Haussler, C. A., Whitfield, G. K., and Haussler, M. R. (1998) *J. Biol. Chem. 273*, 8483−8491.

BI980561R