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A Novel Principle for Partial Agonism of Liver X Receptor Ligands

*COMPETITIVE RECRUITMENT OF ACTIVATORS AND REPRESSORS**

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Partial, selective activation of nuclear receptors is a central issue in molecular endocrinology but only partly understood. Using LXRs as an example, we show here that purely agonistic ligands can be clearly and quantitatively differentiated from partial agonists by the cofactor interactions they induce. Although a pure agonist induces a conformation that is incompatible with the binding of repressors, partial agonists such as GW3965 induce a state where the interaction not only with coactivators, but also corepressors is clearly enhanced over the unliganded state. The activities of the natural ligand 22(*R*)-hydroxycholesterol and of a novel quinazolinone ligand, LN6500 can be further differentiated from GW3965 and T0901317 by their weaker induction of coactivator binding. Using biochemical and cell-based assays, we show that the natural ligand of LXR is a comparably weak partial agonist. As predicted, we find that a change in the coactivator to corepressor ratio in the cell will affect NCoR recruiting compounds more dramatically than NCoR-dissociating compounds. Our data show how competitive binding of coactivators and corepressors can explain the tissue-specific behavior of partial agonists and open up new routes to a rational design of partial agonists for LXRs.

Nuclear receptors are a family of transcriptional regulators whose activity can be modulated by their binding to small molecule compounds, such as hormones and metabolites. For many members of the family, this property has allowed their use as drug targets (1). In most cases, however, full activation or inhibition of the receptor is not desired. Instead, agonists are required that only partially activate the receptor. Partial agonists can display tissue-specific activation or repression of nuclear receptors, as has been shown for the estrogen receptor partial agonist raloxifen (2). However, little is known about the molecular mechanisms leading to partial *versus* full agonism.

Another example for nuclear receptors, which, if they are to be used as drug targets, require partial agonists, are the liver X receptors α (LXRα,[2] NR1H3) and β (LXRβ, NR1H2). They have been shown to play a central role in the transcriptional regulation of lipid and cholesterol homeostasis and inflammation (3–7). Activation of LXR-dependent transcription leads to increased expression of cholesterol transporters (8–11) and has been shown to enhance the efflux of cholesterol from macrophages (10–12), reducing the formation of atherosclerotic plaques (13–15). This observation has raised hopes that the manipulation of LXR activity would be of therapeutic value in the treatment of lipid disorders and atherosclerosis. However, activation of LXRs by agonistic compounds induces the expression of enzymes involved in the synthesis of fatty acids in liver cells (16–19). As a consequence, agonists for LXRs cause liver steatosis and elevated serum triglyceride levels in mice (19, 20). Thus, to develop LXR ligands as drugs for the treatment of atherosclerosis, partial, selective activators of LXRs are needed that induce cholesterol efflux in macrophages but do not induce fatty acid synthesis in liver.

Among the currently known ligands for LXRs, some compounds, such as the sulfonamide T0901317, appear to have a purely agonistic activity, whereas others, such as GW3965, have been reported to be more selective in their activation of LXR function (19, 21). Based on structural data, it has been predicted that the cofactor interactions induced by T0901317 and a natural ligand would differ from each other (22). We present here a detailed study of the mechanisms of LXR activation by synthetic agonists, including a novel chemotype and a natural LXR ligand. We show that partial activation of LXRs can be brought about by inducing a receptor conformation that enhances the affinity for both activating and repressing cofactors at the same time. We show that the net effect of the activated receptor on transcription depends on the prevalence of coactivators and corepressors in the cell and propose a novel classification scheme for partial nuclear receptor agonists.

EXPERIMENTAL PROCEDURES

Yeast Two-hybrid Assays—To measure the ligand-dependent binding of cofactor fragments to LXRs, proteins and protein fragments were expressed in GATEWAY-compatible versions of pGBT9 and pGAD424 in the haploid yeasts CG1945 and Y187 (Clontech), respectively. The following protein fragments were used: DAX1, full-length protein; NCoA3, amino acids 391–748; NCoA3-part, amino acids 622–748; RAP250, amino acids 546-end; NCoR, amino acids 1906–2313; TRAP220, amino acids 433–803; NCoR, amino acids 2157–2181; FKHRL, amino acids 411-end; PGC1, amino acids 1–676; SRC1, amino acids 381-end; TIF2, amino acids 548–878. In addition, two fragments from NCoA3 and SMRT were used that had been isolated in yeast two-hybrid screens (23). Combinations of the various constructs were generated in diploid cells by mating of the respective strains. Diploids were passaged twice in 96-well plates in liquid medium and diluted 1:20 into selective medium containing LXR-ligands before fluorescence was measured as described (23) after 22 h for T0901317 and after 38 h for all other compounds. To remove background signals, *e.g.* from the fluorescence of LXR ligands, relative values were generated by subtracting the signals of strains expressing only the cofactors but an empty vector

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[2] The abbreviations used are: LXRα, liver X receptor α; FRET, fluorescence resonance energy transfer; LBD, ligand-binding domain; 22R-HC, 22(*R*)-hydroxycholesterol; NCoR, nuclear receptor corepressor.

TABLE 1

Origin of peptides used in this study

Numbers of amino acid position refer to the indicated data base entry from RefSeq.

Protein	Identifier (RefSeq)	Amino acids	Sequence
NcoA3	NP_006525	672–695	SNMHGSLLQEKHRILHKLLQNGNSP
SMRT	NP_006303	2138–2156	RVVTLAQHISEVITQDYTR
NCoR	NP_006302	2253–2277	SFADPASNLGLEDIIRKALMGSFDD
DAX	NP_000466	132–156	CCFCGEDHPRQGSILYSLLTSSKQT
TRAP220	NP_038662	631–655	PVSSMAGNTKNHPMLMNLLKDNPAQ

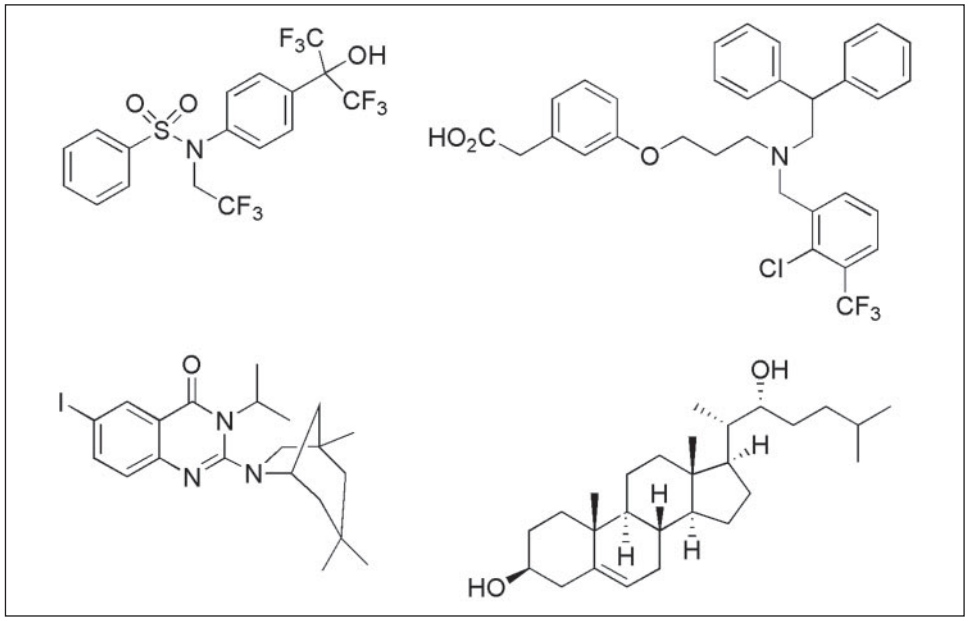

FIGURE 1. **Structures of the compounds used in this study.** *Left top*, T0901317; *right top*, GW3965; *left bottom*, LN6500; *right bottom*, 22R-HC.

instead of the receptors from the signals of strains expressing both cofactors and receptors in the presence of compounds.

Fluorescence Resonance Energy Transfer (FRET) Assays—The ligand-binding domain (LBD) of LXRα (AAH41172, Gln^{201}-Glu^{448}) was expressed as fusion protein with GST in BL-21 cells using the vector pDEST15. The LXRβ LBD (BC033500, Glu^{156}-Glu^{460}) was expressed as a GST fusion from a recombinant baculovirus in SF9 cells. Cells were lysed by sonication, and the fusion proteins purified over glutathione-Sepharose (Pharmacia Corp.) according to the manufacturer's instructions. Assays were done in a final volume of 25 μl in a 384-well plate in a buffer containing Tris/HCl, pH 6.8, 5 mM $MgCl_2$, 400 mM KCl, 0.9 μg/μl bovine serum albumin, 215 ng of Streptavidin-xlAPC Conjugate PJ25 S (Prozyme), 2 ng of europium-labeled anti-GST antibody AD0064 (PerkinElmer Life Science), roughly 20 ng of the purified receptor and varying concentrations of amino-terminally biotinylated cofactor peptides and low molecular weight ligands for LXR. The mix was equilibrated for 1 h at room temperature and measured in a Victor V (PerkinElmer Life Science) fluorometer using 340 nm as excitation and 615 and 665 nm as emission wavelengths. The origin and sequences of the peptides used are shown in Table 1. The coactivator peptides were chosen from relevant LXR-coactivators such that they represent a range of affinities for LXR. For corepressors, we selected the higher affinity peptide from the two published nuclear receptor-binding motifs found each on NCoR and SMRT.

RNA Isolation and Analysis of Gene Expression by Real Time Quantitative PCR—Cultures of the monocyte-macrophage cell line THP-1 and the hepatocytes HepG2 were obtained from the American Type Tissue Culture Collection, Rockville, MD and were grown in RPMI 1640 medium supplemented with 10% fetal bovine serum, 10 mM HEPES, 2 mM pyruvate, 50 μM β-mercaptoethanol (THP-1), and minimal essential medium (Eagle) with 2 mM L-glutamine and Earle's balanced salt solution supplemented with 10% fetal bovine serum, 2 mM glutamine, 0.1 mM non-essential amino acids, 1 mM sodium pyruvate (HepG2) at 37 °C in 5% CO_2. THP-1 cells were differentiated into macrophages by the addition of 100 nM phorbol 12-myristate 13-acetate (Sigma P8139) for 36–72 h, and phorbol 12-myristate 13-acetate was included in the medium of all subsequent experiments to maintain differentiation. All treatments were done in triplicate, and experiments were repeated at least twice.

THP-1 cells were seeded in 24-well plates at 3×10^5 cells/well in RPMI 1640 medium containing 10% fetal calf serum and 100 nM 12-*O*-tetradecanoylphorbol-13-acetate for 24 h. HepG2 cells were seeded in poly-L-Lysine-coated 24-well plates at 1×10^6 cells/well in Eagle's minimal essential medium containing 10% fetal calf serum and grown to 60% confluency.

Before treatment with LXR compounds, growth medium was changed to medium containing 10% charcoal/dextran-stripped fetal calf serum for 12 h. Treatment was done for 12 h (THP-1 cells) and 24 h (HepG2 cells), respectively, in medium containing 10% charcoal/dextran-stripped fetal calf serum (and 100 nM phorbol 12-myristate 13-acetate in the case of THP-1 cells). LXR compounds were dissolved in Me_2SO, with the final solvent concentration not exceeding 0.125%. Total RNA was extracted using the Qiagen Rneasy mini kit and treated with DNase (DNAfree kit, Ambion). RNA was reverse-transcribed with Oligo(dT) primer and real time reverse transcription PCR (TaqMan) was performed using the ABI Prism 7900HT Sequence Detection System and reagents supplied by Applied Biosystems. mRNA steady state levels were normalized to H3 histone (H3F3A) expression levels. The

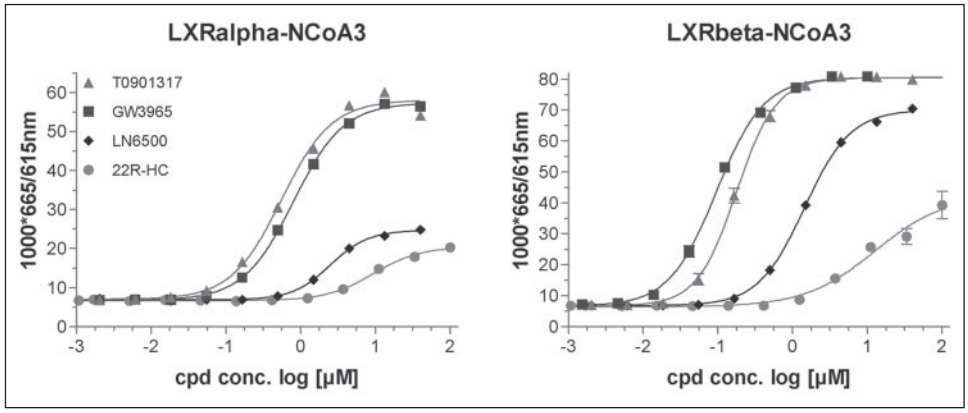

FIGURE 2. **Activity of LXR-ligands in cell-free cofactor binding assays.** The ligand-dependent binding of a peptide modeled on a receptor interacting motif from NCoA3 was measured by FRET (see "Experimental Procedures" for details). *Error bars* are S.D. derived from triplicates.

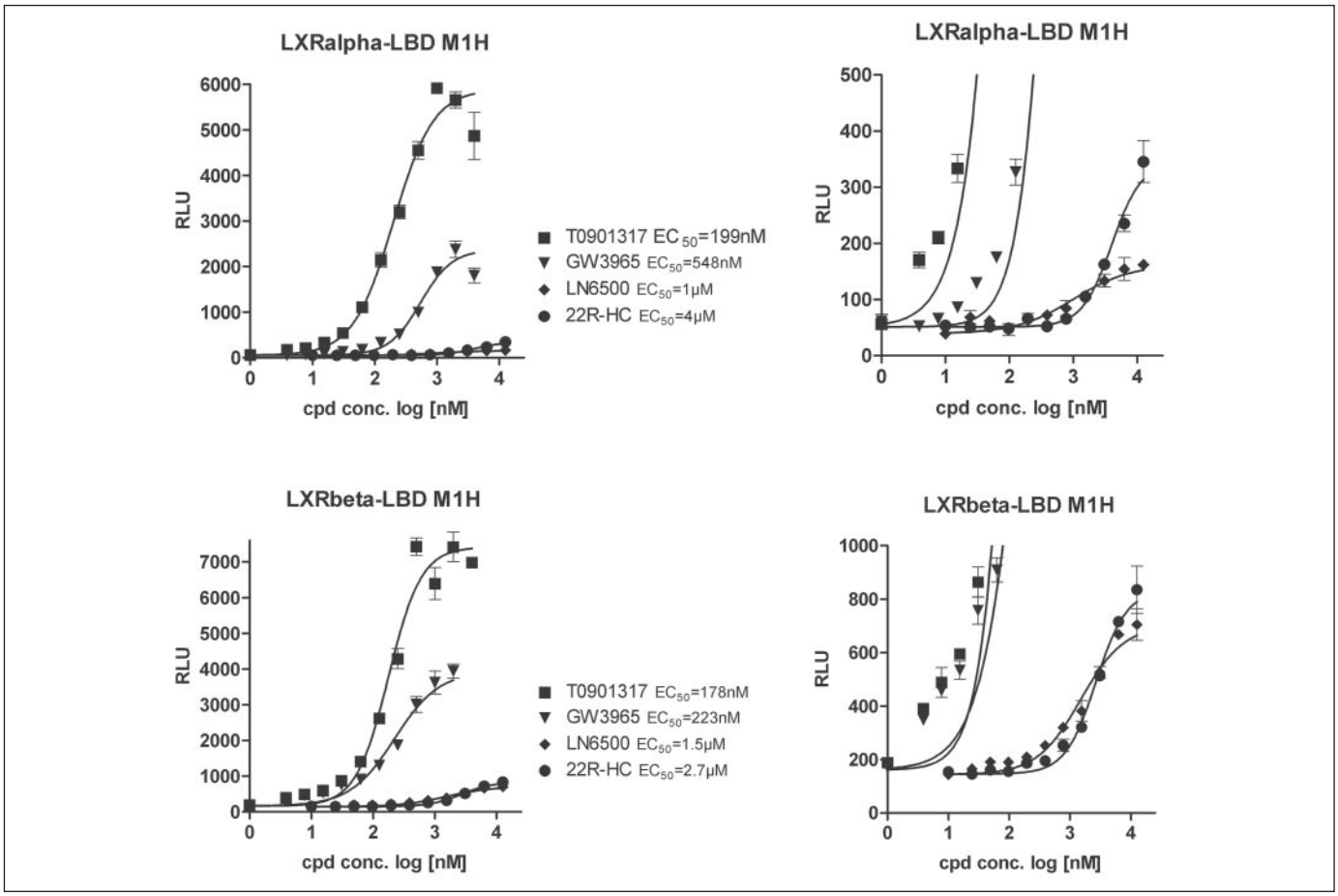

FIGURE 3. **The activity of LXR agonists was determined in mammalian one-hybrid cellular reporter assays as described under "Experimental Procedures."** The *right panel* shows the same data as the *left* with an increased resolution of the *y*-axis, to display the activity of the partial agonist more clearly. *Error bars* are S.D. derived from biological triplicates.

sequences of forward primers, reverse primers and TaqMan probes were as follows: LXRα, CAGCTCAGCCCGGAACAAC, GGAGCGCCGGTTACACTGT, FAM-CGAGAAGCTCGTCGCTGCCCAGTAMRA; FAS, CTGAGACGGAGGCCATATGCT, GCTGCCACACGCTCCTCTAG, FAM-CAGCAGTTCACGGACATGGAGCACAATAMRA; ABCG1, GACGTGCCCTTTCAGATCATGT, GACGGCTGCGACGTCATC, FAM-CCAGTGGCCTACTGCAGCATCGTGTACT-TAMRA; ABCA1, TCCTGTGGTGTTTCTGGATGAAC, CTTGACAACACTTAGGGCACAATTC, FAM-ACCACAGGCATGGATCCCAAAGCC-TAMRA; SCD, TGGCATTCCAGAATGATGTCTATG, CTGGGTGTTTGCGCACAAG, FAM-TGCTGATCCTCATAATTCCCGACGTGG-TAMRA.

Transient Transfection Assays—All transient transfections were done in HEK293 cells (obtained from DSMZ (German Collection of Microorganisms and Cell Cultures), Braunschweig, Germany) grown in minimum essential medium (Eagle) with 2 mM L-glutamine and Earle's balanced salt solution supplemented with 10% fetal bovine serum, 2 mM glutamine, 0.1 mM non-essential amino acids, 1 mM sodium pyruvate, at 37 °C in 5% CO_2. For transfection, 4×10^4 cells were plated per well in 96-well plates in growth medium supplemented with 10% charcoal/dextran-treated fetal bovine serum (HyClone, South Logan, Utah) and transiently transfected the following day at >90% confluency by polyethylene-imine-based transfection. Compound stocks were prepared in Me_2SO, prediluted in medium, and added 4 h after the addition of the

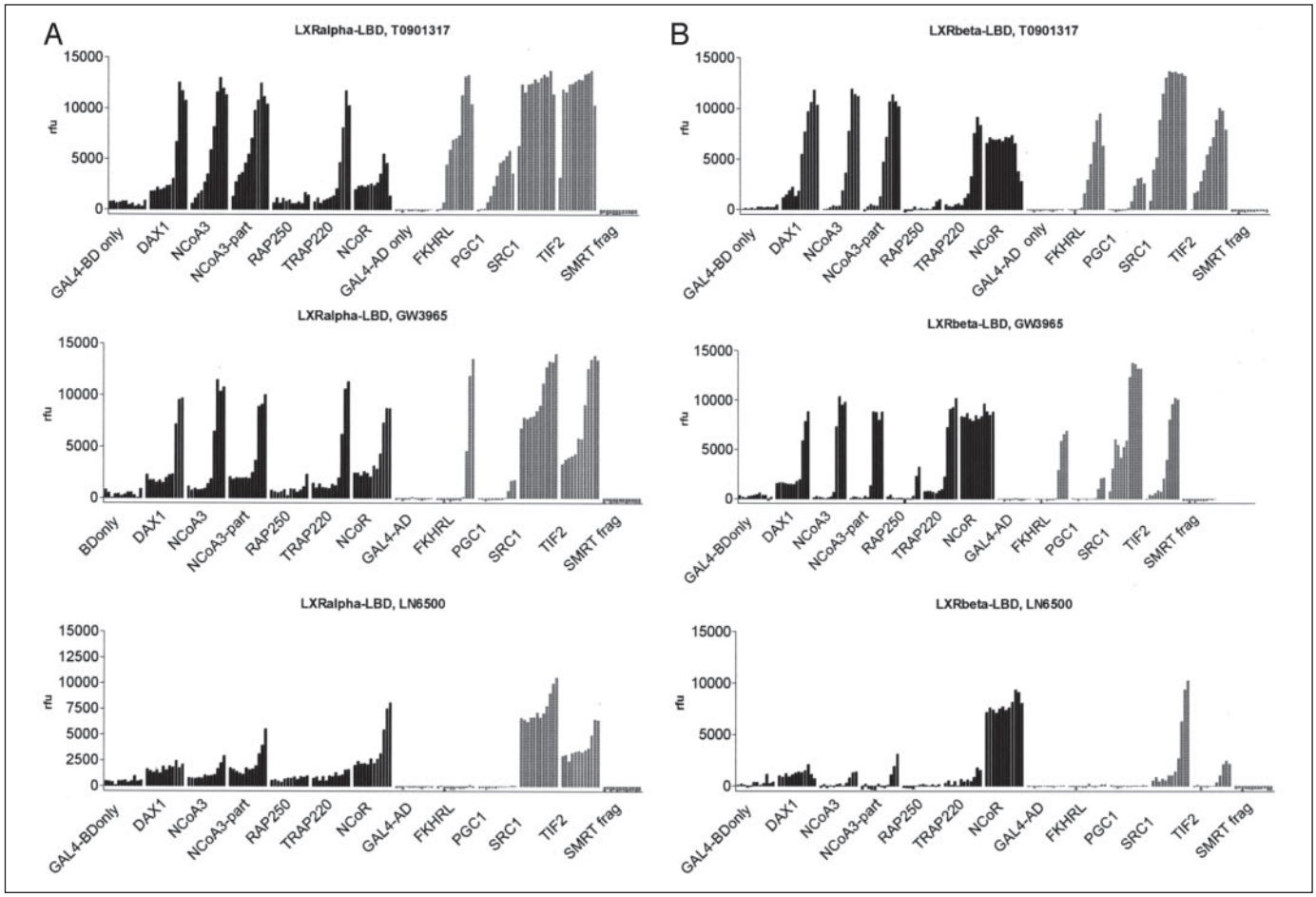

FIGURE 4. **Induction of LXR-cofactor interactions by different synthetic ligands as measured in a quantitative yeast two-hybrid assay.** *A*, LXRα; *B*, LXRβ. In the left half of each panel (*black bars*), cofactors were used as fusions to the DNA-binding domain (*BD*) of GAL4, and tested for interaction with receptor LBDs fused to the GAL4 activation domain (*AD*). In the right half of each panel (*gray bars*), the orientation was switched. Cofactors are indicated below each dose-response curve. The left-most bar of each cofactor curve is the signal generated in the absence of compound. Each cofactor was measured in the presence of the compound indicated, at concentrations increasing 3-fold from left to right. Top concentrations of the compounds are 50 μM for T0901317 and GW3965, and 100 μM for LN6500. See "Experimental Procedures" for details.

transfection mixture (final vehicle concentration not exceeding 0.05%). Cells were incubated for additional 16 h before firefly and *Renilla* luciferase activities were measured sequentially in the same cell extract using buffers according to Ref. 24. Transfection efficiency was controlled via the pRL-TK *Renilla reniformis* luciferase reporter (Promega). The ligand-binding domains of LXRα (Leu^{155}-Glu^{447}) and LXRβ (Glu^{156}-Glu^{461}) were expressed from a GATEWAY (Invitrogen)-compatible version of pCMV-BD (Stratagene) as fusions to the DNA-binding domain of the yeast protein GAL4. pFR-Luc (Stratagene) was used as a reporter plasmid. The NCoR expression plasmid pCMX-NCoR was kindly provided by Andreas Hörlein.

RESULTS

Natural and Synthetic LXR Ligands Displaying Partial Agonism—We used HTR-FRET assays to screen for novel LXR-activating compounds in a collection of combinatorial compound libraries. Among the active structures identified was a group of quinazolinone compounds, such as LN6500 (Fig. 1), that activated LXRα and LXRβ activities in HTR-FRET assays (Fig. 2) with an EC_{50} of 2.5 and 1.4 μM, respectively. When compared with the well characterized LXR agonist T0901317, the efficacy of LN6500 in HTR-FRET assays is in the range of 35% for LXRα and 80% for LXRβ. These efficacies of LN6500 are comparable to or slightly higher than those of the natural agonist (22R-HC). Similar activities are observed when these compounds are used in cellular reporter assays that have a stably integrated LXR-dependent promoter driving the expression of the luciferase reporter (data not shown).

However, when the same compounds are assayed in a cellular reporter assay that uses the LBD of LXRα fused to the GAL4-DNA-binding domain, differences in the efficacies of the compounds become apparent. The natural agonist and the quinazolinone compound only reach efficacies in the range of 2–12% of T0901317. Also GW3965 clearly causes only a partial activation of the receptors, reaching 37 and 62% of the activity of T0901317 for LXRα and LXRβ, respectively (Fig. 3). Thus, it appears that in these assays the three compounds behave as partial agonists, although to different extents. This difference can be detected in several cellular and biochemical assays (see below) but appears to be, for unclear reasons, very pronounced in the mammalian one hybrid assays.

Differential Recruitment of Cofactors Induced by LXR Agonists—Activation of nuclear receptors by small molecule ligands triggers the formation of a binding surface for cofactor proteins on the LBD, termed AF2. In the presence of agonistic ligands, most coactivators bind to the AF2 site of nuclear receptors via a canonical leucine-rich motif (25–28). In the absence of ligands, or in the presence of antagonistic ligands, many nuclear receptors, including LXRs, preferentially interact with corepressors via a different leucine-rich motif, which is found on the two nuclear receptor corepressors NCoR and SMRT (29–32). The switch in binding preference is brought about in part by a 90° turn of

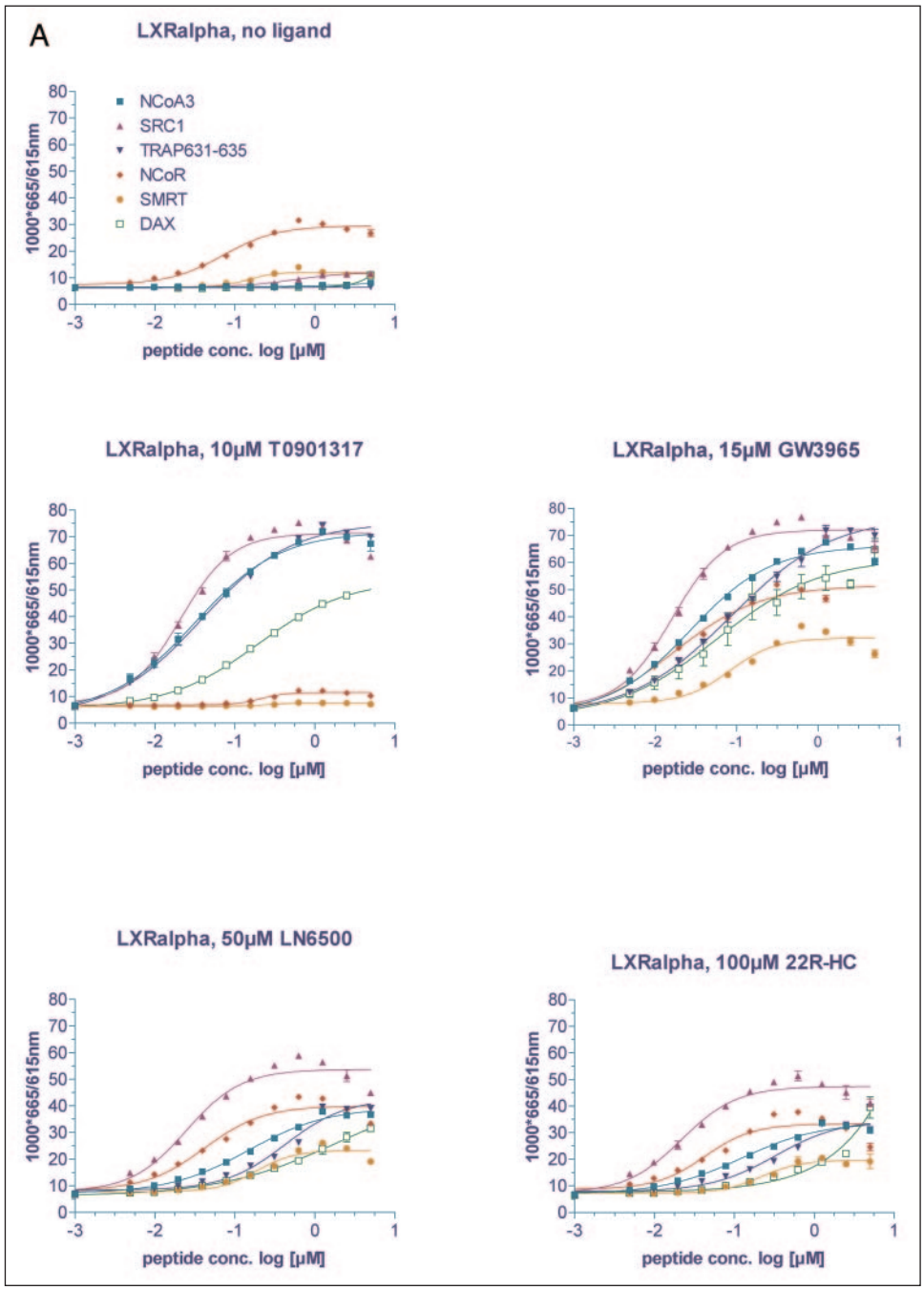

FIGURE 5. **Dose-dependent binding of cofactor peptides to the LBDs of LXRs at constant amounts of ligands detected by FRET assays.** *A*, LXRα; *B*, LXRβ. The type and concentration of the ligand used is indicated above each set of curves. Note that the maximum binding achieved with the corepressor peptides and LXRα is higher in the presence of the GW3965, LN6500, and 22R-HC than in the absence of ligand. *Error bars* are S.D. of triplicates.

helix 12 of the LBD, which opens up a larger groove for the longer corepressor interaction motif (33). Selective interaction of activated receptors with subsets of cofactors has been put forward as a possible explanation for selective activities of nuclear receptor ligands (34, 35).

To see whether differential cofactor recruitment correlates with partial agonism for LXR, we used an array of cofactor constructs and tested their ligand-induced interactions with LXRs. Initial experiments were done at single doses using a quantitative yeast two-hybrid assay (data not shown). Of 100 different cofactor constructs tested, 29 were induced to bind to LXR by T0901317 (data not shown). GW3965 induced the binding of the same spectrum of cofactors. A subset of these cofactors was further analyzed for dose-dependent recruitment to the full-length receptors (not shown) and to the LXR LBD by T0901317, GW3965 and LN6500, as displayed in Fig. 4.

In these experiments, several compound-specific effects can be observed: (i) T0901317 and GW3965 are comparable in their ability to induce the binding of coactivators to LXRs, the only difference being a less efficient recruitment of PGC1 to LXRα by GW3965; (ii) LN6500 induces the binding only of a subset of the cofactors recruited by T0901317 and GW3965; and (iii) surprisingly, both GW3965 and LN6500 lead to an increased binding of LXRs not only to coactivators, but also to the corepressor NCoR. In contrast T0901317, causes only a modest binding of corepressors to LXRα at intermediate concentrations, and represses NCoR binding at higher concentrations.

The latter observation is surprising because, according to the structural information available, the conformations required for the binding of coactivators do not allow the binding of corepressors and *vice versa* (33). Thus, compounds are expected to enhance either the binding of

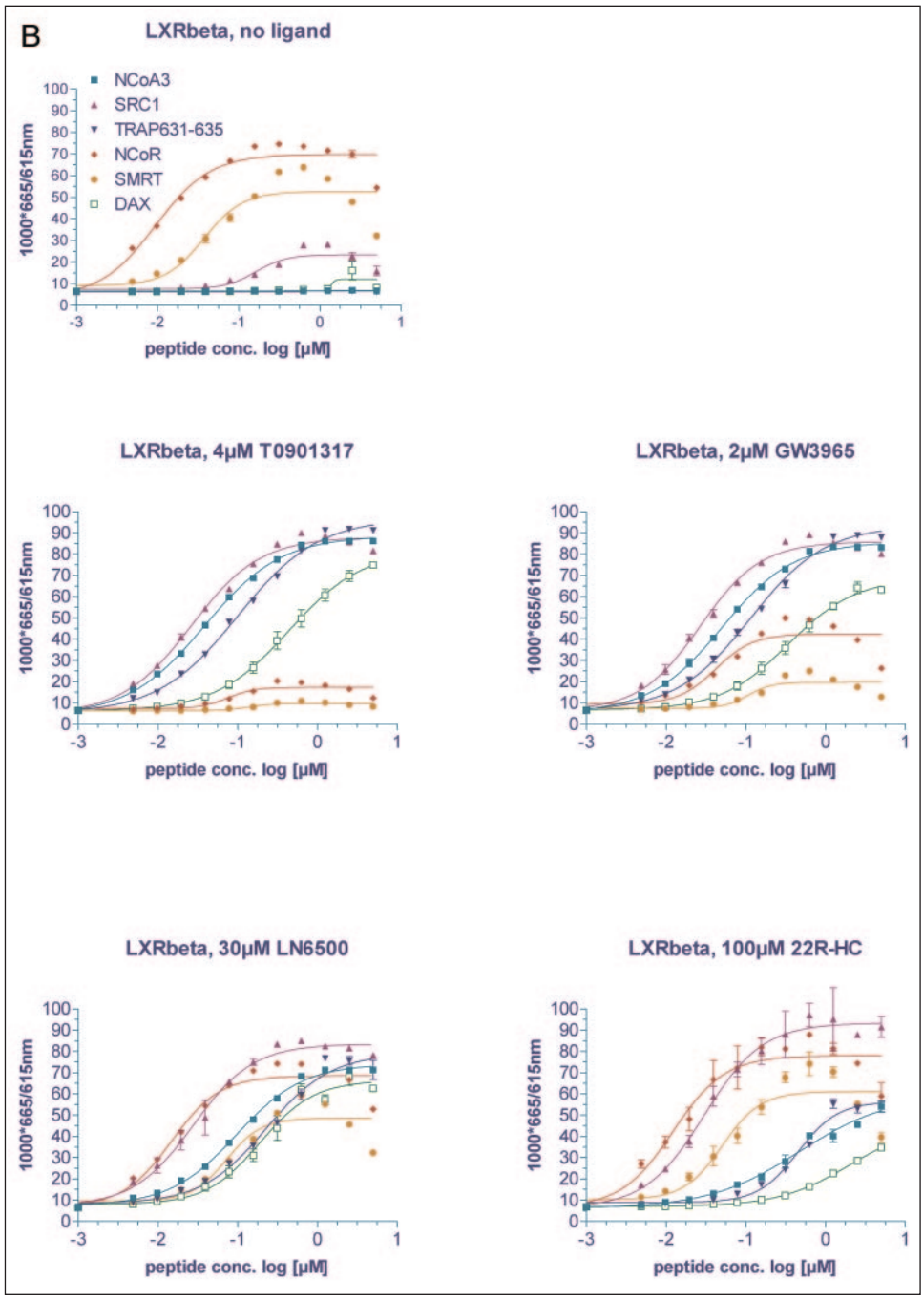

FIGURE 5—*continued*

coactivators or of corepressors, but not enhance the binding of both at the same time.

Partial Agonists Induce a Different Conformation than T0901317—To study the effects of the compounds on the receptor in greater detail, we turned to cell-free binding assays. The affinity of a nuclear receptor's LBD for various peptides can be used to differentiate receptor conformations (36–39). We therefore measured the binding of varying concentrations of cofactor peptides to the receptors at saturating amounts of ligands. As can be seen in Fig. 5*A*, the unliganded LXRα displays a significant affinity for corepressor peptides (EC_{50} for NCoR: 25 nM), whereas it hardly interacts with the coactivator peptides. Inclusion of T0901317 in this assay produced the expected increased affinity of the receptor for coactivator peptides and a repression of corepressors binding, reflecting the well known conformational changes around helix 12 of the LBD. In contrast, GW3965 displayed a drastically different behavior; although the affinity for coactivator peptides was increased as expected, the binding to corepressors also was strongly enhanced compared with the apo-receptor, confirming data from the yeast two-hybrid experiments. Similarly, LN6500 and 22R-HC induced the binding of corepressors, however, these two compounds induced coactivator binding more weakly than T0901317 and GW3965.

Consistent with the yeast two-hybrid data, LXRβ displays a greater affinity for corepressors in the absence of compounds (Fig. 5*B*). When compared with LXRα, the compounds display a similar rank-order with respect to the degree of coactivator and corepressor binding they induce. However, corepressor affinity is not significantly enhanced over the unliganded receptor by any of the compounds. Judged by the induction of corepressor binding, GW3965 appears to be more agonistic to LXRβ than to LXRα, as reflected in the cellular assays shown in Fig. 4.

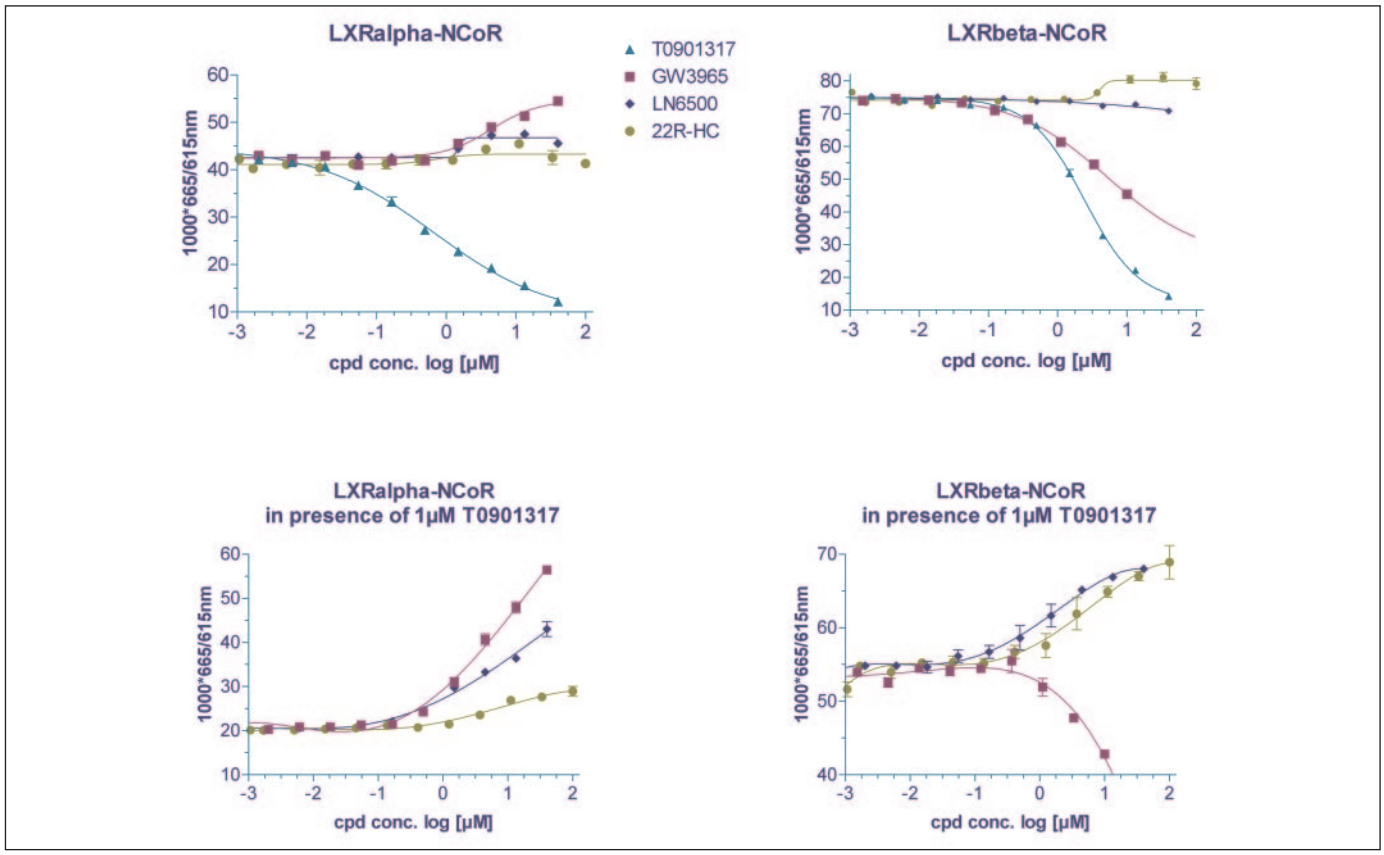

FIGURE 6. **Binding of NCoR peptide to LXR LBDs at increasing amounts of LXR ligands.** In the *lower panel*, T0901317 was additionally included in the assay to show the binding of the corepressor peptide induced by the weaker partial agonists more clearly. *Error bars* are S.D. derived from triplicates.

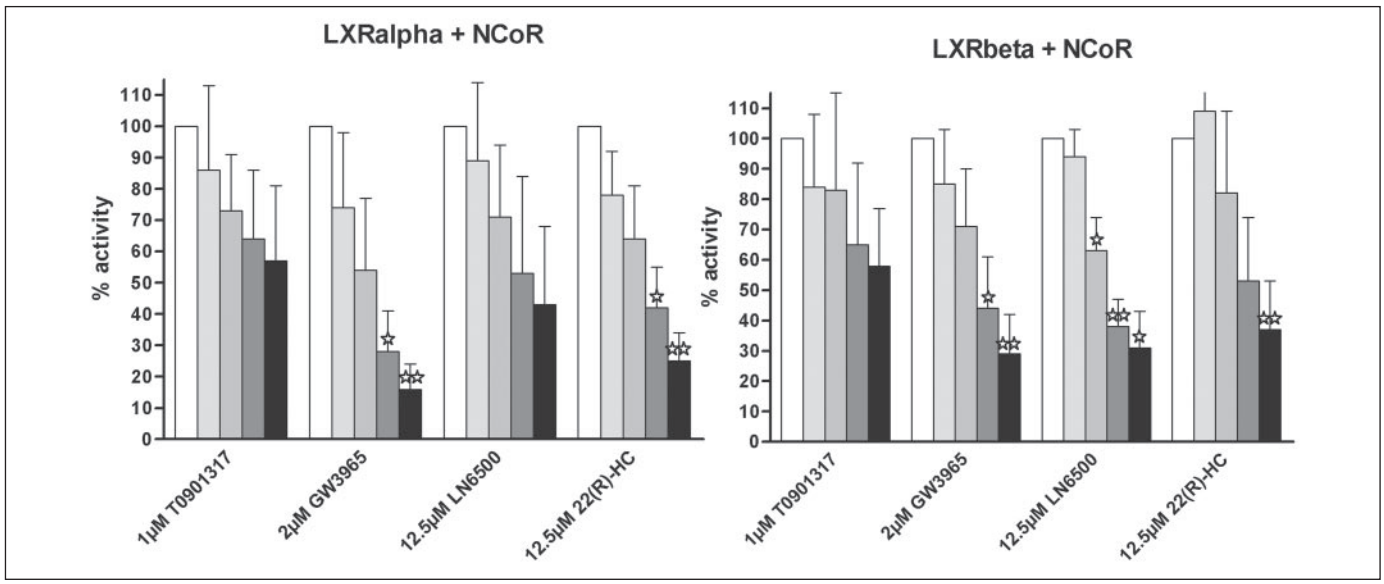

FIGURE 7. **Sensitivity of LXR-activity induced by full and partial agonists to increasing amounts of NCoR in the cell.** The activity of LXR agonists was determined in mammalian one-hybrid cellular reporter assays, with increasing amounts of NCoR expression plasmid included in the transfection mix from left (*light gray*) to right (*black*), as follows: 0, 0.2, 1, 5, and 20 ng. Data normalized for transfection efficiency are shown. Maximal levels of induction differ strongly between the compounds (*cf* Fig. 3) and have been set to 100% for each compound. The experiment represents the average of three independent experiments done in duplicate on different days. *Error bars* are S.D. of the normalized data. Significance of the reduction of LXR activity was determined in a paired *t* test. Significance is indicated as * and ** at the level of 0.05 and 0.01, respectively.

The differential behavior of receptors in the presence of the four compounds can also be observed in assays in which the compound concentration is varied, as shown in Fig. 6. Whereas the full agonist T0901317 inhibits NCoR-peptide binding with increasing concentrations, the partial agonists allow or induce the binding to the corepressor. The different effects of GW3965 on LXRα and LXRβ are again clearly discernible; whereas for LXRα, the binding to NCoR is induced over that of the unliganded receptor, it is inhibited for LXRβ but to a lesser extent than for T0901317.

We conclude that T0901317 induces a conformation that allows only very weak binding of corepressors and strong binding of coactivators, as

expected for a pure agonist. In contrast, GW3965, LN6500, and the natural ligand 22R-HC behave as partial agonists, as they induce a state of the receptor, which is flexible to adapt an agonistic or antagonistic conformation. Interacting cofactor peptides help to stabilize either of the two conformations in an "induced fit"-like mechanism. These observations correlate well with the effects of the compounds in cellular assays and provide an explanation for the partial agonism displayed by GW3965, LN6500, and 22R-HC. We would like to stress that the cofactor peptides used here serve merely as biochemical tools and may not be representative of the behavior of the full-length cofactor proteins.

Sensitivity of LXR Agonists to the Cellular Cofactor Environment—Given the above data, it may be expected that the relative prevalence and availability of coactivators and corepressors in a cell would determine the net effect of a ligand on LXR-dependent transcription. Compounds that induce conformations that allow an interaction with corepressors should be more sensitive to increased amounts of corepressors than compounds that allow only weak corepressor binding. This prediction was tested in transient transfection assays. As shown in Fig. 7, the effects of partial agonists are indeed more sensitive to increasing amounts of NCoR than those of T0901317. This effect is most pronounced for GW3965-induced LXRα activity and weaker for LXRβ, which is in excellent accordance with the cofactor profiles observed in the FRET assays. At least with respect to LXRβ, the effects of LN6500 and 22R-HC are also more sensitive to an increased amount of NCoR in the cell than those of T0901317. These data suggest that the activities of the compounds should respond differently to changes in the cofactor context of cells and are likely to be cell type-dependent.

Therefore, we tested whether the different sensitivity of the activities of the synthetic ligands to corepressor concentrations would be reflected in a cell type-dependent behavior, by measuring the induction of LXR-regulated genes in the liver-derived cell line HepG2 and in differentiated THP-1 macrophage cells. Fig. 8 shows that despite their different affinities for LXRs all three compounds induced the cholesterol efflux pumps ABCA1 and ABCG1 and the LXRα gene in THP-1 cells to comparable maximal levels. In HepG2 cells, T0901317 strongly stimulates the expression of all genes tested, including genes involved in lipid synthesis, FAS and SCD, whereas GW3965 induces these genes more weakly, which is most apparent for FAS. LN6500 appears to be barely active in HepG2 cells. Thus, the rank order of partial agonism observed in the cofactor-binding experiments seems to be reflected in the cell type-specific activity of the compounds. The reduced activity of GW3965 in HepG2 cells is in line with studies on the effects of GW3965 and T0901317 on lipid metabolism in animals (21, 40). In a recent paper, GW3965 was reported to exert similar effects on the expression of ABCA1 in intestine but to failed to significantly induce triglyceride synthesis in the liver (40). We would like to propose that this selective effect is because of the differential effect of GW3965 on the recruitment of coactivators and corepressors, defining a new route to selectively modulate LXR activity.

DISCUSSION

In the development of drugs directed against nuclear receptors, pure agonism or antagonism are only rarely the goal. In most cases, partial and selective agonists are desired, which activate the receptor in a tissue-specific manner. The prototypic examples for such selective nuclear receptor modulators are tamoxifen and raloxifen, which have been shown to activate the estrogen receptor in a tissue-specific way (reviewed in Ref. 41). Responses to raloxifen and tamoxifen are sensitive to the amount of coactivators and corepressors in a cell, such that the balance between agonism and antagonism can be tilted by changes in the expression of coactivators and corepressors (34, 35, 42, 43). From these and other studies, a well accepted explanation for the tissue-selective behavior of estrogen receptor ligands has been formulated, which argues that the relative availability of corepressors and coactivators in a cell will determine the agonistic or antagonistic behavior of partial agonists (34). According to the model, a spectrum of conformations can be adopted by the receptor between the two extremes of a purely antagonistic state and a purely agonistic state (reviewed in Ref. 44). Partial agonists would induce conformations that are to be placed in intermediate positions between the two extremes on a linear scale. Our studies clearly show that a similar explanation should hold true for tissue-specific effects of LXR ligands. Indeed, our data indicate that the conformations displayed by LXRβ are compatible with such a model.

However, the present study also shows that the spectrum of conformations that LXRα can adopt goes beyond a linear scale; GW3965 induces binding of LXRα to coactivators comparably to T0901317, but differs dramatically in the effect on corepressor binding. GW3965 and LN6500, on the other hand, have similar effects on corepressor binding, but LN6500 induces coactivator binding much more weakly. Thus, we would like to propose a classification of partial agonists according to their cofactor recruitment properties, as depicted in Fig. 9. In addition to pure agonists and pure antagonists four principal modes of partial agonism are possible: (i) simultaneous full induction of corepressor as well as coactivator binding; (ii) partial induction of coactivator binding without induction of corepressor binding; (iii) partial induction of both coactivator and repressor binding; and (iv) inhibition of the binding of any cofactor, resulting in derepression as has been seen in LXR-knockout mice (45).

Because the EC_{50} for the binding of cofactors to the receptors can be determined, it provides an objective, quantitative parameter to position a compound regarding this spectrum of receptor conformations. Importantly, the measurement can be done at saturating amounts of ligand and is therefore independent of the potency of the compound. To demonstrate the utility of this classification of partial agonists, we have tentatively mapped the conformational positions of LXRα and -β based on the measured EC_{50}s into that graph. From these graphs, it is clearly apparent that all compounds map to a straight line for LXRβ, being representative of a linear spectrum of conformations. For LXRα, however, the competitive induction of cofactor binding is evident for GW3965.

Structural data suggest that the nuclear receptor conformations required for coactivator and repressor binding are mutually exclusive (33). In the crystal structures of GW3965-bound LXRβ and a binding coactivator peptide, the receptor adopts an agonistic conformation (22, 46, 47). Crystal structures of LXRα in the presence of GW3965 have not been reported, but our data clearly show that GW3965 will enhance the binding to both activators and repressors compared with the unliganded receptor. It has to be assumed that GW3965 stabilizes a conformation in which the receptor is ready for cofactor binding, but helix 12 is flexible to adopt several positions. The presence of a coactivator or corepressor will then stabilize the receptor in an agonistic or antagonistic conformation. This assumption is well in accordance with the fact that T0901317 coordinates helix 12 tightly through direct contacts to histidine 421, which in turn fixes tryptophan 443 in a state that allows coactivators to bind to the receptor. GW3965, on the other hand, does not directly coordinate helix 12 in LXRβ (46), which is probably the reason for the higher affinity of the receptor for corepressors as shown here. Our data suggest that the AF2 will be even more flexible for LXRα and should be free to adapt its position to both type of cofactors. We would predict that it should be possible to obtain structures of agonistic

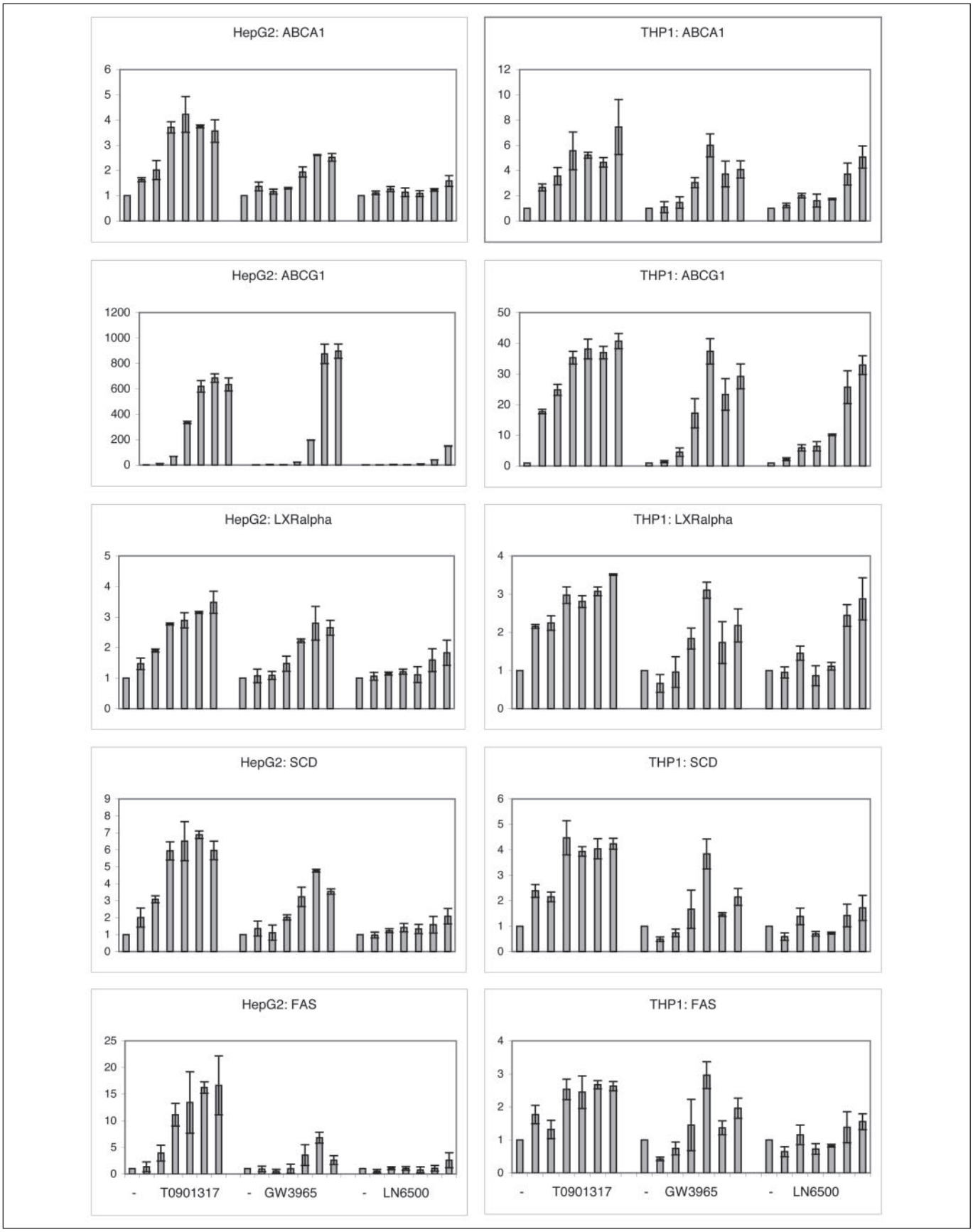

FIGURE 8. **Induction of LXR-response genes by LXR ligands in HepG2 cells (*left panels*) and differentiated THP1-macrophage cells (*right panels*).** Groups of seven columns represent increasing concentrations of the compounds indicated in the lowest panel. The left-most columns of each group represent mock-treated cells. Increments are 4-fold for T0901317 and GW3965 with top concentrations of 10 μM, and 2.5-fold for LN6500 with a top concentration of 25 μM. *Error bars* are S.D. from three biological replicates. At saturating concentrations of compound (2.5 μM), the difference in gene induction between T0901317 and GW3965 at 2.5 μM compound observed in HepG2 cells for FAS, SCD, and ABCA1 is significant at a level of $p = 0,1$.

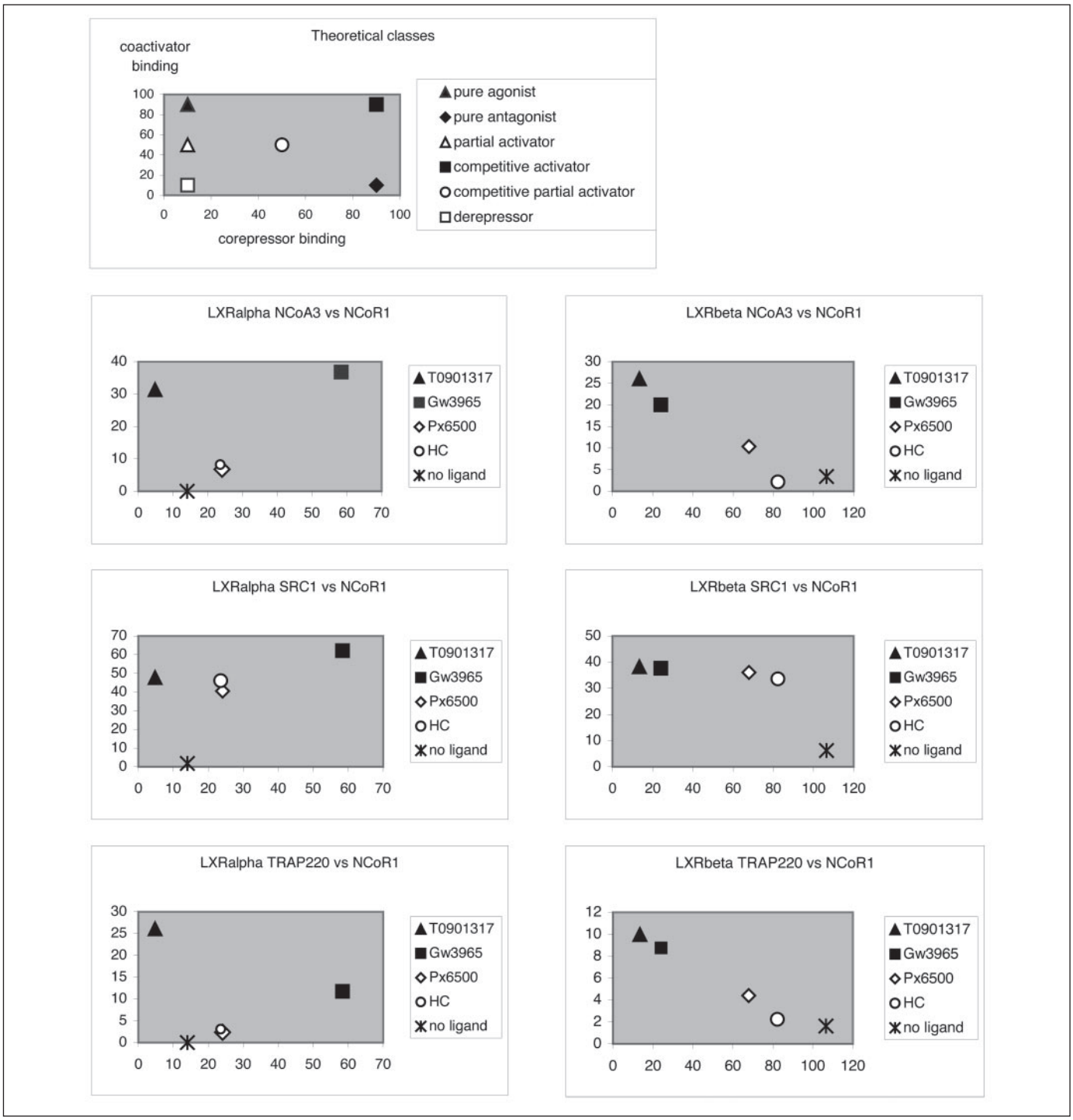

FIGURE 9. **Classification of LXR ligands according to their cofactor recruitment properties.** Both axes represent the affinities of the indicated cofactor peptides for the liganded receptor expressed as the inverse of the EC_{50} in μM. Thus, low affinities are placed near the origin of the graph, whereas high affinities are placed at the *top* and *right-most* part of the graph. Diagrams represent a theoretical classification of agonists classes (*top*) as well as diagrams created with the EC_{50} data from various cofactor peptides as determined by FRET and presented in this paper. Pure agonists that induce only coactivator binding are found on the *top left*, whereas pure antagonists are found at the *bottom right*. Note that for LXRβ, all compounds tested are found roughly on a straight line, suggestive of a linear spectrum of conformations in which the higher the affinity for coactivators, the less it is for corepressors. For LXRα, GW3965 is found off this linear scale of conformations, because it induces the interaction with both types of cofactors.

and antagonistic conformations, depending on the type of cofactor present. Similar effects of partial agonists on peroxisome proliferator-activated receptor α and -δ (48) and retinoic acid receptor α (49) have been reported.

According to our data, the natural ligand 22R-HC is a partial agonist that does not fully activate the transcriptional potential of LXR. In accordance with our data, mutational analysis (50), and structural data (22) have suggested that the coordination of helix 12 by the natural ligands of LXR is less tightly than by T0901317. This would explain why superactivation of the receptor by more agonistic synthetic ligands is possible, as has been shown (12). Also, the amplitude of LXR activation by 22R-HC is likely to depend on the relative abundance of coactivators and corepressors in a tissue. This would also allow for tissue-specific tuning of LXR activity by changes in the cellular cofactor environment.

Use of LXRs as drug targets has been hampered by the fact that agonists not only prevent the formation of atherosclerotic plaques but also increase serum and liver triglyceride levels. Recently, GW3965 has been shown to have a much milder effect on triglyceride levels in mice than T0901317 (40), which fits well to the mechanisms of partial agonism we observe here. However, it is clear that the remaining effects are still prohibitive for drug development, and a potential drug will have to be more selective in its effects. Although we have shown that compounds such as LN6500 can be found that display even less agonistic properties than GW3965, it remains to be seen whether these properties can be conserved in derivatives with increased potencies. It is not clear at present whether a highly potent compound can be found that does not coordinate the AF2 tightly and thus freezes the receptor in one of the two extreme states of agonism or antagonism. Also, the type of partial agonism described in this paper may not be the only way to selectively modulate LXR activity, *e.g.* subtype-specific compounds may allow to dissociate the various effects of LXR activation. Nevertheless, the increased understanding of partial agonism of LXR and the availability of quantitative assays to distinguish different modes of LXR activation paves the way to further explore use of this interesting potential drug target.

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REFERENCES

1. Gronemeyer, H., Gustafsson, J. A., and Laudet, V. (2004) *Nat. Rev. Drug Discov.* **3,** 950–964
2. Delmas, P. D., Bjarnason, N. H., Mitlak, B. H., Ravoux, A. C., Shah, A. S., Huster, W. J., Draper, M., and Christiansen, C. (1997) *N. Engl. J. Med.* **337,** 1641–1647
3. Li, A. C., and Glass, C. K. (2004) *J. Lipid Res.* **45,** 2161–2173
4. Lund, E. G., Menke, J. G., and Sparrow, C. P. (2003) *Arterioscler. Thromb. Vasc. Biol.* **23,** 1169–1177
5. Lala, D. S. (2004) *IDrugs* **7,** 563–569
6. Steffensen, K. R., and Gustafsson, J. A. (2004) *Diabetes* **53,** Suppl. 1, 36–42
7. Joseph, S. B., and Tontonoz, P. (2003) *Curr. Opin. Pharmacol.* **3,** 192–197
8. Whitney, K. D., Watson, M. A., Goodwin, B., Galardi, C. M., Maglich, J. M., Wilson, J. G., Willson, T. M., Collins, J. L., and Kliewer, S. A. (2001) *J. Biol. Chem.* **276,** 43509–43515
9. Repa, J. J., Turley, S. D., Lobaccaro, J. A., Medina, J., Li, L., Lustig, K., Shan, B., Heyman, R. A., Dietschy, J. M., and Mangelsdorf, D. J. (2000) *Science* **289,** 1524–1529
10. Schwartz, K., Lawn, R. M., and Wade, D. P. (2000) *Biochem. Biophys. Res. Commun.* **274,** 794–802
11. Venkateswaran, A., Laffitte, B. A., Joseph, S. B., Mak, P. A., Wilpitz, D. C., Edwards, P. A., and Tontonoz, P. (2000) *Proc. Natl. Acad. Sci. U. S. A.* **97,** 12097–12102
12. Sparrow, C. P., Baffic, J., Lam, M. H., Lund, E. G., Adams, A. D., Fu, X., Hayes, N., Jones, A. B., Macnaul, K. L., Ondeyka, J., Singh, S., Wang, J., Zhou, G., Moller, D. E., Wright, S. D., and Menke, J. G. (2002) *J. Biol. Chem.* **277,** 10021–10027
13. Tangirala, R. K., Bischoff, E. D., Joseph, S. B., Wagner, B. L., Walczak, R., Laffitte, B. A., Daige, C. L., Thomas, D., Heyman, R. A., Mangelsdorf, D. J., Wang, X., Lusis, A. J., Tontonoz, P., and Schulman, I. G. (2002) *Proc. Natl. Acad. Sci. U. S. A.* **99,** 11896–11901
14. Joseph, S. B., Castrillo, A., Laffitte, B. A., Mangelsdorf, D. J., and Tontonoz, P. (2003) *Nat. Med.* **9,** 213–219
15. Terasaka, N., Hiroshima, A., Koieyama, T., Ubukata, N., Morikawa, Y., Nakai, D., and Inaba, T. (2003) *FEBS Lett.* **536,** 6–11
16. Yoshikawa, T., Shimano, H., Amemiya-Kudo, M., Yahagi, N., Hasty, A. H., Matsuzaka, T., Okazaki, H., Tamura, Y., Iizuka, Y., Ohashi, K., Osuga, J., Harada, K., Gotoda, T., Kimura, S., Ishibashi, S., and Yamada, N. (2001) *Mol. Cell. Biol.* **21,** 2991–3000
17. Repa, J. J., Liang, G., Ou, J., Bashmakov, Y., Lobaccaro, J. M., Shimomura, I., Shan, B., Brown, M. S., Goldstein, J. L., and Mangelsdorf, D. J. (2000) *Genes Dev.* **14,** 2819–2830
18. Joseph, S. B., Laffitte, B. A., Patel, P. H., Watson, M. A., Matsukuma, K. E., Walczak, R., Collins, J. L., Osborne, T. F., and Tontonoz, P. (2002) *J. Biol. Chem.* **277,** 11019–11025
19. Schultz, J. R., Tu, H., Luk, A., Repa, J. J., Medina, J. C., Li, L., Schwendner, S., Wang, S., Thoolen, M., Mangelsdorf, D. J., Lustig, K. D., and Shan, B. (2000) *Genes Dev.* **14,** 2831–2838
20. Chisholm, J. W., Hong, J., Mills, S. A., and Lawn, R. M. (2003) *J. Lipid Res.* **44,** 2039–2048
21. Quinet, E. M., Savio, D. A., Halpern, A. R., Chen, L., Miller, C. P., and Nambi, P. (2004) *J. Lipid Res.* **45,** 1929–1942
22. Williams, S., Bledsoe, R. K., Collins, J. L., Boggs, S., Lambert, M. H., Miller, A. B., Moore, J., McKee, D. D., Moore, L., Nichols, J., Parks, D., Watson, M., Wisely, B., and Willson, T. M. (2003) *J. Biol. Chem.* **278,** 27138–27143
23. Albers, M., Kranz, H., Kober, I., Kaiser, C., Klink, M., Suckow, J., Kern, R., and Koegl, M. (2005) *Mol. Cell Proteomics* **4,** 205–213
24. Dyer, B. W., Ferrer, F. A., Klinedinst, D. K., and Rodriguez, R. (2000) *Anal. Biochem.* **282,** 158–161
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. Heery, D. M., Kalkhoven, E., Hoare, S., and Parker, M. G. (1997) *Nature* **387,** 733–736
27. Chang, C., Norris, J. D., Gron, H., Paige, L. A., Hamilton, P. T., Kenan, D. J., Fowlkes, D., and McDonnell, D. P. (1999) *Mol. Cell. Biol.* **19,** 8226–8239
28. Torchia, J., Rose, D. W., Inostroza, J., Kamei, Y., Westin, S., Glass, C. K., and Rosenfeld, M. G. (1997) *Nature* **387,** 677–684
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,** 397–404
31. Perissi, V., Staszewski, L. M., McInerney, E. M., Kurokawa, R., Krones, A., Rose, D. W., Lambert, M. H., Milburn, M. V., Glass, C. K., and Rosenfeld, M. G. (1999) *Genes Dev.* **13,** 3198–3208
32. Hu, X., and Lazar, M. A. (1999) *Nature* **402,** 93–96
33. Xu, H. E., Stanley, T. B., Montana, V. G., Lambert, M. H., Shearer, B. G., Cobb, J. E., McKee, D. D., Galardi, C. M., Plunket, K. D., Nolte, R. T., Parks, D. J., Moore, J. T., Kliewer, S. A., Willson, T. M., and Stimmel, J. B. (2002) *Nature* **415,** 813–817
34. Smith, C. L., Nawaz, Z., and O'Malley, B. W. (1997) *Mol. Endocrinol.* **11,** 657–666
35. Shang, Y., and Brown, M. (2002) *Science* **295,** 2465–2468
36. Paige, L. A., Christensen, D. J., Gron, H., Norris, J. D., Gottlin, E. B., Padilla, K. M., Chang, C. Y., Ballas, L. M., Hamilton, P. T., McDonnell, D. P., and Fowlkes, D. M. (1999) *Proc. Natl. Acad. Sci. U. S. A.* **96,** 3999–4004
37. Bramlett, K. S., Wu, Y., and Burris, T. P. (2001) *Mol. Endocrinol.* **15,** 909–922
38. Iannone, M. A., Simmons, C. A., Kadwell, S. H., Svoboda, D. L., Vanderwall, D. E., Deng, S. J., Consler, T. G., Shearin, J., Gray, J. G., and Pearce, K. H. (2004) *Mol. Endocrinol.* **18,** 1064–1081
39. Iannone, M. A., Taylor, J. D., Chen, J., Li, M. S., Rivers, P., Slentz-Kesler, K. A., and Weiner, M. P. (2000) *Cytometry* **39,** 131–140
40. Miao, B., Zondlo, S., Gibbs, S., Cromley, D., Hosagrahara, V. P., Kirchgessner, T. G., Billheimer, J., and Mukherjee, R. (2004) *J. Lipid Res.* **45,** 1410–1417
41. McDonnell, D. P. (2003) *Endocrinology* **144,** 4237–4240
42. Schulz, M., Eggert, M., Baniahmad, A., Dostert, A., Heinzel, T., and Renkawitz, R. (2002) *J. Biol. Chem.* **277,** 26238–26243
43. Wang, Q., Blackford, J. A., Jr., Song, L. N., Huang, Y., Cho, S., and Simons, S. S., Jr. (2004) *Mol. Endocrinol.* **18,** 1376–1395
44. Smith, C. L., and O'Malley, B. W. (2004) *Endocr. Rev.* **25,** 45–71
45. Wagner, B. L., Valledor, A. F., Shao, G., Daige, C. L., Bischoff, E. D., Petrowski, M., Jepsen, K., Baek, S. H., Heyman, R. A., Rosenfeld, M. G., Schulman, I. G., and Glass, C. K. (2003) *Mol. Cell. Biol.* **23,** 5780–5789
46. Farnegardh, M., Bonn, T., Sun, S., Ljunggren, J., Ahola, H., Wilhelmsson, A., Gustafsson, J. A., and Carlquist, M. (2003) *J. Biol. Chem.* **278,** 38821–38828
47. Hoerer, S., Schmid, A., Heckel, A., Budzinski, R. M., and Nar, H. (2003) *J. Mol. Biol.* **334,** 853–861
48. Stanley, T. B., Leesnitzer, L. M., Montana, V. G., Galardi, C. M., Lambert, M. H., Holt, J. A., Xu, H. E., Moore, L. B., Blanchard, S. G., and Stimmel, J. B. (2003) *Biochemistry* **42,** 9278–9287
49. Zechel, C. (2002) *J. Recept. Signal Transduct. Res.* **22,** 31–61
50. Svensson, S., Ostberg, T., Jacobsson, M., Norstrom, C., Stefansson, K., Hallen, D., Johansson, I. C., Zachrisson, K., Ogg, D., and Jendeberg, L. (2003) *EMBO J.* **22,** 4625–4633