0888-8809/02/$15.00/0
Printed in U.S.A.

Molecular Endocrinology 16(9):2065–2076

doi: 10.1210/me.2001-0194

The Small Heterodimer Partner Interacts with the Liver X Receptor α and Represses Its Transcriptional Activity

CAROLE BRENDEL, KRISTINA SCHOONJANS, ORONZA A. BOTRUGNO, ECKARDT TREUTER, AND JOHAN AUWERX

Institut de Génétique et de Biologie Moléculaire et Cellulaire (C.B., K.S., O.A.B., J.A.), Centre National de la Recherche Scientifique/Institut National de la Santé et de la Recherche Médicale/Université Louis Pasteur, BP 1042, 67404 Illkirch, France; and Department of Biosciences at Novum (E.T.), Karolinska Institute, S-14157 Huddinge/Stockholm, Sweden

The small heterodimer partner SHP (NR0B2) is an unusual nuclear receptor that lacks the typical DNA binding domain common to most nuclear receptors. SHP has been reported to act as a corepressor for several nuclear receptors, but its exact mechanism of action is still elusive. Here we show that SHP can interact with the liver X receptors LXRα (NR1H3) and LXRβ (NR1H2), as demonstrated by glutathione-*S*-transferase pull-down assays, mammalian two-hybrid, and coimmunoprecipitation experiments. In transfection assays, SHP inhibits the expression of an artificial reporter driven by an LXR-response element and represses the transcriptional activation by LXR of the human ATP-binding cassette transporter 1 (*ABCA1*) promoter. Treatment of Caco-2 cells with bile acids, which activate farnesoid X receptor and subsequently induce SHP, leads to the repression of the human *ABCG1* gene, an established LXR target gene. These results demonstrate that SHP is able to interact with LXR and to modulate its transcriptional activity. (*Molecular Endocrinology* 16: 2065–2076, 2002)

NUCLEAR RECEPTORS CONSTITUTE a large family of transcription factors that affect many physiological pathways. When unliganded, some nuclear receptors are actively repressing transcription by associating with corepressors. Upon binding of ligands, the nuclear receptors release corepressors and concomitantly recruit cofactors (1), which in turn, through chromatin remodeling and histone modification, facilitate transcription of target genes.

Liver X receptors (LXRs) are nuclear receptors that are activated by oxysterols (2–6). LXRα (NR1H3, RLD-1) (2, 7) and LXRβ (NR1H2, UR, NER1, OR-1, RIP15) (8–11) are encoded by different genes and bind both to DNA as a heterodimer with retinoid X receptor (RXR). Whereas LXRβ is ubiquitously expressed, LXRα is mainly localized in the liver, intestine, kidney, and adipose tissue. LXRα modulates the expression of multiple genes involved in cholesterol homeostasis such as the cholesterol-7-α-hydroxylase (CYP7A1) gene that encodes the rate limiting enzyme of the bile acid synthesis pathway (4, 12), the ATP-binding cassette transporters ABCA1, ABCG1, ABCG5, and ABCG8 (13–19), the cholesteryl ester transfer protein (CETP) (20), the apolipoprotein E (21), and the sterol regulatory element-binding protein-1c (6, 22, 23). Due to the coordinate regulation of several of these target genes, LXR has been suggested to play an important role in reverse cholesterol transport. For some genes, optimal activity of the LXR-RXR heterodimer is achieved when the liver receptor homolog-1 (LRH-1), an orphan nuclear receptor, is also bound to an adjacent site on the promoter of target genes, such as reported for *CYP7A1* (24) or *CETP* (25). Therefore, LRH-1 is considered as a competence factor for the LXR-RXR heterodimer.

The small heterodimer partner (SHP) is an atypical nuclear receptor that lacks a DNA binding domain (26). It has been shown to repress the transcriptional activity of several nuclear receptors, such as the constitutive androstane receptor (26), thyroid receptor (26), RXR, retinoic acid receptor (RAR) (26), estrogen receptors (ERs) (27, 28), hepatocyte nuclear factor 4α (HNF-4α) (29), peroxisome proliferator-activated receptors (30), and LRH-1 (24, 31). SHP is closely related to dosage-sensitive sex reversal-adrenal hypoplasia congenita critical region on the X chromosome gene 1 (DAX-1), a corepressor for the steroidogenic factor-1 (32, 33), the paralog of LRH-1. An illustration for the functional relevance of the interaction of SHP with nuclear receptors has been provided by the existence of an autoregulatory loop controlling bile acid homeostasis involving several nuclear receptors. In fact, bile acids activate the farne-

Abbreviations: ABCA1, ABCG1, ABCG5, and ABCG8, ATP-binding cassette transporters; CDCA, chenodeoxycholic acid; CETP, cholesteryl ester transfer protein; CYP7A1, cholesterol-7-α-hydroxylase; DAX-1, dosage-sensitive sex reversal-adrenal hypoplasia congenita critical region on the X chromosome gene 1; DBD, DNA binding domain; DMSO, dimethylsulfoxide; ER, estrogen receptor; FCS, fetal calf serum; FXR, farnesoid X receptor; GST, glutathione-*S*-transferase; HNF, hepatocyte nuclear factor; LBD, ligand binding domain; LPDS-DCC, lipoprotein-deficient and charcoal-stripped FCS; LRH, liver receptor homolog; Luc, luciferase; LXR, liver X receptor; RXR, retinoid X receptor; SDS, sodium dodecyl sulfate; SHP, small heterodimer partner (NR0B2); TFIID, general transcription initiation factor II D; TFIIE, general transcription initiation factor II E; TK, thymidine kinase; UAS, upstream activating sequence.

soid X receptor (FXR)/bile acid receptor, which on its turn induces the expression of SHP (24, 31, 34, 35). SHP will repress on the one hand LRH-1 activity, ultimately decreasing CYP7A1 expression (24, 31), and on the other hand RXR/RAR activity, decreasing the expression of the principal hepatic bile acid transporter sodium/taurocholate cotransporting polypeptide (36). This will coordinately down-regulate bile acid import and synthesis, thereby protecting the liver from bile acid-mediated cellular damage.

Although the expression patterns of LXRα and LRH-1 overlap and these receptors coregulate several target genes, it has not been clearly demonstrated whether SHP is also able to interact with LXR. Therefore, we compared the interaction of SHP with LXR on the one hand and with LRH-1 on the other hand. We demonstrate here that SHP is able to bind to LXR. Helix 12 of LXR is indispensable for the interaction with SHP, whereas both the central and carboxy terminus of SHP are required for its interaction with LXR. SHP strongly represses LXR activation of an artificial reporter gene controlled by multiple LXR response elements and represses also, although to a lesser extent, the promoter of the LXR target gene *ABCA1*. In Caco-2 cells, the LXR-dependent induction of *ABCG1* mRNA expression is repressed after induction of SHP by chenodeoxycholic acid. The finding that SHP represses the activity of both LRH-1 and LXR suggests that it is an important integrator of bile acid metabolism.

RESULTS

SHP Interacts *in Vitro* with LXR

The optimal efficacy of LXRα to activate its target genes, such as *CYP7A1* or *CETP,* is usually obtained when LRH-1, a competence factor for LXRα, is also bound to an adjacent site on the promoter (20, 24, 25). Recently it has been shown that SHP inhibits LRH-1 activity, thereby attenuating the *CYP7A1* promoter and establishing an inhibitory feed-back loop controlling bile acid synthesis by its end products. We investigated here the possibility that LXRα might also be a molecular target for SHP. To this end, we analyzed whether LXR and SHP interact in glutathione-*S*-transferase (GST)-pull-down assays. Full-length LXRα- and LRH-1-GST fusion proteins were incubated with ^{35}S-radiolabeled SHP. SHP interacted to a similar extent with the LXRα- and LRH-1-GST fusion proteins but was unable to bind to the GST protein itself, which served as a negative control (Fig. 1A). Full-length GST-SHP fusion protein also interacted with ^{35}S-radiolabeled full-length LXRβ (Fig. 1B). SHP interacted with LXRs in the absence of ligand. The efficiency of the interaction of SHP with LXRα was, however, significantly increased by the addition of T0901317, a synthetic ligand for LXRα (6) (Fig. 1C). The binding between LXRα and SHP, in this particular experiment, could not be detected anymore in the absence of ligand because the vehicle to dissolve T0901317, *i.e*. dimethylsulfoxide (DMSO), strongly inhibited the interaction between LXRα and SHP (compare Fig. 1, A and C). Likewise and consistent with previous reports, the interaction between SHP and RXRα was also enhanced in the presence of the synthetic rexinoid, LG100268 (26) (Fig. 1D).

LXRα Coimmunoprecipitates with Endogenous SHP

To provide further evidence that SHP directly interacts with LXRα *in vivo,* coimmunoprecipitation assays were carried out. An LXRα-flag fusion protein expression vector or an empty expression vector were transfected

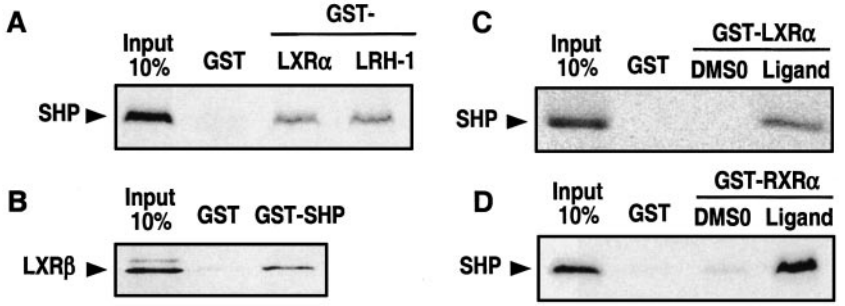

Fig. 1. SHP Interacts *in Vitro* with LXRα and LXRβ

A, ^{35}S-radiolabeled SHP protein was incubated with GST, LXRα-GST, or LRH-1-GST fusion proteins bound to glutathione-Q sepharose beads. The beads were then washed and the samples separated on a 15% SDS-polyacrylamide gel. Protein interactions were detected by autoradiography. B, ^{35}S-radiolabeled LXRβ protein was incubated with GST or SHP-GST fusion proteins bound to glutathione-Q sepharose beads. The beads were then washed and the samples separated on a 10% SDS-polyacrylamide gel. Protein interactions were detected by autoradiography. C, ^{35}S-radiolabeled SHP protein was incubated with GST or LXRα-GST fusion proteins in the presence (Ligand) of T0901317 (10^{-4} M) or vehicle (DMSO). The beads were then washed and the samples separated on a 15% SDS-polyacrylamide gel. Protein interactions were detected by autoradiography. D, ^{35}S-radiolabeled SHP protein was incubated with GST or RXRα-GST fusion proteins in the absence or presence of the rexinoid LG100268 (10^{-6} M). The beads were then washed and the samples separated on a 15% SDS-polyacrylamide gel. Protein interactions were detected by autoradiography.

in RK-13 cells containing endogenous SHP. Cells were treated for 20 h with 10^{-5} M 22*(R)*-hydroxycholesterol, an LXR ligand, before harvesting and lysis. The lysate was then incubated with an irrelevant antibody (Fig. 2A, lanes 3 and 5) or an anti-SHP antibody (Fig. 2A, lanes 4 and 6) and the precipitated proteins were analyzed by immunoblotting with an anti-flag antibody. No band was detected in the immunoprecipitate when an irrelevant antibody was used (Fig. 2A, lane 5). In contrast, the LXRα-flag protein could be detected when an anti-SHP antibody was used for immunoprecipitation (Fig. 2A, lane 6). The amount of endogenous SHP present in the cells transfected with the empty pCMX expression vector or pCMX-LXRα-flag and detected by anti-SHP immunoblot was similar (Fig. 2B).

Multiple Domains of SHP and the Ligand Binding Domain (LBD) of Both LXRα and LRH-1 Are Necessary and Sufficient for *in Vitro* Interaction

We next localized the exact regions of SHP involved in the interaction with LXRα. The interaction domain with LRH-1 has been recently localized to the amino-terminal part of SHP (37). Various deletion mutants of SHP fused to the GST protein were generated as outlined in Fig. 3A. The fusion proteins were subsequently incubated with ^{35}S-radiolabeled full-length RXRα or LXRα. Consistent with previous reports (38), the SHP mutant containing region 1 + 2 still interacted with RXRα in the presence of the RXR ligand, LG100268 (Fig. 3B). In contrast to the situation for RXRα, region 1 + 2 of SHP seemed less critical for the interaction with LXRα (Fig. 3B). Interestingly, the carboxy-terminal region 3 of SHP by itself interacted with LXRα, although the interaction was weaker than when domains 2 and 3 of SHP were combined.

A similar experiment was performed next, now using GST-LXRα and a series of *in vitro* translated ^{35}S-radiolabeled SHP mutants (Fig. 3C). In this experiment, SHP domain 1+2 (SHP1 + 2) still interacted with LXRα, albeit more weakly than the wild-type (full length) protein (SHPFL) (Fig. 3, D and E). Mutation of the two LXXLL motifs in domains 1 and 2 of the wild-type protein (SHPmut1+2) did not abolish the interaction but weakened it. This result confirms that domain 3 also contributes to the interaction between SHP and LXRα and that region 1 + 2 is dispensable to some extent. To determine the exact domains of LXRα and LRH-1 that interact with SHP, deletion mutants of LXRα and LRH-1 fused to GST (outlined in Fig. 4, A and C) were incubated with ^{35}S-radiolabeled full-length SHP. For both receptors, the LBD (or DE domain) was necessary and sufficient for the interaction with SHP (Fig. 4, B and D). This result is consistent with a previous report delimitating the LRH-1 interaction domain to the AF-2 region (37).

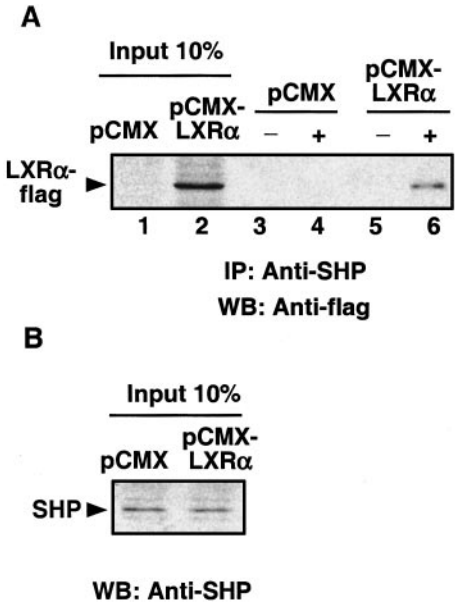

Fig. 2. LXRα-Flag Fusion Protein Coimmunoprecipitates with Endogenous SHP

A, RK-13 cells were transfected with 10 μg of either the pCMX or the pCMX-LXRα-flag expression vector and treated with 22*(R)*-hydroxycholesterol at a final concentration of 10^{-5} M. LXRα-flag protein was then detected after coimmunoprecipitation (IP) with endogenous SHP using either an irrelevant antibody (−) or an anti-SHP antibody (+). Samples were separated on a 7.5% SDS-polyacrylamide gel, and a Western blot (WB) was performed using an anti-flag antibody. B, Cellular extracts corresponding to 10% of the input were separated on a 12% SDS-PAGE gel, and WB was performed using the anti-SHP antibody.

SHP Interacts with LXRα in an AF-2-Dependent Manner and Interacts *in Vitro* with RNA Polymerase II

To confirm that SHP directly interacts with the LBD of LXRα *in vivo*, we used the mammalian two-hybrid system in which the LXRα LBD was fused to the DNA binding domain (DBD) of the Gal4 protein and in which the full-length SHP protein was fused to the activation domain of VP16. As the consequence of a direct interaction between LXRα and SHP, increased transcriptional activity of a reconstituted Gal4-VP16 transcription factor was monitored by transfecting the luciferase reporter vector under the control of a multimerized Gal4 response element (upstream activating sequence, UAS).

The ectopic expression of VP16-SHP in the presence of a DBD-Gal4-LXRα_{LBD} resulted in a ±3-fold induction in luciferase activity compared with the empty vector VP16 (Fig. 5, A and B). As for the interaction observed in GST pull-down assays between LXRα and SHP, the interaction was increased in the presence of the LXRα ligand, 22*(R)*-hydroxycholesterol. In a parallel experiment, the interaction between DBD-Gal4-LRH-1_{LBD} and VP16-SHP was shown to lead to a more moderate (±2-fold) induction of luciferase activity, which is consistent with previously reported results (24) (Fig. 5, C and D). We also repeated

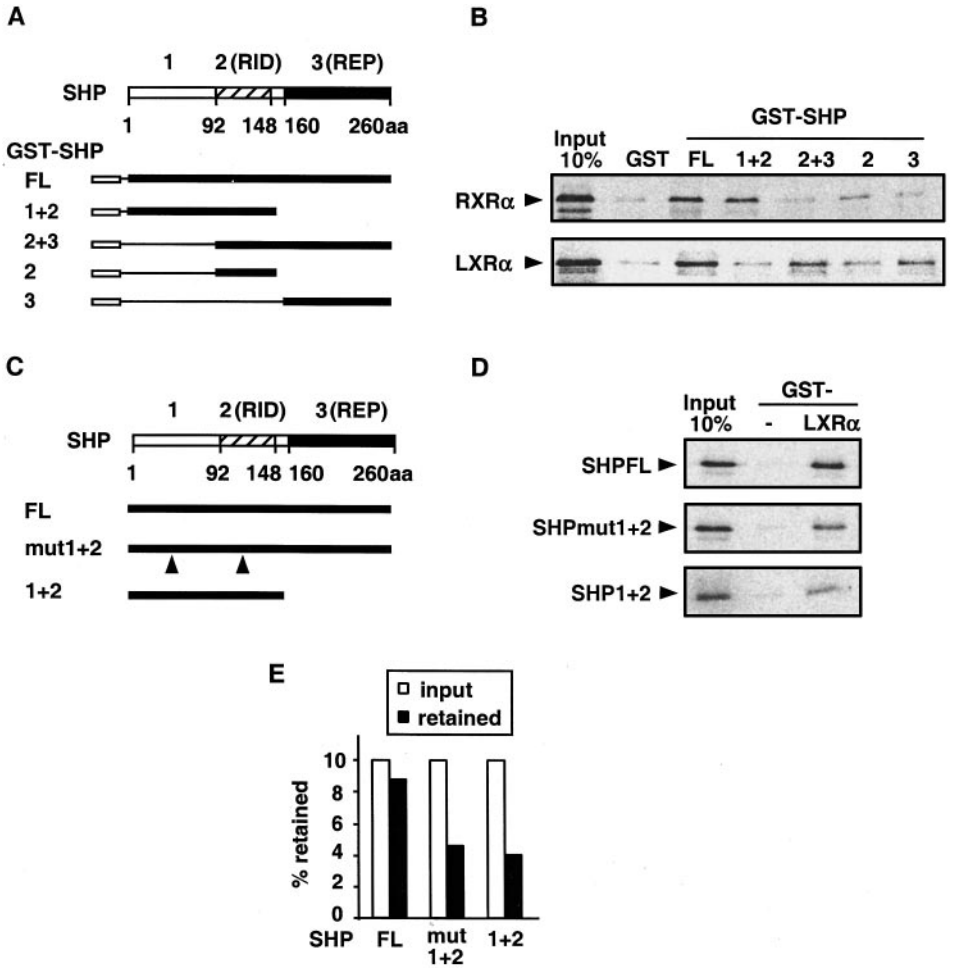

Fig. 3. The Carboxy-Terminal Part of SHP Is Necessary and Sufficient for *in Vitro* Interaction with Both LXRα and LRH-1

A and C, Schematic representation depicting the SHP domain structure and the various constructs used in the GST pull-down assays. *Black bars* represent parts of the SHP protein, whereas the parts of the fusion proteins derived from GST are depicted in *white*. The two point mutations in SHPmut1+2 are indicated by *arrowheads*. RID, Receptor interaction domain, REP, repression domain. B, ^{35}S-radiolabeled RXRα or LXRα proteins were incubated with GST or with various deletion mutants of SHP-GST fusion proteins bound to glutathione-Q sepharose beads. The pull-down assays with RXRα were carried out in the presence of LG100268 (10^{-6} M). After pull-down, the beads were washed and the samples separated on a 10% SDS-PAGE gel and protein interactions were detected by autoradiography. D, ^{35}S-radiolabeled SHP or SHPmut1+2 or SHP1+2 were incubated with GST or with LXR-GST fusion protein bound to glutathione-Q sepharose beads. After pull-down, the beads were washed and the samples separated on a 12% SDS-PAGE gel and protein interactions were detected by autoradiography. E, Quantification of the amount of *in vitro* translated SHP proteins retained by the LXR-GST column. The intensities of the specific bands were quantitated using a PhosphorImager (Molecular Dynamics, Saclay, France). The percent retention was determined by comparison to the 10% input lane.

the mammalian two-hybrid experiments using more detailed deletion mutants of the LBD of LXRα and LRH-1 fused to the DBD of Gal4. Deletion of helices 11 and 12 completely abrogated the interaction of VP16-SHP with LXRα in the presence of 22*(R)*-hydroxycholesterol (Fig. 5E). The deletion of helix 12 alone had the same effect as deletion of both helices 11 and 12, which confirms that helix 11, that is thought to be part of the corepressor anchoring site in some nuclear receptors (39), is not crucial for the interaction with SHP. Likewise, deletion of helix 12 of LRH-1 also abolished completely the capacity of LRH-1 to interact with VP16-SHP (Fig. 5F).

In another set of experiments, pCMX-Gal4-LXRα$_{LBD}$ was cotransfected with either the empty expression vector pCDM8 or the pCDM8-SHP vector that codes for the full-length SHP protein without the activation domain of VP16. In the absence of the VP16 activation domain, we would hence expect SHP to decrease the capacity of LXR to activate the reporter gene. As expected, a reduction of the UAS-driven reporter activity could be observed when pCDM8-SHP was cotransfected instead of pCDM8 (Fig. 6A). This reduction in UAS-driven reporter activity was somewhat more pronounced in the presence of an LXR ligand. In general, these data are consistent with the reported moderate

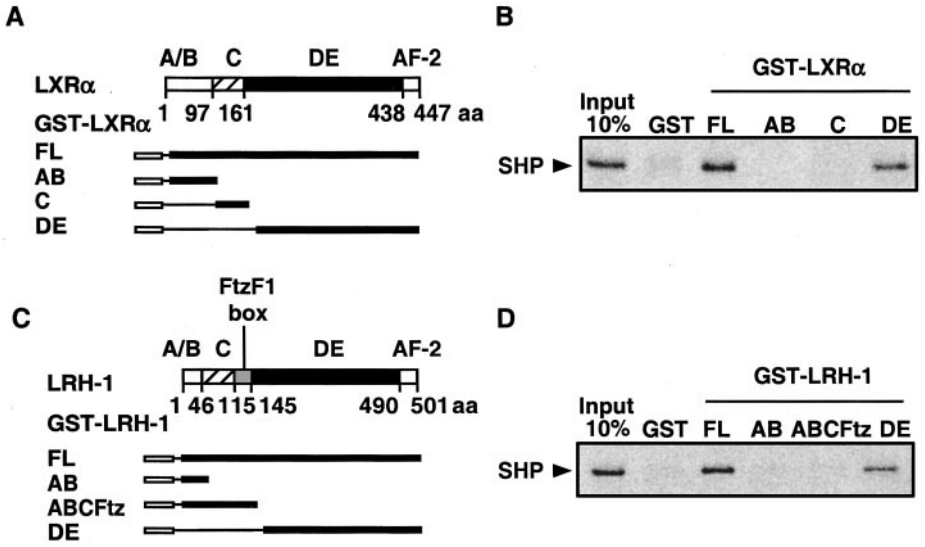

Fig. 4. The LBD of LXRα and LRH-1 Is Necessary for the *in Vitro* Interaction with SHP

A and C, Schematic representation of the LXRα (A) or LRH-1 (C) domains, as well as the various constructs used in the GST pull-down assays. *Black bar*s represent parts of the LXRα or LRH-1 protein, whereas the parts of the fusion proteins derived from GST are depicted in *white*. B and D, ^{35}S-radiolabeled SHP proteins were incubated with GST, LXRα-GST (B), or LRH-1-GST (D) fusion proteins bound to glutathione-Q sepharose beads. After pull-down, the beads were washed and the samples separated on a 15% SDS-polyacrylamide gel and protein interactions were detected by autoradiography.

reduction of ERα activity by SHP cotransfection (28). As the basal transcriptional activity of DBD-Gal4-LXRα_{LBD} was only slightly activated by the addition of 22*(R)*-hydroxycholesterol (Fig. 5A), we sought to increase the activation of the reporter gene by cotransfecting an expression vector encoding the coactivator TIF2. Cotransfection of TIF2 increased the amplitude of the ligand-induced activation of the UAS-driven reporter by the DBD-Gal4-LXR$_{LBD}$ fusion protein. Also, under these conditions, SHP was able to repress TIF2-mediated coactivation (Fig. 6B).

SHP seemed therefore to repress LXR transcriptional activity by competing with cofactors such as TIF2. The exact mechanism of the repressive action of SHP is, however, still unclear. We have tested in GST pull-down assays whether SHP is capable to interact with several components of the basal transcription machinery, *i.e.* general transcription initiation factor II D (TFIID), general transcription factor II E (TFIIE), and RNA polymerase II. Interestingly, no interaction could be detected between SHP and TFIID or TFIIE, whereas RNA polymerase II was efficiently retained by a GST-SHP column (Fig. 6C).

SHP Represses LXR Transcriptional Activity on an Artificial and a Natural Promoter

Because SHP interacts with LXRα both *in vitro* and *in vivo* and because this interaction is associated with moderate repressive effect on transcriptional activity in our mammalian two-hybrid assays, we investigated next the possibility that LXRα transactivation might be attenuated by SHP. We therefore transfected CV-1 cells with a luciferase reporter gene under the control of a multimerized response element for LXR. When the heterodimer LXR/RXR was cotransfected with increasing amounts of SHP, transcriptional activity was strongly decreased (Fig. 7A). This repression was abrogated when a SHP construct containing mutations in the LXXLL motifs present in domain 1 and 2 was used (Fig. 7B). These data support the concept that these motifs, although dispensable for the interaction between SHP and LXRα, are crucial for the repressive activity of SHP on LXRα.

We also tested whether SHP could also inhibit LXR transactivation of a well established LXR activated promoter, such as the promoter of the *ABCA1* gene. This promoter was selected because it was reported to be primarily responsive to human LXRα but not to human LRH-1 (25). CV-1 cells were therefore cotransfected with an *ABCA1* reporter luciferase vector containing the region between −400 and +181 harboring the LXR/RXR response element. In the presence of increasing amounts of cotransfected SHP, a repression of *ABCA1* promoter was observed (Fig. 7C). Under similar conditions, we demonstrated that increasing amounts of SHP blocked the capacity of LRH-1 to activate the SHP promoter, as previously published (24) (Fig. 7D).

SHP Represses LXR Transcriptional Activity *in Vivo*

To validate our *in vitro* data *in vivo*, we performed an experiment in differentiated Caco-2 cells derived from a human colon adenocarcinoma. Caco-2 cells express

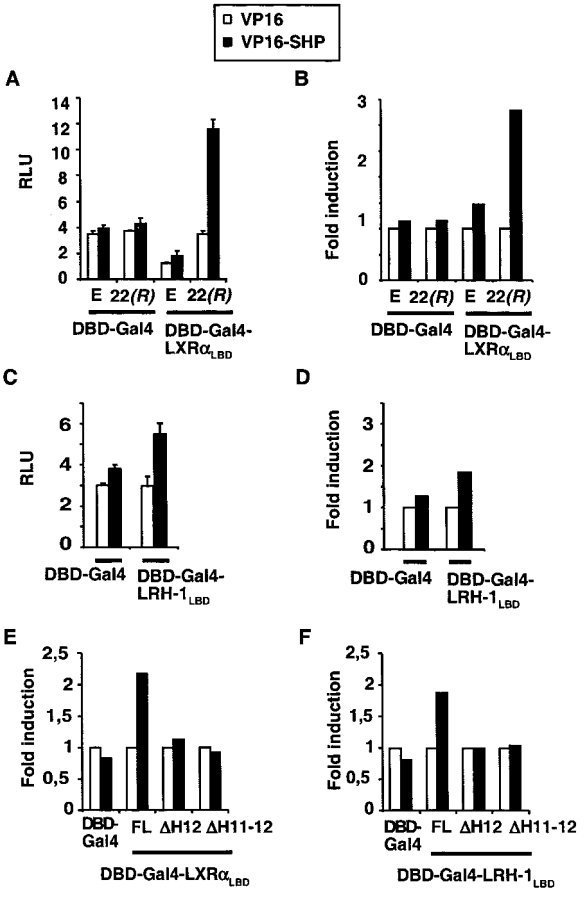

Fig. 5. SHP Interacts with LXRα and LRH-1 LBD in a Mammalian Two-Hybrid System

A, RK-13 cells were cotransfected with expression vectors for the DBD-Gal4 or DBD-Gal4-LXRα_{LBD} fusion proteins (200 ng/well), an expression vector encoding VP16 or VP16-SHP (1 μg/well) and the reporter construct pGL3-(UAS)$_5$TK-Luc (1 μg/well). Cells were grown 24 h in the absence (EtOH) or presence (22*(R)*-HC) of 10^{-5} M 22*(R)*-hydroxycholesterol. The histogram represents the transcriptional activity of the DBD-Gal4-LXRα_{LBD} fusion protein in the presence of cotransfected pCMX-VP16 vector or pCMX-VP16-SHP. B, The results of A are represented as fold induction. The histogram represents the transcriptional activity of the DBD-Gal4-LXRα_{LBD} fusion protein in presence of pCMX-VP16-SHP relative to its activity in presence of the pCMX-VP16 control vector. C, A similar experiment as in A was performed using DBD-Gal4-LRH-1$_{LBD}$ fusion protein. D, The results of C are represented as fold induction. E, RK-13 cells were cotransfected with expression vectors encoding the DBD-Gal4, DBD-Gal4-LXRα_{LBD}, or the indicated deletion mutants of the DBD-Gal4-LXRα_{LBD} fusion proteins (200 ng/well), an expression vector for VP16-SHP (1 μg/well), and the pGL3-(UAS)$_5$TK-Luc reporter construct (1 μg/well). Cells were then grown 24 h in the presence of 10^{-5} M 22*(R)*-hydroxycholesterol. The histogram in fold induction represents the transcriptional activity of the various DBD-Gal4-LXRα_{LBD} fusion proteins in presence of pCMX-VP16-SHP relative to their activity in presence of the pCMX-VP16 control vector. The activity of the chimeric protein was arbitrarily set to 1 when no SHP is added. F, A similar experiment as in E was performed using various DBD-Gal4-LRH-1$_{LBD}$ deletion mutants.

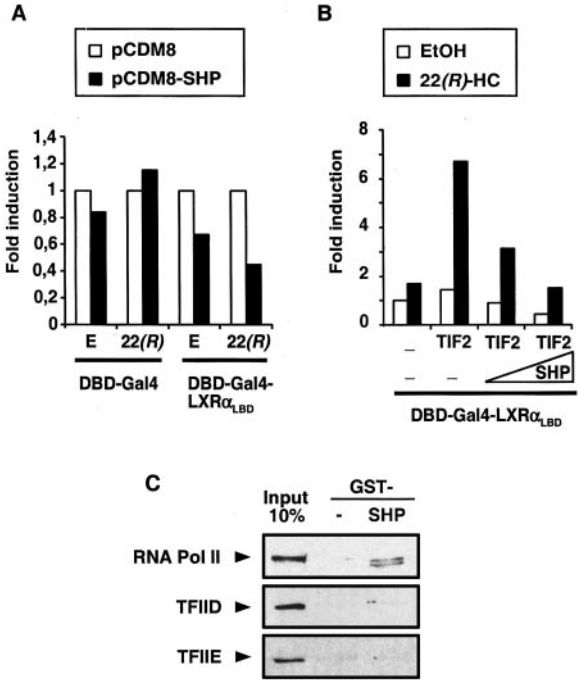

Fig. 6. SHP Inhibits the AF-2 Activity of LXR and Interacts *in Vitro* with RNA Polymerase II

A, RK-13 cells were cotransfected with expression vectors for the DBD-Gal4 or DBD-Gal4-LXRα_{LBD} fusion proteins (200 ng/well), an empty expression vector (pCDM8), or the same vector encoding SHP (pCDM8-SHP) (200 ng/well), and the pGL3-(UAS)$_5$TK-Luc reporter construct (1 μg/well). Cells were then grown 24 h in the presence or absence of 10^{-5} M 22*(R)*-hydroxycholesterol. The histograms represent the difference in the transcriptional activity of the DBD-Gal4-LXRα_{LBD} fusion protein in presence of the pCDM8-SHP and pCDM8 control vectors. B, RK-13 cells were cotransfected with expression vectors for the DBD-Gal4 or DBD-Gal4-LXRα_{LBD} fusion protein (200 ng/well), an expression vector for TIF2 (1 μg/well), increasing amounts of an expression vector for SHP (0, 1, 2 μg/well), and the pGL3-(UAS)$_5$TK-Luc reporter construct (1 μg/well). Cells were then grown 24 h in the absence (*white bars*) or presence (*black bars*) of 10^{-5} M 22*(R)*-hydroxycholesterol. The histograms represent the relative change in transcriptional activity of the DBD-Gal4-LXRα_{LBD} fusion protein relative to the activity of the DBD-Gal4 protein, in the presence of cotransfected TIF2 and SHP. C, Purified RNA polymerase II, TFIID, or TFIIE complexes were incubated with GST or SHP-GST fusion proteins bound to glutathione-Q sepharose beads. The beads were then washed and the samples separated on a 7.5% SDS-polyacrylamide gel. Protein interactions were detected by Western blot using anti-RPB-1, anti-TBP, and anti-His antibodies, respectively.

LXR, FXR, and SHP (Fig. 8A and data not shown), and are an adequate model to study certain steps of cholesterol absorption and bile acid metabolism. Caco-2 cells were therefore treated with T0901317 (10^{-5} M), a synthetic LXR agonist, and/or chenodeoxycholic acid (CDCA; 250 μM), an FXR ligand that induces SHP. RNA was isolated from these cells and analyzed by Northern blot hybridization (Fig. 8A). Interestingly, T0901317

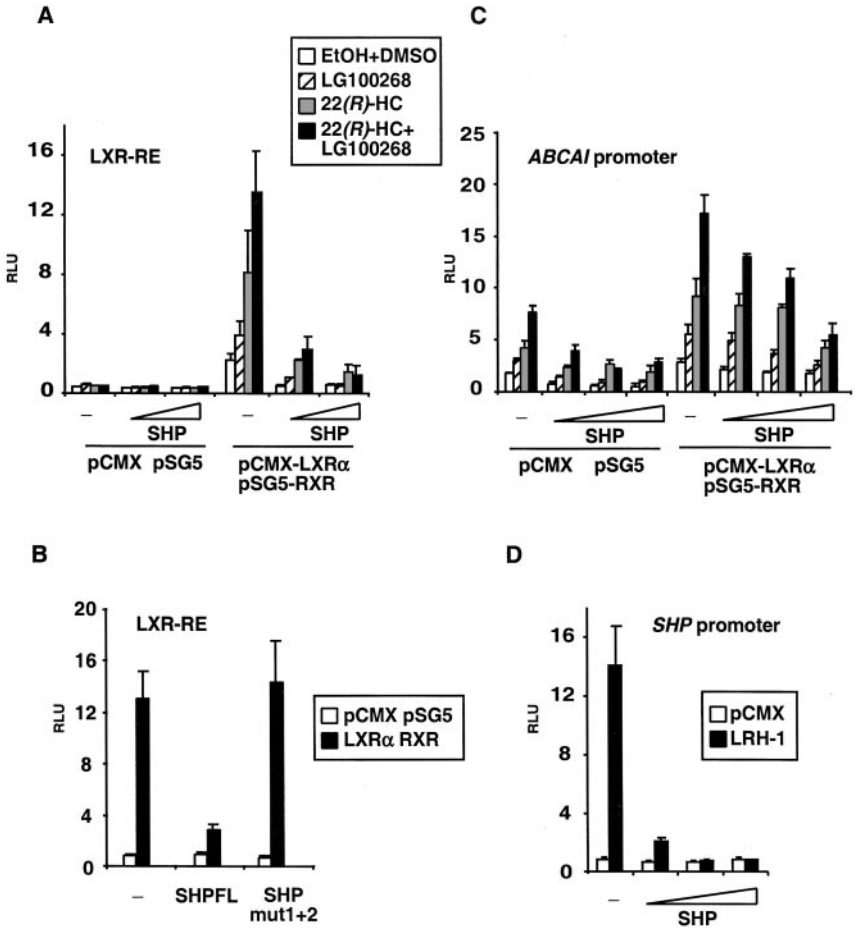

Fig. 7. SHP Represses LXR Transcriptional Activity on the pGL3-$(LXRE)_5$TK-Luc Reporter Construct and on the Human *ABCA1* Promoter

A, CV-1 cells were cotransfected with the reporter construct pGL3-$(LXRE)_5$TK-Luc (0.5 μg/well), pCMX-LXRα (100 ng/well), pSG5-RXRα (10 ng/well), or the corresponding empty pCMX and pSG5 vectors, and increasing amounts (0.1- or 0.3-fold molar ratio) of pCDM8-SHP (0, 10, 30 ng/well). After transfection, cell were grown during 24 h in medium containing LPDS-DCC, in the presence or absence of 10^{-6} M LG100268, 10^{-5} M 22*(R)*-hydroxycholesterol, or both activators together, before harvesting for luciferase assay. B, CV-1 cells were cotransfected with the reporter construct pGL3-$(LXRE)_5$TK-Luc (0.5 μg/well), pCMX-LXRα (100 ng/well), pSG5-RXRα (10 ng/well) or the corresponding empty pCMX and pSG5 vectors, and pSG5-SHPFL encoding for full-length SHP (100 ng/well) or pSG5-SHPmut1+2 (100 ng/well) or the corresponding empty pSG5 vector. After transfection, cell were grown during 24 h in medium containing LPDS-DCC, in the presence of 10^{-6} M LG100268 and 10^{-5} M 22*(R)*-hydroxycholesterol before harvesting for luciferase assay. C, CV-1 cells were cotransfected with the human pGL3-hABCA1-Luc reporter construct (1 μg/well), pCMX-LXRα (100 ng/well), pSG5-RXRα (10 ng/well) or the corresponding empty pCMX and pSG5 vectors, and increasing amounts (0.1-, 0.3-, or 1-fold molar ratio) of pCDM8-SHP (0, 10, 30, 100 ng/well). After transfection, cell were grown during 24 h in medium containing LPDS-DCC, in the presence or absence of 10^{-6} M LG100268, 10^{-5} M 22*(R)*-hydroxycholesterol, or both activators together, before harvesting for luciferase assay. D, CV-1 cells were cotransfected with the reporter construct pGL3-hSHP (569)-Luc (1 μg/well), a mouse LRH-1 expression vector (500 ng/well), and increasing amounts (0.1-, 0.3-, or 1-molar ratio) of the expression vector pCDM8-SHP (0, 50, 150, 500 ng/well). Cells were then grown during 24 h before harvesting for luciferase assay.

addition induced the expression of *SHP* (12-fold induction) (Fig. 8A, lane 2). When CDCA and T0901317 were added together to the Caco-2 cells, *SHP* mRNA was, however, induced to a much higher level (40-fold induction) (Fig. 8A, lane 4). Unexpectedly, *ABCA1* mRNA was induced by CDCA (30-fold induction), and no clear conclusions could be made regarding its expression (Fig. 8A, lane 3). In absence of the bile acids, treatment with T0901317 induced *ABCG1* mRNA (Fig. 8A, lane 2), another LXR target gene (16, 21). The up-regulation of *ABCG1* mRNA levels by T0901317 was completely abrogated when the Caco-2 cells were treated with CDCA, which coincided with stronger *SHP* expression (Fig. 8A, lane 4). The fact that *ABCG1* expression was independent of LRH-1 was established by the demonstration that *ABCG*1 expression was not up-regulated in mouse hepatic (BNL-CL.2) cells that were infected with a retrovirus that expressed LRH-1 (Fig. 8C, lane 2). In this case, a RT-PCR was used because of the low expression of

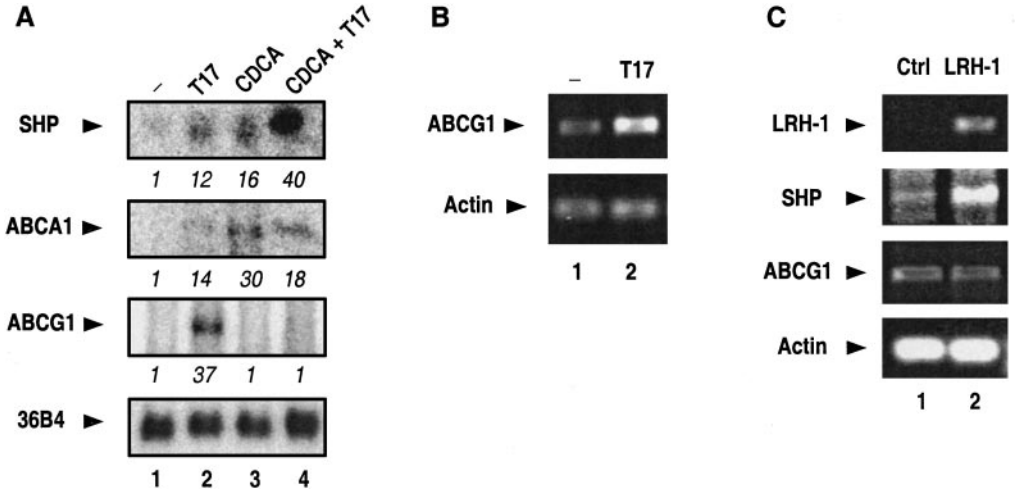

Fig. 8. SHP Represses LXR Transcriptional Activity *in Vivo*

A, Differentiated Caco-2 cells (colon adenocarcinoma cells) were treated with 10^{-5} M T0901317 (T17) or vehicle (DMSO) during 4 h before the addition of 250 μM CDCA or vehicle (EtOH) during 24 h. RNA was extracted and Northern blots were hybridized with cDNA probes for SHP, ABCA1, ABCG1, or 36B4. The intensities of the specific bands were quantified using a PhosphorImager. Numbers represent signal intensities relative to nontreated cells (lane 1). B, BNL-CL.2 cells were treated with 10^{-5} M T0901317 (T17) or vehicle (DMSO) during 24 h. RNA was extracted and a RT-PCR was performed to amplify ABCG1 and β-actin. C, BNL-CL.2 cells were infected with an empty retrovirus (Ctrl) or a retrovirus expressing mouse LRH-1 (LRH-1). RNA was extracted and a RT-PCR followed by a PCR was performed to amplify LRH-1, SHP, ABCG1 and β-actin.

the different genes. *SHP*, a well-established LRH-1 target gene, was induced in these cells (Fig. 8C, lane 2). We have also treated noninfected BNL-CL.2 cells with T0901317 to demonstrate that ABCG1 mRNA can be induced upon treatment with an LXR agonist (Fig. 8B, lane 2). These reults hence confirm our *in vitro* data and claim a role of SHP in the direct down-regulation of specific LXR target genes.

DISCUSSION

SHP is an atypical nuclear receptor highly expressed in many tissues (26, 40). SHP interacts with several nuclear receptors, and this interaction impedes the transcriptional activity of these receptors. Recently, it has been shown that SHP is able to decrease the promoter activity of CYP7A1, the rate-limiting enzyme for bile acid synthesis, by repressing the activity of LRH-1, which acts as a competence factor for LXR/RXR on the *CYP7A1* gene (24, 31). However, whether SHP also interacts with LXRα has never been fully explored. The evaluation of an eventual interaction of SHP and LXRα is confounded by the fact that most of the currently known promoters that are activated by LXRα also require the presence of LRH-1 as a competence factor [*CYP7A1, CETP* (24, 25)].

In the present study, we provide evidence for a direct interaction of LXR and SHP in the absence and presence of ligand. A solid binding between both subtypes of LXR and SHP was demonstrated by GST pull-down assays *in vitro* in the absence of ligand. In addition, the efficiency of interaction between LXRα and SHP was significantly increased in the presence of a synthetic LXR ligand. Consistent with these *in vitro* interaction data, a direct *in vivo* interaction between LXRα and endogenous SHP was also demonstrated by immunoprecipitation in cells. The carboxy-terminal domain of SHP, in combination with the central domain, is implicated in the interaction with LXRα. It was shown previously that the carboxy-terminal domain of SHP was necessary for the repressive activity of SHP but most likely dispensable for the interaction with nuclear receptors, which required mainly its central domain (38). Consistent with these reports, we also demonstrated that the central domain of SHP, and not the carboxy-terminal region, is necessary for the interaction with RXR. The fact that LXRα interacts with two different domains in SHP, including the C-terminal domain, which is not implicated in the binding of other nuclear receptors such as RXRα, ER and HNF-4α (27, 29, 38, 41), hence suggests that the interaction domains between SHP and its partners slightly differs according to the nature of the nuclear receptor. It is likely that the carboxy-terminal region of SHP is more involved in the ligand-independent interaction with LXRα, whereas the LXXLL motifs, present in the N-terminal and central part of SHP, could be responsible for the ligand-dependent interaction. In support of this hypothesis, SHP inhibition of LXRα on a synthetic promoter is more effective when the two LXXLL motifs of SHP, found in domains 1 and 2, are intact.

On the receptor side, the LBDs of both LXRα and LRH-1 are necessary for the interaction with SHP in both GST pull-down and mammalian two-hybrid assays. The interaction of SHP with the LBD of LXRα was

significantly stronger than the interaction of SHP with the LBD of LRH-1. This binding was reinforced by the presence of a ligand. Consistent with a role of ligand binding in the SHP/LXRα interaction is the fact that the interaction between SHP and LXRα or between SHP and LRH-1 is completely abrogated when the minimal AF-2 domain (contained within helix 12) of both nuclear receptors is deleted. This is in line with previous reports where it has been hypothesized that SHP acts as a corepressor by competing with the binding of coactivators to helix 12, which constitutes an interaction surface for coactivators in ligand-bound nuclear receptors (28).

SHP acts as a corepressor of LXR transactivation both on a heterologous reporter construct responsive to LXR and on a reporter construct controlled by the human ABCA1 promoter. We have chosen to work with the ABCA1 promoter because, unlike the promoter of other LXR target genes, such as CYP7A1 and CETP (24, 25), the expression of the human ABCA1 promoter activity is unaffected by LRH-1. Therefore, this promoter is a bona fide system to characterize an eventual effect of SHP on LXR activity (25). Although the inhibition of the LXR-mediated activation of the ABCA1 promoter by SHP is weaker compared with its capacity to inhibit the LRH-1-activated SHP promoter, the molar ratio of SHP necessary to observe a repression is in accordance with what was reported in the literature for other nuclear receptors, such as ERα (27, 28, 41) and HNF-4α (29).

We have tried to validate the relevance of the LXR-SHP interaction in differentiated human colon adenocarcinoma (Caco-2) cells treated with CDCA, a bile acid that activates FXR and hence induces SHP. In these cells, the expression of two reported LXR target genes, ABCA1 and ABCG1 was analyzed. ABCA1 mRNA was weakly induced after treatment with a synthetic LXR ligand, T0901317, whereas a more robust induction was seen with the FXR ligand CDCA. This therefore suggests that FXR might also directly regulate the expression of ABCA1. Hence, no clear conclusions concerning the effect of SHP on the *in vivo* regulation of ABCA1 by LXR could be made. This direct induction of ABCA1 expression by FXR agonists is, however, not very surprising on a physiological point of view because it has been reported that FXR modulates the expression of other transporters such as the bile salt export pump (BSEP) (42, 43) and ABCC2 (44). Interestingly, ABCG1 mRNA levels were robustly induced upon treatment with T0901317 in Caco-2 and mouse hepatic (BNL-CL.2) cells. This induction was abrogated when Caco-2 cells were treated with CDCA, which induces SHP expression. Importantly, we established that the expression of the ABCG1 gene is not affected by LRH-1 because its expression does not change in BNL-CL.2 cells that retrovirally overexpress LRH-1. In contrast, classical LRH-1 target genes, such as SHP, are induced in the LRH-1 infected cells. This effect of FXR activation on an LXR (but not LRH-1) target gene is consistent with our *in vitro* data and supports the notion that SHP can directly down-regulate specific LXR target genes.

The mechanism by which SHP represses the activity of nuclear receptors is still not completely elucidated. SHP was first thought to inhibit the DNA binding of receptors via dimerization, such as was suggested for the RAR/RXR heterodimer (26), but this hypothesis was discarded for ERs as SHP interacts with their LBD (27). SHP was then shown to form a ternary complex with dimeric ERβ on DNA (41). It has thus been hypothesized that SHP recruits corepressor complexes, or even directly represses transcription. SHP does not seem to be able to interact with the nuclear receptor corepressor N-CoR and probably does not recruit histone deacetylases (29, 38). In contrast, SHP can recruit the mouse E1A-like inhibitor of differentiation 1, which could antagonize the CBP/p300-dependent coactivator functions (45). DAX-1, a nuclear receptor that resembles SHP both structurally and in its function as a corepressor, is thought to recruit corepressor complexes such as N-CoR and/or Alien (46, 47). In addition, it has been suggested that DAX-1 acts by binding to hairpin-loop structures of DNA (48). In another study, DAX-1 was shown to bind to RNA and exert regulatory functions at a posttranscriptional level (49). More recently, it has been also suggested that DAX-1 inhibits the androgen receptor function by cytoplasmic tethering (50).

Our data, which show that SHP decreases TIF2 stimulation of LXRα activity in the presence of a ligand, would be rather consistent with the hypothesis that the repression function of SHP is at least in part linked to competition with cofactor binding, as previously proposed for ER and HNF-4α (29, 41). Nevertheless, the fold induction (with and without ligand) is affected to a relatively low extent; therefore, it cannot be excluded that the results shown on Fig. 6B are due to a more general inhibition of the basal transcription rate by SHP. Indeed, in an attempt to explore other possible molecular mechanisms underlying SHP activity we have found that SHP can interact *in vitro* with RNA polymerase II. This interaction could be an additional way how SHP could inhibit both basal and induced transactivation. Further experiments are actually ongoing to explore how this interaction could mediate repression by SHP.

In conclusion, we report the interaction of LXR with SHP and mapped the interaction domain to the AF-2 domain of LXR. SHP can inhibit the expression of an artificial reporter gene, whose expression is under the control of LXR, as well as the human *ABCA1* promoter, a natural LXR target gene. Furthermore, the induction of SHP by CDCA in differentiated Caco-2 cells leads to a dramatic down-regulation of the expression of the LXR target gene *ABCG1*. In combination, our data suggest that the interaction between SHP and LXR is relevant *in vivo* and that this interaction leads to the repression of LXR transcriptional activity on specific LXR target genes.

MATERIALS AND METHODS

Materials

22*(R)*-hydroxycholesterol was purchased at Sigma (St. Louis, MO). LG100268 was a kind gift of R. Heyman at X-ceptor Therapeutics (San Diego, CA). T0901317 was a kind gift of O. Morand at Hoffman-LaRoche Inc. (Basel, Switzerland). Purified TFIIE, TFIID, and RNA polymerase II were kind gifts of P. Chambon (Institut de Génétique et de Biologie Moléculaire et Cellulaire, Illkirch, France). Anti-flag antibodies (anti-FLAG M2) were purchased at Sigma. The polyclonal rabbit anti-SHP antibody has been produced as previously described (41). Protease inhibitor cocktail was purchased at ICN Biochemicals (Orsay, France).

Plasmids

pCMX-SHP was obtained by insertion of a PCR product corresponding to the mouse SHP cDNA into the pCMX vector using *Eco*RI and *Xma*I restriction sites. pCMX-LRH-1 was produced by insertion of a PCR product, corresponding to the human LRH-1 cDNA, into the pCMX vector. For protein expression, cDNA fragments encoding the deletion mutant proteins were inserted in the pGex-4T-1 bacterial expression vector (Amersham Pharmacia Biotech, Orsay, France). The various full-length cDNA or deletion mutants of SHP, LXRα, and LRH-1 cloned into pGex-4T-1 were obtained by restriction digestion or PCR amplification. The SHP mutants obtained by PCR were cloned using *Eco*RI and *Xma*I sites. SHP "1+2" mutant was obtained by digestion of pGex-4T-1-SHP by *Eco47III* and *Sma*I. The LXRα mutants obtained by PCR were cloned into pGex-4T-1 using *Bam*HI and *Xma*I sites. The LRH-1 mutants obtained by PCR were cloned into pGex-4T-1 using *Eco*RI and *Xma*I sites. LRH-1 "AB" mutant was obtained by digestion of pGex-4T-1-LRH-1 with *Bgl*II and *Xma*I.

For the mammalian two-hybrid assays, a full-length mouse SHP cDNA PCR product was inserted downstream the pCMX-VP16 activation domain with *Bam*HI and *Nhe*I to generate the pCMX-VP16-SHP expression vector. The LBDs of human LXRα and human LRH-1 (or their deletion mutants) were cloned in pCMX-BDGal4 vector, downstream of the Gal4 DNA binding domain, using *Kpn*I and *Xma*I for LXRα, and *Eco*RI and *Xma*I for LRH-1.

The pGL3-$(LXRE)_5$TK-Luc reporter construct contains five tandem repeats of the DR-4 LXR response element (5′-gcggt-tcccag**GGTTTA**AATA**AGTTCA**tctagat) cloned upstream of the herpes simplex virus thymidine kinase (TK) promoter and the luciferase (Luc) reporter gene. pGL3-hABCA1-Luc was obtained by amplifying the −400/+181 part of the human *ABCA1* promoter by PCR using human genomic DNA as a template. The luciferase reporter construct pGL3-$(UAS)_5$TK-Luc comprises five tandem repeats of the Gal4 UAS cloned in front of the TK promoter. pCMV-βGal was used as an internal control for transfection efficiency. All constructs were verified by DNA sequencing. pCDM8-mSHP, pCMX-hLXRα, pBluescript-mLXRβ, pCMX-LXRα-flag, pSG5-mRXRα, pGex-2T-mRXRα, pSG5-hTIF2, and the human LRH-1 PCR product were gifts. pSG5-SHP, pSG5-SHP mut1+2, pSG5-SHP1+2 (also called pSG5-SHP159) and pGL3-hSHP (569)-Luc were described elsewhere (24, 41).

Cell Culture, Transient Transfection Assays, and Retroviral Infection

CV-1, RK-13, BNL-CL.2, and Caco-2 cells (ATCC, Manassas, VA) were maintained at 37 C, 5% CO_2 and respectively grown in DMEM supplemented with 5 or 10% fetal calf serum (FCS), MEM supplemented with 10% FCS, or MEM supplemented with 20% FCS, L-glutamine, and antibiotics (penicillin-streptomycin-seromed A2213). Caco-2 cells were differentiated as described (51). Unless specified, cells were transfected by the calcium phosphate-DNA coprecipitation technique as described (52). In general, cells were transfected in six-well plates. Empty expression vectors were used to maintain equivalent amounts of expression vector for each transfection condition. Luciferase data were normalized to an internal β-galactosidase control and represent the mean (±SD) of triplicate assays. When indicated, lipoprotein-deficient and charcoal-stripped FCS (LPDS-DCC) was used as described (53). For retroviral infection, 293 cells stably expressing Moloney *gag* and *pol* (293 gp) were maintained in DMEM supplemented with 10% fetal bovine serum in an atmosphere of 5% CO_2 at 37 C. Virus production for infection was performed by cotransfection with lipofectamine (Life Technologies, Inc., Gaithersburg, MD) of the 293 gp packaging cell line with 15 μg of pLPCX retroviral vector containing the cDNA of mouse LRH-1, or the empty vector, and 5 μg of the ecotropic vector SV-E-MLV-env containing the Moloney virus envelope cDNA downstream of the Simian virus 40 promoter enhancer. After 48 h, the medium was removed and filtered, polybrene (Sigma) was then added (8 μg/ml). Infection of BNL-CL.2 cells was performed by adding 3 ml of supernatant containing the viruses to approximately 10^5 cells for 8 h. The cells were then allowed to expand for 48 h and the infected cells were selected using 2.5 μg/ml of puromycin (Sigma).

Protein Production and GST Pull-Down Assays

GST fusion proteins were expressed in *Escherichia coli* and purified on a glutathione affinity matrix (Amersham Pharmacia Biotech). Purified proteins (TFIIE, TFIID, and RNA polymerase II) or *in vitro* ^{35}S-radiolabeled translated proteins (TNT T7 Quick Rabbit Reticulocyte, Promega Corp., Madison, WI) were incubated 1 h at 25 C in pull-down buffer (PBS 1×, glycerol 10%, Triton 0.1%, protease inhibitor cocktail) with GST or the GST fusion proteins linked to glutathione-Q sepharose beads in the presence of vehicle or T0901317 (10^{-4} M final concentration) or LG100268 (10^{-6} M final concentration). Beads were then washed five times in pull-down buffer and boiled in SDS-PAGE sample buffer. Samples were separated on a 7.5–15% sodium dodecyl sulfate (SDS) polyacrylamide gel and protein interaction was visualized by Western blot or autoradiography.

Coimmunoprecipitation and Western Blot Analysis

RK-13 cells were grown in 100-mm dishes and were transfected with 10 μg of either the pCMX or the pCMX LXRα-flag expression vectors, using lipofectamine (Life Technologies, Inc.). After 4 h, medium was changed and cells were treated for another 20 h with 22*(R)*-hydroxycholesterol (10^{-5} M). Cells from were lysed in 400 μl of IP buffer (NaCl 150 mM; Nonidet P-40 1%; Tris, pH 8; protease inhibitor cocktail) for 30 min at 4 C. Supernatant was incubated 1 h at 25 C with rabbit preimmune serum and 2.5 mg of protein A sepharose (Amersham Pharmacia Biotech) to preclear the samples. Half of the supernatant was first incubated with the anti-SHP antibody or an irrelevant antibody, and consecutively incubated with 2.5 mg of protein A sepharose overnight at 4 C. Beads were washed three times in lysis buffer and three times in wash buffer (KCl 250 mM, dithiothreitol 1 mM, PBS 1×). Beads were then boiled in SDS-PAGE sample buffer. Proteins were separated on a 7.5% (12% for the SHP input) SDS acrylamide gel and electroblotted to a nitrocellulose membrane. Membranes were incubated in PBS Tween (0.05%) containing 5% nonfat dry milk 1 h at 25 C and subsequently with the anti-flag primary antibody (1/500 dilution) or the anti-SHP antibody (1/2000 dilution) in PBS Tween (0.05%) containing 5% nonfat dry milk overnight at 4 C. Membranes were washed in PBS Tween and incubated with a secondary goat antimouse or antirabbit antibody coupled to horseradish peroxidase (1/5000 dilution) for 1 h at 25 C. Membranes were

washed in PBS Tween and proteins were visualized with the enhanced chemiluminescence (ECL) kit (Amersham Pharmacia Biotech).

Cell Treatment, Northern Blot Analysis, and RT-PCR

Caco-2 cells were first treated with 10^{-5} M T0901317 or DMSO during 4 h and then with 250 μM chenodeoxycholic acid (Sigma) or ethanol during 24 h in normal medium supplemented with 20% LPDS-DCC. RNA extraction and Northern blot analysis of RNA were performed as described (52). A human acidic ribosomal phosphoprotein 36B4 cDNA clone was used as control (54). All probes were labeled by random priming (Roche Molecular Biochemicals, Mannheim, Germany). BNL-CL.2 cells were treated with 10^{-5} M T0901317 or DMSO during 24 h in normal medium supplemented with 10% LPDS-DCC. RNA extraction was performed as described (52). RT-PCR was performed using random hexanucleotides and Superscript reverse transcriptase (Invitrogen, Cergy Pontoise, France).

Acknowledgments

We acknowledge the gift of materials by P. Chambon, J.-M. Egly, R. Heyman, D. Lala, D. M. Lazar, D. Moore, and O. Morand. We also thank Céline Haby and Karen Van Herck for excellent technical support and L. Fajas and L. Gelman for fruitful discussions.

Received August 17, 2001. Accepted May 30, 2002.

Address all correspondence and requests for reprints to: Johan Auwerx, Institut de Génétique et de Biologie Moléculaire et Cellulaire, 1 rue Laurent Fries, 67404 Illkirch, France. E-mail: auwerx@igbmc.u-strasbg.fr.

This work was supported by grants from the Centre National de la Recherche Scientifique (CNRS)/Institut National de la Santé et de la Recherche Médicale (INSERM), Hôpitaux Universitaires de Strasbourg, the Juvenile Diabetes Foundation, Association pour la Recherche contre le Cancer, the National Institute of Health, the Human Frontier Science Program and the European Community Quality of Life Program. J.A. is a research director with CNRS, and K.S. is a research associate with INSERM. C.B. was supported by grants from the Société de Nutrition et de Diététique de Langue Française and Nestlé.

REFERENCES

1. Glass CK, Rosenfeld MG 2000 The coregulator exchange in transcriptional functions of nuclear receptors. Genes Dev 14:121–141
2. Willy PJ, Umesono K, Ong ES, Evans RM, Heyman RA, Mangelsdorf D J 1995 LXR, a nuclear receptor that defines a distinct retinoid response pathway. Genes Dev 9:1033–1045
3. Janowski BA, Willy PJ, Devi TR, Falck JR, Mangelsdorf DJ 1996 An oxysterol signalling pathway mediated by the nuclear receptor LXRα. Nature 383:728–731
4. Lehmann JM, Kliewer SA, Moore LB, Smith-Oliver TA, Oliver BB, Su JL, Sundseth SS, Winegar DA, Blanchard DE, Spencer TA, Willson TM 1997 Activation of the nuclear receptor LXR by oxysterols defines a new hormone response pathway. J Biol Chem 272:3137–3140
5. Janowski BA, Grogan MJ, Jones SA, Wisely GB, Kliewer SA, Corey EJ, Mangelsdorf DJ 1999 Structural requirements of ligands for the oxysterol liver X receptors LXRα and LXRβ. Proc Natl Acad Sci USA 96:266–271
6. Schultz JR, Tu H, Luk A, Repa JJ, Medina JC, Li L, Schwendner S, Wang S, Thoolen M, Mangelsdorf DJ, Lustig KD, Shan B 2000 Role of LXRs in control of lipogenesis. Genes Dev 14:2831–2838
7. Apfel R, Benbrook D, Lernhardt E, Ortiz MA, Salbert G, Pfahl M 1994 A novel orphan receptor specific for a subset of thyroid hormone-responsive elements and its interaction with the retinoid/thyroid hormone receptor subfamily. Mol Cell Biol 14:7025–7035
8. Shinar DM, Endo N, Rutledge SJ, Vogel R, Rodan GA, Schmidt A 1994 NER, a new member of the gene family encoding the human steroid hormone nuclear receptor. Gene 147:273–276
9. Song C, Kokontis JM, Hiipakka RA, Liao S 1994 Ubiquitous receptor: a receptor that modulates gene activation by retinoic acid and thyroid hormone receptors. Proc Natl Acad Sci USA 91:10809–10813
10. Seol W, Choi HS, Moore DD 1995 Isolation of proteins that interact specifically with retinoid X receptor: two novel orphan receptors. Mol Endocrinol 9:72–85
11. Teboul M, Enmark E, Li Q, Wikström AC, Pelto-Huikko M, Gustafsson J-A 1995 OR-1, a member of the nuclear receptor superfamily that interacts with the 9-cis-retinoic acid receptor. Proc Natl Acad Sci USA 92:2096–2100
12. Peet DJ, Turley SD, Ma W, Janowski BA, Lobaccaro JM, Hammer RE, Mangelsdorf DJ 1998 Cholesterol and bile acid metabolism are impaired in mice lacking the nuclear oxysterol receptor LXR α. Cell 93:693–704
13. Repa JJ, Turley SD, Lobaccaro JA, Medina J, Li L, Lustig K, Shan B, Heyman RA, Dietschy JM, Mangelsdorf DJ 2000 Regulation of absorption and ABC1-mediated efflux of cholesterol by RXR heterodimers. Science 289: 1524–1529
14. Costet P, Luo Y, Wang N, Tall AR 2000 Sterol-dependent transactivation of the human ABC1 promoter by liver X receptor/retinoid X receptor. J Biol Chem 275: 28240–28240
15. Schwartz K, Lawn R, Wade DP 2000 ABC1 gene expression and apoA-I-mediated cholesterol efflux are regulated by LXR. Biochem Biophys Res Commun 274:794–802
16. Venkateswaran A, Repa JJ, Lobaccaro J-MA, Bronson A, Mangelsdorf DJ 2000 Human White/murine ABC8 mRNA levels are highly induced in lipid-loaded macrophages. J Biol Chem 275:14700–14707
17. Chawla A, Boisvert WA, Lee C, Laffitte BA, Barak Y, Joseph SB, Liao D, Nagy L, Edwards PA, Curtiss LK, Evans RM, Tontonoz P 2001 A PPARγ-LXR-ABCA1 pathway in macrophages is involved in cholesterol efflux and atherogenesis. Mol Cell 7:161–171
18. Claudel T, Leibowitz MD, Fievet C, Tailleux A, Wagner B, Repa JJ, Torpier G, Lobaccaro JM, Paterniti JR, Mangelsdorf DJ, Heyman RA, Auwerx J 2001 Reduction of atherosclerosis in apolipoprotein E knockout mice by activation of the retinoid X receptor. Proc Natl Acad Sci USA 98:2610–2615
19. Repa JJ, Berge KE, Pomajzl C, Richardson JA, Hobbs HH, Mangelsdorf DJ 2002 Regulation of ATP-binding cassette sterol transporters, ABCG5 and ABCG8, by the oxysterol receptors, LXRα and β. J Biol Chem 277: 18793–18800
20. Luo Y, Tall AR 2000 Sterol upregulation of human CETP expression *in vitro* and in transgenic mice by an LXR element. J Clin Invest 105:513–520
21. Laffitte BA, Repa JJ, Joseph SB, Wilpitz DC, Kast HR, Mangelsdorf DJ, Tontonoz P 2001 LXRs control lipid-inducible expression of the apolipoprotein E gene in macrophages and adipocytes. Proc Natl Acad Sci USA 98:507–512
22. Repa JJ, Liang G, Ou J, Bashmakov Y, Lobaccaro JM, Shimomura I, Shan B, Brown MS, Goldstein JL, Mangelsdorf DJ 2000 Regulation of mouse sterol regulatory element-binding protein-1c gene (SREBP-1c) by oxysterol receptors, LXRα and LXRβ. Genes Dev 14:2819–2830

23. Yoshikawa T, Shimano H, Amemiya-Kudo M, Yahagi N, Hasty AH, Matsuzaka T, Okazaki H, Tamura Y, Iizuka Y, Ohashi K, Osuga J, Harada K, Gotoda T, Kimura S, Ishibashi S, Yamada N 2001 Identification of liver X receptor-retinoid X receptor as an activator of the sterol regulatory element-binding protein 1c gene promoter. Mol Cell Biol 21:2991–3000
24. Lu TT, Makishima M, Repa JJ, Schoonjans K, Kerr TA, Auwerx J, Mangelsdorf DJ 2000 Molecular basis for feedback regulation of bile acid synthesis by nuclear receptors. Mol Cell 6:507–515
25. Luo Y, Liang CP, Tall AR 2001 The orphan nuclear receptor LRH-1 potentiates the sterol-mediated induction of the human CETP gene by LXR. J Biol Chem 276: 24767–24773
26. Seol W, Choi HS, Moore DD 1996 An orphan nuclear hormone receptor that lacks a DNA binding domain and heterodimerizes with other receptors. Science 272: 1336–1339
27. Seol W, Hanstein B, Brown M, Moore DD 1998 Inhibition of estrogen receptor action by the orphan receptor SHP (short heterodimer partner). Mol Endocrinol 12:1551–1557
28. Johansson L, Thomsen JS, Damdimopoulos AE, Spyrou G, Gustafsson JA, Treuter E 1999 The orphan nuclear receptor SHP inhibits agonist-dependent transcriptional activity of estrogen receptors ERα and ERβ. J Biol Chem 274:345–353
29. Lee YK, Dell H, Dowhan DH, Hadzopoulou-Cladaras M, Moore DD 2000 The orphan nuclear receptor SHP inhibits hepatocyte nuclear factor 4 and retinoid X receptor transactivation: two mechanisms for repression. Mol Cell Biol 20:187–195
30. Masuda N, Yasumo H, Tamura T, Hashiguchi N, Furusawa T, Tsukamoto T, Sadano H, Osumi T 1997 An orphan nuclear receptor lacking a zinc-finger DNA-binding domain: interaction with several nuclear receptors. Biochim Biophys Acta 1350:27–32
31. Goodwin B, Jones SA, Price RR, Watson MA, McKee DD, Moore LB, Galardi C, Wilson JG, Lewis MC, Roth ME, Maloney PR, Willson TM, Kliewer SA 2000 A regulatory cascade of the nuclear receptors FXR, SHP-1, and LRH-1 represses bile acid biosynthesis. Mol Cell 6:517–526
32. Ito M, Yu R, Jameson JL 1997 DAX-1 inhibits SF-1-mediated transactivation via a carboxy-terminal domain that is deleted in adrenal hypoplasia congenita. Mol Cell Biol 17:1476–1483
33. Nachtigal MW, Hirokawa Y, Enyeart-VanHouten DL, Flanagan JN, Hammer GD, Ingraham HA 1998 Wilms' tumor and Dax-1 modulate the orphan nuclear receptor SF-1 in sex-specific gene expression. Cell 93:445–454
34. Makishima M, Okamoto AY, Repa JJ, Tu H, Learned RM, Luk A, Hull MV, Lustig KD, Mangelsdorf DJ, Shan B 1999 Identification of a nuclear receptor for bile acids. Science 284:1362–1365
35. Sinal CJ, Tohkin M, Miyata M, Ward JM, Lambert G, Gonzalez FJ 2000 Targeted disruption of the nuclear receptor FXR/BAR impairs bile acid and lipid homeostasis. Cell 102:731–744
36. Denson LA, Sturm E, Echevarria W, Zimmerman TL, Makishima M, Mangelsdorf DJ, Karpen SJ 2001 The orphan nuclear receptor, shp, mediates bile acid-induced inhibition of the rat bile acid transporter, ntcp. Gastroenterology 121:140–147
37. Lee YK, Moore DD 2002 Dual mechanisms for repression of the monomeric orphan receptor liver receptor homologous protein-1 by the orphan small heterodimer partner. J Biol Chem 277:2463–2467
38. Seol W, Chung M, Moore DD 1997 Novel receptor interaction and repression domains in the orphan receptor SHP. Mol Cell Biol 17:7126–7131
39. Renaud JP, Harris JM, Downes M, Burke LJ, Muscat GE 2000 Structure-function analysis of the Rev-erbA and RVR ligand-binding domains reveals a large hydrophobic surface that mediates corepressor binding and a ligand cavity occupied by side chains. Mol Endocrinol 14:700–717
40. Lee HK, Lee YK, Park SH, Kim YS, Park SH, Lee JW, Kwon HB, Soh J, Moore DD, Choi HS 1998 Structure and expression of the orphan nuclear receptor SHP gene. J Biol Chem 273:14398–14402
41. Johansson L, Bavner A, Thomsen JS, Farnegardh M, Gustafsson JA, Treuter E 2000 The orphan nuclear receptor SHP utilizes conserved LXXLL-related motifs for interactions with ligand-activated estrogen receptors. Mol Cell Biol 20:1124–1133
42. Ananthanarayanan M, Balasubramanian N, Makishima M, Mangelsdorf DJ, Suchy FJ 2001 Human bile salt export pump promoter is transactivated by the farnesoid X receptor/bile acid receptor. J Biol Chem 276:28857–28865
43. Schuetz EG, Strom S, Yasuda K, Lecureur V, Assem M, Brimer C, Lamba J, Kim RB, Ramachandran V, Komoroski BJ, Venkataramanan R, Cai H, Sinal CJ, Gonzalez FJ, Schuetz JD 2001 Disrupted bile acid homeostasis reveals an unexpected interaction among nuclear hormone receptors, transporters, and cytochrome P450. J Biol Chem 276: 39411–39418
44. Kast HR, Goodwin B, Tarr PT, Jones SA, Anisfeld AM, Stoltz CM, Tontonoz P, Kliewer S, Willson TM, Edwards PA 2002 Regulation of multidrug resistance-associated protein 2 (ABCC2) by the nuclear receptors pregnane X receptor, farnesoid X-activated receptor, and constitutive androstane receptor. J Biol Chem 277:2908–2915
45. Bavner A, Johansson L, Toresson G, Gustafsson JA, Treuter E 2002 A transcriptional inhibitor targeted by the atypical orphan nuclear receptor SHP. EMBO Rep 3:478–484
46. Crawford P A, Dorn C, Sadovsky Y, Milbrandt J 1998 Nuclear receptor DAX-1 recruits nuclear receptor corepressor N-CoR to steroidogenic factor 1. Mol Cell Biol 18:2949–2956
47. Altincicek B, Tenbaum SP, Dressel U, Thormeyer D, Renkawitz R, Baniahmad A 2000 Interaction of the corepressor Alien with DAX-1 is abrogated by mutations of DAX-1 involved in adrenal hypoplasia congenita. J Biol Chem 275:7662–7667
48. Zazopoulos E, Lalli E, Stocco DM, Sassone-Corsi P 1997 DNA binding and transcriptional repression by DAX-1 blocks steroidogenesis. Nature 390:311–315
49. Lalli E, Ohe K, Hindelang C, Sassone-Corsi P 2000 Orphan receptor DAX-1 is a shuttling RNA binding protein associated with polyribosomes via mRNA. Mol Cell Biol 20:4910–4921
50. Holter E, Kotaja N, Makela S, Strauss L, Kietz S, Janne OA, Gustafsson JA, Palvimo JJ, Treuter E 2002 Inhibition of androgen receptor (AR) function by the reproductive orphan nuclear receptor DAX-1. Mol Endocrinol 16:515–528
51. Lefebvre AM, Paulweber B, Fajas L, Woods J, McCrary C, Colombel JF, Najib J, Fruchart JC, Datz C, Vidal H, Desreumaux P, Auwerx J 1999 Peroxisome proliferator-activated receptor γ is induced during differentiation of colon epithelium cells. J Endocrinol 162:331–340
52. Schoonjans K, Peinado-Onsurbe J, Lefebvre AM, Heyman RA, Briggs M, Deeb S, Staels B, Auwerx J 1996 PPARα and PPARγ activators direct a tissue-specific transcriptional response via a PPRE in the lipoprotein lipase gene. EMBO J 15:5336–5348
53. Schoonjans K, Gelman L, Haby C, Briggs M, Auwerx J 2000 Induction of LPL gene expression by sterols is mediated by a sterol regulatory element and is independent of the presence of multiple E boxes. J Mol Biol 304:323–334
54. Masiakowski P, Breathnach R, Bloch J, Gannon F, Krust A, Chambon P 1982 Cloning of cDNA sequences of hormone-regulated genes from MCF-7 human breast cancer cell line. Nucleic Acids Res 10:7895–7903