Involvement of corepressor complex subunit GPS2 in transcriptional pathways governing human bile acid biosynthesis

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Edited by David W. Russell, University of Texas Southwestern Medical Center, Dallas, TX, and approved August 16, 2007 (received for review July 18, 2007)

Coordinated regulation of bile acid biosynthesis, the predominant pathway for hepatic cholesterol catabolism, is mediated by few key nuclear receptors including the orphan receptors liver receptor homolog 1 (LRH-1), hepatocyte nuclear factor 4α (HNF4α), small heterodimer partner (SHP), and the bile acid receptor FXR (farnesoid X receptor). Activation of FXR initiates a feedback regulatory loop via induction of SHP, which suppresses LRH-1- and HNF4α-dependent expression of cholesterol 7α hydroxylase (CYP7A1) and sterol 12α hydroxylase (CYP8B1), the two major pathway enzymes. Here we dissect the transcriptional network governing bile acid biosynthesis in human liver by identifying GPS2, a stoichiometric subunit of a conserved corepressor complex, as a differential coregulator of CYP7A1 and CYP8B1 expression. Direct interactions of GPS2 with SHP, LRH-1, HNF4α, and FXR indicate alternative coregulator recruitment strategies to cause differential transcriptional outcomes. In addition, species-specific differences in the regulation of bile acid biosynthesis were uncovered by identifying human CYP8B1 as a direct FXR target gene, which has implications for therapeutic approaches in bile acid-related human disorders.

cholesterol 7α hydroxylase | sterol 12α hydroxylase | farnesoid X receptor | small heterodimer partner

Bile acids (BAs) are cholesterol derivatives essential for absorption of dietary lipids and fat-soluble vitamins and maintenance of cholesterol BA homeostasis (1, 2). In humans the major BA biosynthetic pathway is initiated by cholesterol 7α hydroxylase (CYP7A1) to produce two primary BAs, cholic acid and chenodeoxycholic acid (CDCA). Sterol 12α hydroxylase (CYP8B1) catalyzes the synthesis of cholic acid and determines the ratio of cholic acid to CDCA in the bile (1). In addition to emulsification of dietary lipids, cholic acid and CDCA are ligands for farnesoid X receptor (FXR/NR1H4) (3–5). Ligand-bound FXR regulates a number of target genes involving BA transport and metabolism (6). BAs also feedback-regulate BA biosynthesis, where activated FXR induces small heterodimer partner (SHP/NR0B2) gene expression, and SHP in turn inhibits liver receptor homolog 1 (LRH-1/NR5A2) or hepatocyte nuclear factor 4α (HNF4α/NR2A1) activities on the BA response elements (BAREs) of CYP7A1 and CYP8B1 promoters (7–10). BAs can also act via FXR-independent pathways that use PKC (11) and JNK signaling (12, 13) to suppress HNF4α-mediated expression of human CYP8B1 (hCYP8B1) (10, 11, 14). Although the physiological role of SHP in BA biosynthesis is well documented, mechanistic details of repression by SHP remain unclear. Recent studies indicate that SHP may repress its targets (*i*) via direct binding and blocking the coactivator interaction interface of its target nuclear receptors (NRs), (*ii*) by antagonizing CREB binding protein (CBP)/p300-dependent coactivator functions on NRs via recruitment of a coinhibitor protein like EID1, and (*iii*) by recruiting corepressor complexes that include histone deacetylases (HDAC) 1, 3, and 6, Sin3A, and mammalian histone methyltransferase (G9a) (ref. 15 and references therein). Here we identify GPS2 (G protein pathway suppressor 2), a subunit of the NR corepressor (N-CoR) complex (16–18), as a SHP cofactor that, by means of additional interactions with LRH-1, HNF4α, and FXR, participates in the differential regulation of CYP7A1 and CYP8B1 expression in human liver cell lines and primary human hepatocytes. Additionally, we provide insights into the species- and gene-specific regulations of these key enzymes responsible for hepatic BA biosynthesis.

Results and Discussion

Characterization of Functional Interactions of GPS2 with SHP. To identify proteins that cooperate with SHP in corepressing transcription, yeast two-hybrid screens were performed and independent clones encoding GPS2 were isolated. Yeast-based interactions were confirmed by *in vitro* (Fig. 1*A*), *in vivo*, pull-down, and mammalian two-hybrid assays [supporting information (SI) Fig. 7 *A* and *B*]. The minimal SHP-interacting surface of GPS2 was mapped to amino acids 206–327 (Fig. 1*B* and SI Fig. 7*C*). Interestingly, GPS2 deletions displayed contrasting transcriptional activities. Whereas a major repression function was mapped to amino acids 1–105, an activation function was mapped to amino acids 194–281 (Fig. 1*B* and SI Fig. 7 *D* and *E*), consistent with previously suggested connections to N-CoR and p300 (16–19).

Next, the putative role of endogenous GPS2 in transcriptional repression by SHP was investigated in a unique repressor assay (20) combined with RNA interference (Fig. 1*C*). VP16HNF-1-mediated reporter activity was repressed in trans by SHP, and this repression was compromised by siRNA directed against GPS2 (siGPS2) [respective levels of SHP and GPS2 in presence of control siRNA or siGPS2 are shown in SI Fig. 8]. Next, functional interactions of GPS2 and SHP with known corepressor complex components were investigated. Because SHP interacts with HDAC1 and HDAC3 (21, 22), whereas GPS2 via

Author contributions: S.S., C.E., I.T., J.-Å.G., and E.T. designed research; S.S., A.B., A.H., L.-M.N., T.L., I.T., and E.T. performed research; S.R., M.R.W., and I.T. contributed new reagents/analytic tools; S.S., A.B., A.H., L.-M.N., T.L., S.R., M.R.W., C.E., I.T., J.-Å.G., and E.T. analyzed data; and S.S. and E.T. wrote the paper.

Conflict of interest statement: J.-Å.G. is a shareholder, research grant recipient, and consultant of Karo Bio AB.

This article is a PNAS Direct Submission.

Abbreviations: CYP7A1, cholesterol 7α hydroxylase; CYP8B1, sterol 12α hydroxylase; NR, nuclear receptor; BA, bile acid; BARE, BA response element; CDCA, chenodeoxycholic acid; FXR, farnesoid X receptor; FXRE, FXR response element; LRH-1, liver receptor homolog 1; HNF4α, hepatocyte nuclear factor 4α; SHP, small heterodimer partner; HDAC, histone deacetylase; N-CoR, NR corepressor; Pol-II, polymerase II; RTQ-PCR, quantitative real-time PCR; CBP, CREB binding protein.

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This article contains supporting information online at www.pnas.org/cgi/content/full/0706736104/DC1.

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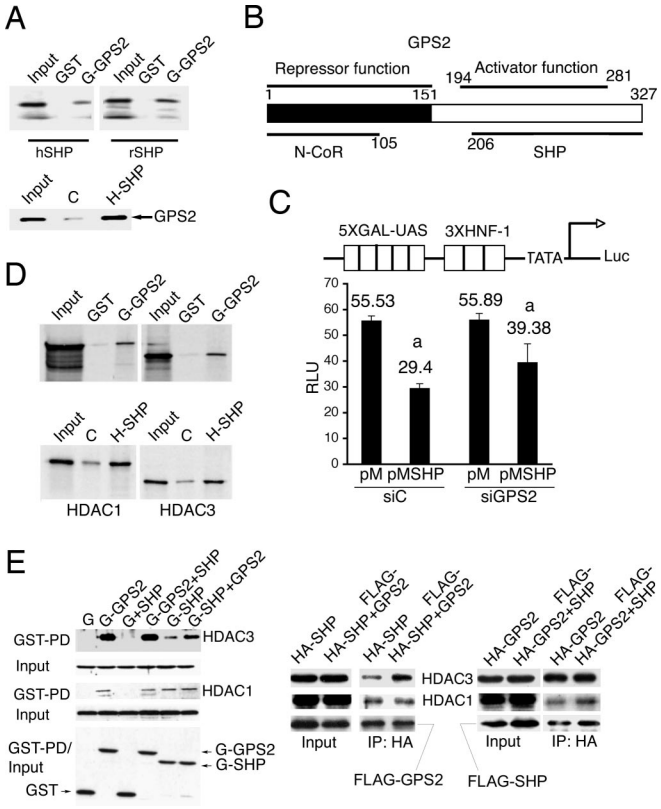

Fig. 1. GPS2 interacts with and augments SHP repression. (*A*) SHP and GPS2 interact *in vitro*. Immobilized GST (*Upper*) or 6XHis (*Lower*) fusion proteins were incubated with ^{35}S-labeled full-length human SHP, rat SHP, or human GPS2. Complexes were resolved by PAGE and autoradiographed. (*B*) A schematic representation of GPS2 and its interaction surfaces for N-CoR and SHP. Activation and repression functions as determined from SI Fig. 7 are indicated. (*C*) GPS2 knockdown relieves SHP repression. Huh7 cells were transfected with indicated siRNAs in quadruplets. After 24 h the indicated luciferase reporter was cotransfected with VP16HNF-1 and pM or pMhSHP. Two wells per experiment were used for reporter assays (*Left*), and the rest were used for RTQ-PCR (SI Fig. 8). a, statistical significance, $P < 0.05$. Data represent means ± SD from three independent experiments. (*D*) GPS2 and SHP physically interact with HDAC1 and HDAC3. GST (*Upper*) and His (*Lower*) pull-down assays. (*E*) GPS2 augments SHP–HDAC3 interaction *in vivo*. As described in the text, Cos-7 cells (*Left*) or Huh7 cells (*Right*) were transfected with indicated expression plasmids. After pull-down or immunoprecipitation the complexes were analyzed by Western blotting. G-GPS2, GST-GPS2; G-SHP, GST-SHP; H-SHP, His-SHP; C, unrelated His-tagged control protein.

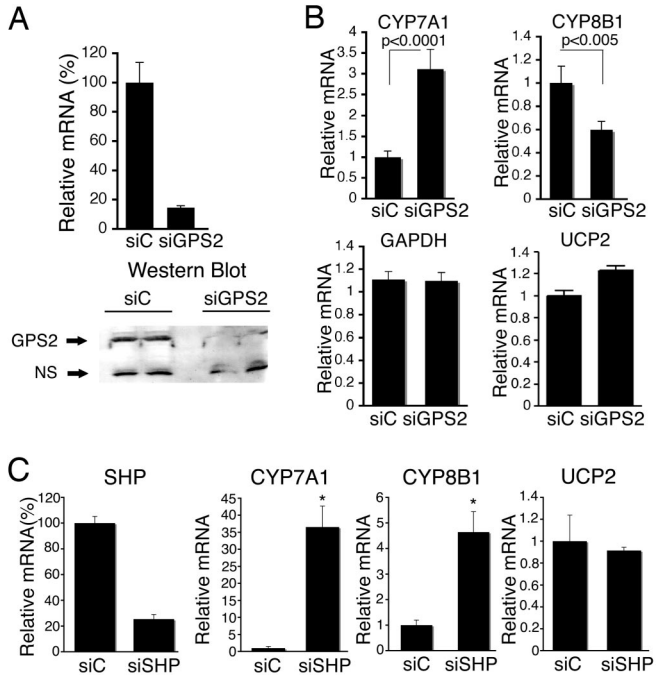

Fig. 2. GPS2 differentially regulates CYP7A1 and CYP8B1 expression. (*A*) siGPS2-mediated knockdown of GPS2 mRNA and protein. (*B*) siGPS2 induces CYP7A1 but represses CYP8B1 mRNA. HepG2 cells were transfected with control siRNA or siGPS2 and analyzed by RTQ-PCR after 24 h. CYP7A1 and CYP8B1 data are means of six independent experiments. GAPDH and UCP2 are means of three independent experiments. (*C*) siSHP induces both CYP7A1 and CYP8B1 expression. HepG2 cells transfected with siSHP were analyzed by RTQ-PCR. Data represent means ± SD from three independent experiments. *, $P < 0.001$.

N-CoR associates with HDAC3 (16), we investigated interactions with these HDACs *in vitro*. As expected, SHP interacted with both HDAC1 and HDAC3 (Fig. 1*D Lower*). Intriguingly, GPS2 also physically interacted with both HDAC1 and HDAC3 (Fig. 1*D Upper*). To confirm such interactions *in vivo* and to determine whether GPS2 could modulate SHP–HDAC1/3 interactions and vice versa, an *in vivo* pull-down assay was performed. Both GPS2 and SHP interacted with endogenous HDAC1 and HDAC3 in Cos-7 cells (Fig. 1*E Left*). Ectopic expression of GPS2 increased the SHP–HDAC3 interaction without altering the SHP–HDAC1 interaction. In contrast, ectopic expression of SHP did not alter GPS2–HDAC1/3 interactions. This GPS2-mediated augmentation of the SHP–HDAC3 interaction was also replicated in human liver Huh7 cells using coimmunoprecipitation (Fig. 1*E Right*).

Together, our results indicate that GPS2 modulates SHP repression via augmenting interaction with HDAC3 and suggest GPS2 as a candidate bridging factor between SHP and the N-CoR complex. Indeed, recent reports indicate that SHP associates with N-CoR *in vivo* despite the absence of a physical interaction (21) and that binding of adamantyl-substituted retinoid-related molecules to SHP results in the recruitment of N-CoR and GPS2-containing corepressor complexes (23).

Differential Regulation of CYP7A1 and CYP8B1 Expression by GPS2 but Not SHP. Because SHP is a key player in hepatic BA biosynthesis via negative regulation of both CYP7A1 and CYP8B1 (24), we investigated the effects of GPS2 knockdown on expression of these genes in human liver-derived HepG2 cells. siGPS2 efficiently depleted GPS2 at both mRNA and protein levels (Fig. 2*A*). In support of a role of GPS2 in transcriptional repression, siGPS2 strongly induced CYP7A1 mRNA, whereas expressions of GAPDH and UCP2 remained unchanged (Fig. 2*B*). However, surprisingly, CYP8B1 levels were decreased upon siGPS2 (Fig. 2*B*). In light of these interesting differences regarding GPS2 regulation of CYP7A1 and CYP8B1, we investigated whether SHP regulation of these genes matches the GPS2 action. However, consistent with previous reports, SHP knockdown increased both hCYP7A1 and hCYP8B1 expression (Fig. 2*C*), indicating that GPS2 regulation of these genes to some extent may be independent of SHP.

Differential Regulation of CYP7A1 and CYP8B1 by GPS2 Is Mediated by Direct Interactions with LRH-1 and HNF4α. Because LRH-1, HNF4α, and FXR are implicated in regulation of BA biosynthesis, we next investigated whether GPS2 may interact with these NRs. Mammalian two-hybrid assays revealed that GPS2 interacts with FXR, LRH-1, and HNF4α but not thyroid hormone receptor or retinoid X receptor (RXR) (Fig. 3*A* and data not shown). To identify the precise roles of these NRs on CYP7A1 and CYP8B1 transcription we next studied the regulation of CYP7A1 and

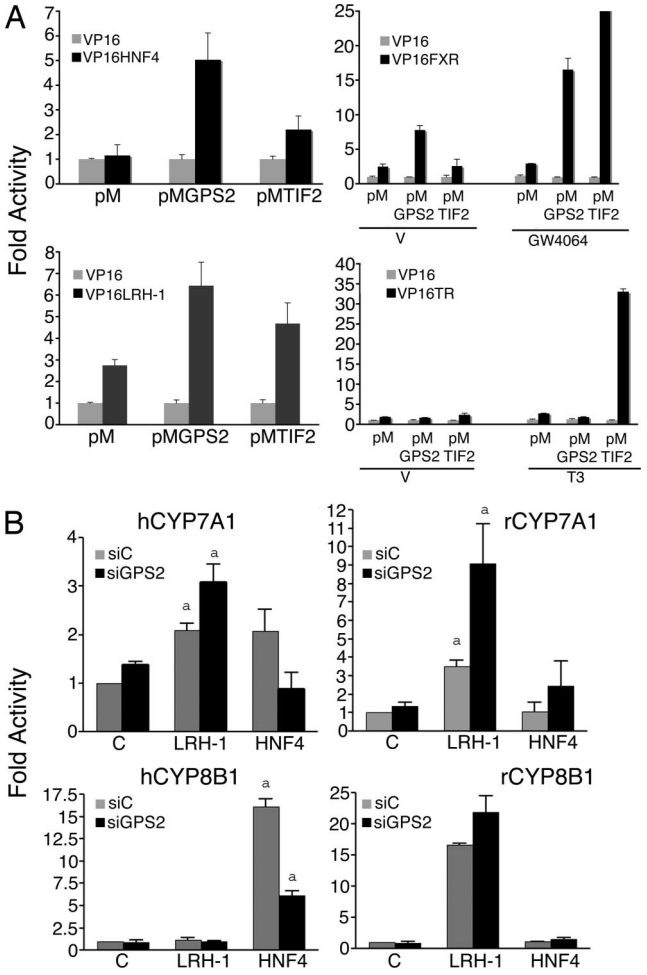

Fig. 3. Gene- and species-specific difference in regulation of CYP7A1 and CYP8B1 by GPS2 is mediated by direct interaction of GPS2 with LRH-1 and HNF4α. (*A*) GPS2 interacts with FXR, LRH-1, and HNF4α. Cos-7 cells were transfected with the indicated pM or VP16 constructs, and fold activities over empty pM and pVP16 transfections were determined and plotted. For FXR and thyroid hormone receptor, cells were treated with 1 μM GW4064, 5 nM T3, or vehicle (V). Data represent means $\pm$ SD from three independent experiments. (*B*) GPS2 differentially affects LRH-1 and HNF4α activity on CYP7A1 and CYP8B1 BAREs. Twenty-four hours after transfection with control siRNA or siGPS2, luciferase reporters were cotransfected in Huh7 cells with the NR expression plasmids or empty vector (C). Normalized luciferase activities were plotted as fold activity over control transfections. Data represent means $\pm$ SD from three independent experiments. a, statistical significance, $P < 0.05$.

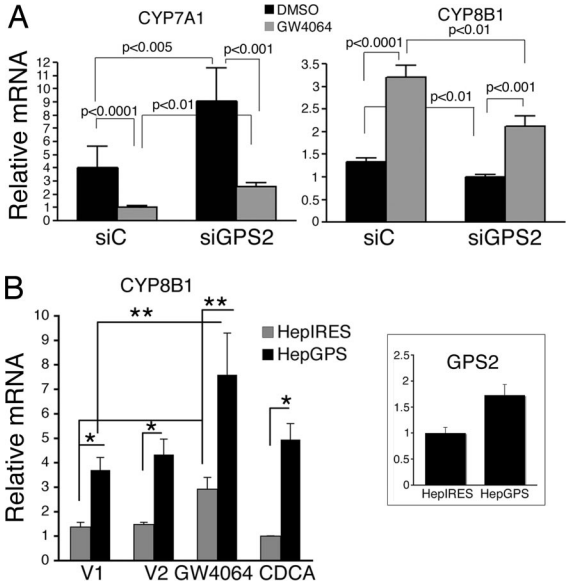

Fig. 4. GPS2 and FXR agonist GW4064 differentially regulate hCYP7A1 and hCYP8B1 expression. (*A*) GPS2 knockdown affects GW4064 regulation of CYP7A1 and CYP8B1 expression. After transfection with the indicated siRNAs, HepG2 cells were treated with DMSO or GW4064 and RTQ-PCRs were performed. (*B*) Stably expressed GPS2 augments FXR activation of CYP8B1. Control (HepIRES) or HepGPS cells were treated with CDCA (100 μM) or GW4064 (1 μM), and RTQ-PCR was performed for the indicated genes. V1, DMSO; V2, ethanol. Data represent means $\pm$ SD from three independent experiments. *, $P < 0.05$; **, $P < 0.01$. (*Inset*) Relative GPS2 expression in HepIRES and HepGPS cells.

CYP8B1 BAREs in detail by using reporter assays in Huh7 cells. We found that, whereas hCYP7A1-luc was activated by both receptors, hCYP8B1-luc was activated by HNF4α only, and both rCYP7A1 and rCYP8B1-luc were responsive to LRH-1 (Fig. 3*B*), pointing at species-dependent and gene-specific differences in the transcriptional regulation of both enzymes. The regulatory interplay with GPS2 was further supported by results after GPS2 depletion. Whereas LRH-1 activation of hCYP7A1, rCYP7A1, and rCYP8B1 was augmented by siGPS2, HNF4α activation of both hCYP7A1 and hCYP8B1 was compromised (Fig. 3*B*). The differences in regulation of hCYP7A1 and hCYP8B1 mRNA expression by GPS2 seen in Fig. 2*B* may thus be explained by the gene-specific differences seen in Fig. 3*B*.

These results indicate that GPS2 inhibits hCYP7A1 mRNA expression by repressing LRH-1 activity on the promoter while it increases hCYP8B1 expression by augmenting HNF4α activity. Such differential regulation is compatible with a dual activator/repressor function of GPS2 observed by us (see above) and in previous studies: In addition to representing a subunit of the N-CoR complex (16–18), GPS2 can potentiate papilloma virus E2 and p53 transactivity by recruiting the histone acetyltransferase p300 (19, 25).

GPS2 Augments FXR Action on CYP7A1 and CYP8B1 Expression. We next investigated whether and to what extent GPS2 would affect the regulation of CYP7A1 and CYP8B1 by SHP via LRH-1 and HNF4α. As demonstrated in Fig. 4*A*, treatment of HepG2 cells with the nonsteroidal FXR agonist GW4064 (26), previously demonstrated to up-regulate SHP expression (27) (SI Fig. 9), suppressed CYP7A1 mRNA. As expected from above-presented interactions of SHP with GPS2, siGPS2 compromised GW4064 suppression of CYP7A1. However, surprisingly, hCYP8B1 mRNA was induced upon GW4064 treatment, and GPS2 depletion compromised this induction, suggesting that FXR somehow activates the hCYP8B1 promoter and GPS2 cooperates with this FXR activation. To further investigate this puzzling observation, we generated a HepG2 cell line stably overexpressing GPS2 (HepGPS) (Fig. 4*B Inset*). In agreement with Fig. 4*A*, HepGPS cells showed an increase in both basal and GW4064-induced CYP8B1 expression. Although the natural BA CDCA modestly repressed CYP8B1 expression in control cells, CYP8B1 mRNA in HepGPS cells was induced (Fig. 4*B*). As expected, both GW4064 and CDCA induced SHP and repressed CYP7A1 mRNA expressions (SI Fig. 9).

Our results are consistent with earlier reports, showing strong repression of CYP7A1 but little or no repression of CYP8B1 by CDCA in human (28, 29), and demonstrate that these regulatory differences can be recapitulated in HepG2 cells.

Identification of a Functional FXR Response Element in the Human CYP8B1 Promoter. To investigate whether FXR could directly activate hCYP8B1 expression and to identify the possible FXR-

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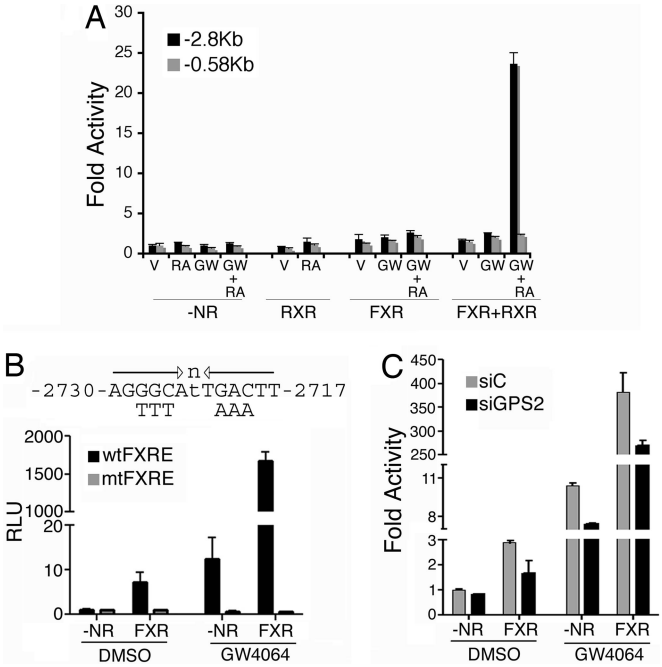

Fig. 5. FXR activates hCYP8B1 promoter via an IR-1 FXRE. (*A*) FXR activates the −2.8-kb hCYP8B1 promoter. Cos-7 cells were cotransfected with the −2.8-kb or the −580-bp hCYP8B1-luc along with indicated expression plasmids. After 24-h treatments with DMSO (V), 1 μM 9-cis RA (RA), 1 μM GW4064, or 1 μM GW+RA, normalized luciferase activities were plotted as fold activity over DMSO-treated samples transfected with empty plasmids. (*B*) An IR-1 element on the distal promoter of hCYP8B1 confers the FXR response. Luciferase reporter derived from predicted IR-1-FXRE sequence was tested for FXR response in Huh7 cells. Sequence and position of the CYP8B1-IR1 on the hCYP8B1 promoter are shown, and mutated nucleotides used for constructing the mutated reporter are indicated. (*C*) GPS2 regulates FXR activity on the CYP8B1-FXRE. Twenty-four hours after siRNA transfection, Huh7 cells were cotransfected with the indicated reporter and expression plasmids followed by ligand treatment. Normalized luciferase activities were plotted as fold activity as above. −NR, empty plasmid. Data represent means ± SD from three independent experiments.

responsive region within the hCYP8B1 promoter, full-length (−2.8 kb) and/or BARE-containing (−580 bp) hCYP8B1 promoter luciferase reporters were tested for FXR response in FXR-negative Cos-7 cells (to avoid endogenous FXR-mediated feedback inhibition). As demonstrated in Fig. 5*A*, ectopic FXR and RXR in the presence of their respective ligands strongly activated the −2.8-kb but not the −580-bp promoter. Therefore, the 2.8-kb hCYP8B1 promoter sequence was scanned with two independent *in silico* NR binding site prediction tools (NUBIscan and NHRscan), and one IR-1 FXR response element (FXRE) common to both the predictions, positioned at −2,730 to −2,717 bp upstream of the transcriptional start site, was identified. This IR-1 element was unique to hCYP8B1 promoter, and corresponding sites were not found in rat or mouse. Next, functionality of this IR-1 element was checked by luciferase reporter assay. Transient transfections in GW4064-responsive Huh7 cells revealed a robust ligand induction of the wild-type but not the mutated 2 copy FXRE-luc in the presence or absence of ectopic FXR, consistent with responsiveness to endogenous FXR activation (Fig. 5*B*). These results clearly demonstrate that FXR activates hCYP8B1 expression via an IR-1 FXRE in the distal promoter region.

Because GPS2 interacted with FXR (Fig. 3*A*), subsequently, the capacity of GPS2 to affect FXR activation of the hCYP8B1-FXRE was tested in Huh7 cells, and the GW4064 response on hCYP8B1-FXRE was significantly compromised by siGPS2 (Fig. 5*C*). Therefore, GPS2 acts as a potentiator of FXR-mediated transcription on hCYP8B1 promoter.

Based on these results we suggest that BA modulation of CYP8B1 expression is tightly regulated in the human liver, where BAs inhibit HNF4α action on the BARE via both SHP-dependent and -independent pathways and positively regulate the distal FXRE. Therefore, these two opposing mechanisms counteract each other and elicit a modest BA response as seen by us and others (28, 29). In contrast, GW4064 triggers the SHP-dependent repression pathway only. Because SHP is believed to exhibit weaker interactions with HNF4α than with LRH-1 (30) and because HNF4α is the sole regulator of the hCYP8B1 BARE (Fig. 3*B*), it is likely that this weak SHP repression is overridden by FXR action on the distal promoter. Indeed, the crucial HepG2 results were replicated in primary human hepatocytes (SI Fig. 10), indicating that the differential regulation of CYP7A1 and CYP8B1 by GPS2 and GW4064 may be relevant for human liver.

Gene-Specific Differential Anchoring Functions of GPS2. Next, to gain further mechanistic insights into the differential role of GPS2 in modulation of CYP7A1 and CYP8B1 transcription, we performed ChIP assays in DMSO- or GW4064-treated control, GPS2-depleted, and SHP-depleted HepG2 cells.

As demonstrated in Fig. 6*A*, in control cells, GPS2 occupied the hCYP7A1 promoter alongside LRH-1, HNF4α, CBP, and RNA polymerase II (Pol-II). As expected, GW4064 treatment resulted in the recruitment of SHP, N-CoR, HDAC1, and HDAC3 and a concomitant reduction of CBP and RNA Pol-II occupancy. Displacement of CBP and RNA Pol-II from the promoter and the recruitment of N-CoR and HDACs were SHP-dependent, because the above changes were not observed in SHP-depleted cells. In GPS2-depleted cells the ChIP signals obtained with CBP and RNA Pol-II antibodies were increased 1.4- and 1.6-fold, respectively, which suggests that the increased CYP7A1 mRNA levels in these cells are due to increased transcription. Importantly, however, although SHP recruitment and displacement of CBP and RNA Pol-II from the promoter in GW4064-treated cells was not affected by GPS2 depletion, N-CoR, HDAC1, and HDAC3 recruitment were greatly compromised. Furthermore, we could detect less GPS2 occupancy in SHP-depleted cells. These results provide further *in vivo* evidence for the role of GPS2 as a bridging factor between SHP and the N-CoR–HDAC complex on CYP7A1 promoter.

Because the novel FXRE is located ≈2.7 kb upstream of the transcription start site of the hCYP8B1 gene, we next analyzed the factor occupancies at both the proximal (Fig. 6*B Upper*) and at the distal (Fig. 6*B Lower*) regulatory regions. In control cells, the proximal region was bound by HNF4α, CBP, RNA Pol-II, and GPS2, whereas only weak ChIP signals for the above factors were evident in the distal enhancer. Upon GW4064 treatment some increase in CBP (1.3-fold) and Pol-II (1.8-fold) occupancy was detected, which is in agreement with the findings of this article that the hCYP8B1 gene is induced by FXR ligands. Several lines of evidence suggest that the mechanism of this induction involves recruitment of FXR to the upstream response element, followed by the formation of a higher-order enhancer–promoter complex by looping out the intervening sequence. First, we observed GW4064-dependent association of FXR with the distal region and also detected sequences corresponding to the proximal promoter in anti-FXR immunoprecipitates. Similarly, we could detect distal region sequences in anti-HNF4α, anti-CBP, and anti-RNA Pol-II immunoprecipitates only in cells treated with GW4064. The simultaneous presence of the two DNA fragments in these immunoprecipitates demonstrates that the upstream and downstream regions come into close proximity, allowing their efficient cross-linking (11, 31). This physical association of the two regions requires GPS2, because HNF4α, CBP, and Pol-II ChIP signals at the upstream

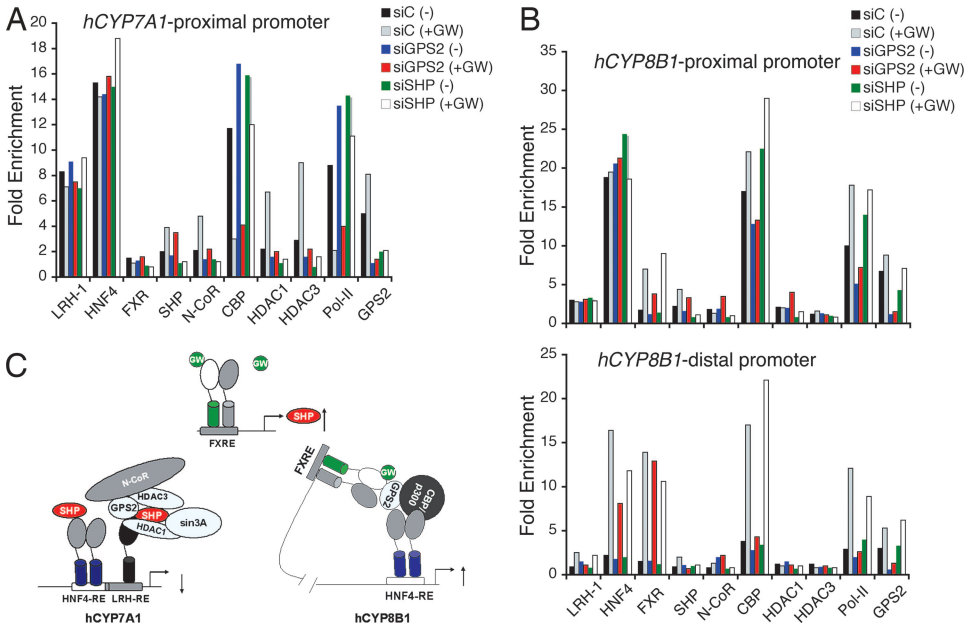

Fig. 6. Promoter-specific recruitment of cofactors by GPS2. HepG2 cells were grown in 15-cm dishes and, 24 h after introduction of indicated siRNAs, were treated with DMSO (−) or GW4064 (+GW). Recruitment of the indicated factors on hCYP7A1-BARE (*A*), hCYP8B1-BARE (*B Upper*), and hCYP8B1 distal promoter containing the FXRE (*B Lower*) were analyzed by ChIP assay. Data show a representative result from two independent experiments exhibiting identical patterns. (*C*) A schematic model depicting the differential regulation of CYP7A1 and CYP8B1 regulatory regions by GPS2.

region or FXR ChIP signal at the downstream region were greatly reduced in GW4064-treated GPS2 knockdown cells. In these latter cells FXR recruitment on the enhancer, HNF4α occupancy of the promoter, and a reduced but substantial promoter occupancy by CBP and Pol-II was still evident, suggesting that, in the absence of GPS2, the CYP8B1 regulatory region adopts a linear configuration.

In control cells GW4064 increased the recruitment of SHP to the promoter, without a parallel association of N-CoR or HDACs (Fig. 6*B*). In contrast to CYP7A1 promoter, at the CYP8B1 regulatory region a somewhat increased N-CoR and HDAC1 ChIP signal was detectable in GW4064-treated GPS2-depleted cells, which disappeared in SHP-depleted cells (Fig. 6*B Upper*). On the other hand, most of the other changes in factor occupancies were only marginally affected by SHP depletion, suggesting that in GPS2 knockdown cells the SHP-recruited repression complex may have a small contribution to the overall modulation of CYP8B1 expression.

Together, these results point to differential gene-specific functions of GPS2. On CYP7A1 promoter it serves as a bridging protein anchoring N-CoR, HDAC1, and HDAC3 to promoter-bound SHP and functions as a repressor. Ligand-dependent transcriptional induction of the CYP8B1 gene involves FXR-mediated long-distance enhancer–promoter communication. GPS2 is required for the stable physical association of the CYP8B1 enhancer and promoter region and therefore, in this context, it functions as an activator (summarized in Fig. 6*C*).

Furthermore, given that LRH-1, FXR, and HNF4α display different affinities for SHP, these results also suggest that the NR-selective activation/repression function of GPS2 can be dictated at least in part by relative affinity of these NRs for SHP.

Conclusions

Our work has identified GPS2 as a regulator of the BA biosynthesis pathway that differentially regulates two major enzymes, CYP7A1 and CYP8B1, via its interaction with SHP, LRH-1, HNF4α, and FXR. Molecular details of these interactions are intriguing for the following reasons: (*i*) GPS2 is likely to recognize an NR surface distinct from the common AF-2 surface (which is absent in SHP). (*ii*) GPS2 provides alternative recruitment strategies for N-CoR–HDAC-containing corepressor complexes, which may be particularly relevant for those NRs (e.g., SHP and LRH-1) that are unlikely to recruit N-CoR directly. (*iii*) Previously reported interactions of GPS2 with p300/CBP may modulate the activity of NRs in a context-dependent manner, as those uncovered in this study regarding differential actions of GPS2 in NR-dependent CYP7A1 and CYP8B1 regulation. (*iv*) Because GPS2 suppresses JNK signaling (32), which also participates in hCYP8B1 regulation (14), it would be of interest to investigate to what extent GPS2 could affect fibroblast growth factor 15/19 regulation of BA biosynthesis, which appears to be SHP- and JNK-dependent (33, 34). Furthermore, our study provides insights into gene- and species-specific differences in the regulation of BA biosynthesis, which are of concern because they add further complexity to previously reported species differences in the oxysterol regulation of BA biosynthesis (35). The identification of human CYP8B1 as a direct FXR target gene has implications for therapeutic approaches of BA-related human disorders. For example, although the documented inhibition of diet-induced cholesterol gallstone disease by FXR agonists in mouse (36) may be translated to human disease, the therapeutic benefits or risks of synthetic FXR ligands in hepatoprotection require future investigations and careful consideration in light of potential differences between rodents and humans.

Materials and Methods

Plasmids, Chemicals, and Antibodies. See *SI Materials and Methods*.

Yeast Two-Hybrid Interaction Screening. Screenings of a *Xenopus* cDNA library (a gift from Christine Dreyer, Max Planck Institute, Tübingen, Germany) using Gal4-rSHP (amino acids 1–260) were performed essentially as described elsewhere (20). Three independent clones encoding GPS2 (amino acids 105–327) were isolated. Interactions of *Xenopus* and mouse GPS2 (amino acids 1–327) with SHP were confirmed by liquid β-galactosidase assays.

BIOCHEMISTRY

Cell Culture, Transfection, and Luciferase Assays. Cos-7, HepG2, and Huh7 cells were from American Type Culture Collection (Manassas, VA) and cultured according to the manufacturer's instructions. Stable cells were generated by transfection of HepG2 cells with pIRESneo3-hGPS2 (HepGPS) or the empty vector (HepIRES). After G 418 Sulfate (Calbiochem, San Diego, CA) selection, colonies were picked and GPS2 levels were determined by using quantitative real-time PCR (RTQ-PCR). Cells expressing 1.5- to 2-fold GPS2 over control were pulled and used. Transfections were performed by using Lipofectamine 2000 (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. Total DNA amounts in all cases were kept to a constant by using empty pcDNA3 vectors. Luciferase assays were performed as described elsewhere (37).

***In Vitro* Interaction Assays.** GST and His pull-down assays were performed as described elsewhere (37).

***In Vivo* Interaction Assays.** Cos-7 or Huh7 cells were transfected in 10-cm dishes with the indicated plasmids. Forty-eight hours after transfection whole-cell extracts were prepared and equal amounts of total protein were used. For *in vivo* GST pull-down assay, whole-cell extracts were immobilized on glutathione Sepharose beads for 2 h and the complexes were resolved by denaturing PAGE followed by Western blot analysis. Coimmunoprecipitation assays are described elsewhere (20).

RNA Interference. SMART pools against GPS2, SHP, or nonsilencing control siRNAs were from Dharmacon (Lafayette, CO). For knockdown, 50 nM indicated siRNAs were introduced to Huh7, HepG2, or primary human hepatocytes by using Dharmafect 1 (Dharmacon) according to the manufacturer's instructions.

Analysis of mRNA Expression. A total of 0.5 μg of isolated RNAs were reverse-transcribed by using the SuperScript II cDNA synthesis kit (Invitrogen) according to the manufacturer's instructions. Primer sequences are available upon request. RTQ-PCR on the basis of SYBR Green I technology were performed with ABI 7500 fast qPCR system (Applied Biosciences, Salt Lake City, UT). Relative changes were calculated by employing the comparative method (ΔCT) using 18S as the reference gene.

ChIP Assays. Formaldehyde cross-linking of cells, ChIPs, and real-time PCR analyses were performed as described previously (38, 39). The immunoprecipitates were analyzed by RTQ-PCR. ChIP data are presented as fold enrichments over the values obtained with immunoprecipitations using control antibody (anti-HA tag). For antibody and primer information see *SI Materials and Methods*.

Promoter Scan Analysis. Two *in silico* NR binding-site prediction tools, NHRscan (http://mordor.cgb.ki.se/cgi-bin/NHR-scan/nhr scan.cgi) and NUBIscan (www.nubiscan.unibas.ch), were used to predict the FXREs on hCYP8B1 promoter, and one common sequence found in both the predictions was used to generate the two-copy FXRE reporter.

Statistics. Data are presented as mean $\pm$ SD unless otherwise indicated. Statistical analyses were performed by two-tailed Student's t test.

We thank Drs. Brett Marshal and Eric Verdin (University of California, San Francisco, CA), Bruce Mayer (Harvard Medical School, Boston, MA), Christine Dreyer, Christopher K. Glass (University of California San Diego, La Jolla, CA), Elliot J. Androphy (Tufts University School of Medicine, Boston, MA), John Y. L. Chiang (Northeastern Ohio Universities College of Medicine, Rootstown, OH), Mitchell A. Lazar (University of Pennsylvania, Philadelphia, PA), and Timophy M. Wilsson (GlaxoSmithKline, Research Triangle Park, NC) for the kind gift of materials. Our gratitude extends to colleagues at the Karolinska Institutet, especially to Drs. Agneta Mode, Anders Ström, Camilla Prafalk, Knut R. Steffensen, Paolo Parini, Pia Kotokorpi, and Shuyang Yu, for sharing materials and knowledge. This study was funded by grants from the Swedish Research Council, the Swedish Cancer Society, the Swedish Diabetes Society, the European Network of Excellence CASCADE, and the KaroBio AB.

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