ACADEMIC PRESS

Biochemical and Biophysical Research Communications 293 (2002) 338–343

BBRC

www.academicpress.com

LXRα is the dominant regulator of CYP7A1 transcription

Seema Gupta,[a] William M. Pandak,[b] and Phillip B. Hylemon[a,*]

[a] *Department of Microbiology and Immunology, Virginia Commonwealth University, MCV Campus, Richmond, VA 23298, USA*
[b] *Department of Medicine–Gastroenterology, Virginia Commonwealth University, MCV Campus, Richmond, VA 23298, USA*

Received 4 March 2002

Abstract

Cholesterol 7α-hydroxylase (CYP7A1) catalyzes the rate-limiting step in the classic pathway of bile acid biosynthesis. Dietary cholesterol stimulates CYP7A1 transcription via activation of oxysterol receptor, LXRα, whereas bile acids repress transcription through FXR-mediated induction of SHP protein. The aim of this study was to determine the quantitative role that LXR- and FXR-regulated pathways play in regulating CYP7A1 and SHP in both rat and hamster models. In rats fed a 2% cholesterol diet, both SHP and CYP7A1 mRNA levels were elevated. The inability to induce CYP7A1 mRNA levels by cholesterol feeding in hamsters led to a decline in SHP mRNA levels. Elimination of hepatic bile acid flux by cholestyramine or bile fistula resulted in a marked repression of rat SHP mRNA levels. These results suggest that under conditions of both SHP and LXRα activation, stimulatory effect of LXRα overrides the inhibitory effect of FXR and results in an induction of rat CYP7A1 mRNA levels.

Keywords: Nuclear receptors; Bile acid biosynthesis; Gene regulation

The liver serves as the primary site for the elimination of cholesterol from the body via conversion to bile acids. Additionally, bile acids stimulate the direct excretion of excess hepatic cholesterol into the bile. Although bile acids play an important role in cholesterol excretion, they are also essential for solubilization and absorption of dietary cholesterol and lipid-soluble vitamins. Cytochrome P450 7A (CYP7A1) is a liver-specific enzyme that catalyzes the rate-limiting step in the classic pathway of bile acid biosynthesis [1]. Transcription of CYP7A1 is inhibited in a feedback mechanism by bile acids and stimulated in a feed-forward mechanism by cholesterol feeding in certain species [2]. Recently, two nuclear hormone receptors, the liver X receptor α (LXRα) and the farnesoid X receptor (FXR), have been implicated in the feed-forward and feedback regulations of CYP7A1, respectively [3]. LXRα heterodimerizes with the retinoid X receptor (RXR) and is activated by specific cholesterol derivatives called oxysterols. In vitro studies have shown that ligand bound LXRα mediates transcriptional up-regulation of CYP7A1 by binding to an LXR regulatory element in the CYP7A1 promoter [4]. Physiological evidence for this process has been provided by the analysis of LXRα knock-out mice ($LXR\alpha^{-/-}$), which fail to up-regulate CYP7A1 in response to cholesterol feeding, and as a result accumulate massive amounts of cholesterol in their livers [5].

Bile acid-mediated repression of CYP7A1 transcription occurs through the ligand-activated regulator, FXR [6–8]. Similar to LXRα, FXR binds to DNA as a heterodimer with RXR, recognizing an inverted hexanucleotide repeat separated by a single base (an IR-1 motif) [9]. The most potent ligands activating FXR are the hydrophobic bile acids: chenodeoxycholic, lithocholic, and deoxycholic acids. Although activated FXR/RXR heterodimer represses CYP7A1 promoter activity, no IR-1 element has been identified in the CYP7A1 promoter [6,10,11]. Recent studies have provided evidence that FXR-mediated repression of CYP7A1 occurs through an indirect mechanism involving two additional orphan nuclear receptors, fetoprotein transcription factor1 (FTF; also called CPF or LRH-1 or NR5A2) and small heterodimer partner (SHP) [12,13]. FTF is required for liver-specific expression of CYP7A1 [14]. FTF is also required for the expression of SHP. SHP is an

* Corresponding author. Fax: +1-804-828-0676.
E-mail address: hylemon@hsc.vcu.edu (P.B. Hylemon).

0006-291X/02/$ - see front matter
PII: S0006-291X(02)00229-2

atypical orphan nuclear receptor that lacks a DNA-binding domain [15]. SHP has been shown to interact with other nuclear receptors and to repress their transcriptional activities [15–18]. Bile acids activate SHP transcription via binding of FXR/RXR heterodimer to an FXR response element in the SHP promoter. Elevated levels of SHP protein then interact with FTF and down-regulate CYP7A1 transcription and that of SHP itself. Evidence for this model is supported by $FXR^{-/-}$ mice, which fail to repress CYP7A1 and are defective in the bile acid induction of SHP [19]. Studies from our laboratory support an additional mechanism for bile acid-mediated repression of CYP7A1 [20]. We have shown that in primary rat hepatocyte cultures, bile acids activate the c-Jun N-terminal kinase (JNK) pathway, which in turn activates the transcription factor c-Jun. Activated c-Jun then binds to an AP-1 element in the SHP promoter and up-regulates its transcriptional activity.

The work discussed above demonstrates the crucial role that LXR and FXR play in regulating cholesterol homeostasis in the body. The current study was designed to evaluate how a balance between the LXR- and FXR-regulated pathways is achieved by studying the regulation of CYP7A1 and SHP in an in vivo rat model where cholesterol feeding increases the bile acid pool size. Additionally, we also performed these studies in hamsters, which in contrast to rats are more susceptible to the cholesterolemic effects of dietary cholesterol as they fail to induce to CYP7A1 expression.

Materials and methods

Animal studies. Male Sprague–Dawley rats and Golden Syrian hamsters (Charles River, Cambridge, MA) weighing between 150–200 and 100–150 g, respectively, were housed under controlled lighting conditions in a 12-h light–dark cycle for 2 weeks. Cholestyramine (5% of diet), cholesterol (2% of diet), or a combination of cholesterol and cholic acid (4% and 1% of diet, respectively) were added to powdered laboratory chow (Ralston Purina, St. Louis, MO). After 14 days of feeding, the animals were killed by decapitation and the livers were harvested for total RNA extraction. For chronic biliary diversion studies, rats were placed under brief methoxyflurane anesthesia; biliary fistulas and intraduodenal canulas were placed as described previously [21]. After surgery, animals were placed in individual metabolic cages with free access to water and laboratory chow. All animals received continuous intraduodenal infusion of glucose–electrolyte replacement solution. After 72 h of biliary diversion, squalestatin was infused at the rate of 15 μg/h for 48 h. At the conclusion of the experiment, livers were harvested for total RNA extraction.

An animal protocol was approved by the Institutional Animal Care and Use Committee of the Medical College of Virginia, Virginia Commonwealth University and in compliance with the institution's guidelines on human care for laboratory animals, as set forth in the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (publication no. 86-23, revised 1985).

Total RNA extraction. Total RNA was prepared from liver tissue using the SV Total RNA isolation kit as per manufacturer's instructions (Promega, Madison, WI) or by the guanidium thiocyanate–CsCl centrifugation method as described previously [22].

Determination of mRNA levels. CYP7A1 mRNA levels were determined by RNase protection assay (RPA) as described previously [22]. Cyclophilin mRNA was used as an internal control.

SHP mRNA levels were determined by Northern blot hybridization. Briefly, 20 μg of total RNA was resolved in a 1% agarose gel containing 7% formaldehyde and then transferred to nitrocellulose membranes by overnight capillary blotting as described by Thomas [23]. The membranes were baked at 80 °C and prehybridized in 4× SSC, 5× Denhardt's solution, 1% SDS, 50% formamide, and 100 μg/ml salmon-sperm DNA at 42 °C for 2–3 h. The rat SHP cDNA was radiolabeled with [α-^{32}P]dCTP using a commercially available random primer labeling kit. About 1.5×10^6 cpm/ml probe was added to the hybridization solution and the membranes were hybridized overnight at 42 °C with shaking. The membranes were washed at room temperature in 2× SSC for 5 min, in 2× SSC and 0.1% SDS for 30 min, and in 0.5× SSC and 0.1% SDS for 15 min. The membranes were exposed to X-ray film overnight at −70 °C. The film was developed and radioactivity levels were quantitated using a Molecular Dynamics densitometer. To standardize liver mRNA, the membranes were reprobed with either a cyclophilin cDNA insert of plasmid p1B15 [24] or with a 28S rRNA oligonucleotide probe.

Statistical analyses. Data were analyzed by Student's *t* test. Level of significance was set at $P < 0.05$.

Results and discussion

CYP7A1 plays an important role in the regulation of bile acid biosynthesis and cholesterol homeostasis. The oxysterol receptor, LXRα, stimulates whereas the bile acid receptor, FXR, inhibits CYP7A1 transcription [3]. Recent evidence suggests that bile acids repress CYP7A1 synthesis by inducing the expression of SHP mRNA via an FXR site in the SHP gene promoter [12,13]. To elucidate the balance between LXR- and FXR-regulated pathways, we performed a series of experiments in rats by feeding a high cholesterol diet that results in the enlargement of the ligand pool for FXR (bile acids) and LXRα (oxysterols). SHP and CYP7A1 mRNA levels were measured to serve as surrogate markers to reflect the activation of FXR and LXR in the liver, respectively. As shown in Figs. 1A and B, feeding a 2% cholesterol diet for 14 days resulted in a threefold increase in rat SHP mRNA levels as well as marked induction (sixfold) of CYP7A1 mRNA. However, FXR mRNA levels showed no change in cholesterol fed animals as compared to controls (data not shown). As would be predicted by supplementing the high cholesterol diet (4% cholesterol) with cholic acid (1% CA), there was a dramatic induction in SHP mRNA levels and a diametric repression of CYP7A1 gene expression. However, it should be noted that even in the presence of cholic acid in the diet, CYP7A1 mRNA levels were still 300% higher than control animals. These data suggest that, in rats, feeding 2% cholesterol for 14 days increases the bile acid pool size and thereby the ligand supply for FXR resulting in induction of SHP mRNA levels. Since FXR mRNA levels did not change upon cholesterol feeding, it

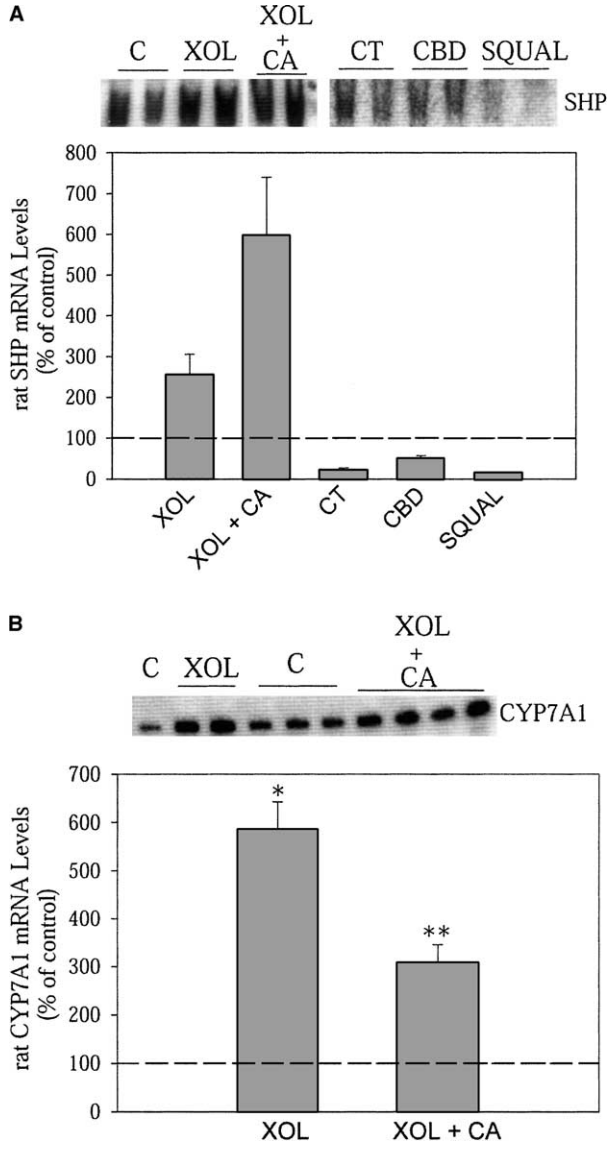

Fig. 1. Cholesterol feeding and bile acids reciprocally regulate SHP and CYP7A1 mRNA levels in rats. (A) Total RNA was isolated from livers of rats fed a 2% cholesterol (XOL; $n = 8$) diet, a cholesterol and cholic acid mix diet (XOL + CA; $n = 4$), a 5% cholestyramine (CT; $n = 3$) diet; from rats with chronic biliary diversion (CBD; $n = 5$), and from CBD rats intraduodenally infused with 15 μg/h of squalestatin for 48 h (SQUAL; $n = 6$). SHP mRNA was quantitated by densitometric analysis of northern blots. Values shown are means ± SE. All values were statistically significant when compared to appropriate controls. Top panel: Representative Northern blot depicting SHP mRNA levels in control rats (C) and rats treated as described above. (B) Total RNA was isolated from livers of rats fed a 2% cholesterol (XOL; $n = 8$) diet and a cholesterol and cholic acid mix diet (XOL + CA; $n = 4$) for 14 days. CYP7A1 mRNA levels were quantitated by RNase protection assay. Values shown are means ± SE; $^{*}P < 0.001$, $^{**}P < 0.005$. Statistical significance was calculated between control diet and XOL or XOL + CA fed rats. Top panel: Representative autoradiogram for RNase protection assay depicting CYP7A1 mRNA levels in control rats (C) and rats treated as described above.

suggests that bile acids are not transcriptional regulators of FXR and only activate FXR in a ligand dependent manner. This is in contrast to what has been reported in rabbits, where depletion of the bile acid pool resulted in reduced FXR mRNA levels [25]. The ligand levels for LXRα (oxysterols) are also concomitantly increased in cholesterol fed animals which then up-regulate CYP7A1 expression. However, in the rat the stimulatory effect of LXRα overrides the inhibitory effect of FXR resulting in the induction of rat CYP7A1 mRNA levels.

Since bile acids repress CYP7A1 synthesis by inducing the expression of SHP, it was of interest to determine if basal levels of SHP would be lower in animals with a disrupted enterohepatic (bile acid) circulation. As expected, in rats treated with cholestyramine that is known to enhance the excretion of bile acids, there was a dramatic reduction in SHP mRNA levels (Fig. 1A). In addition, chronic biliary diversion (CBD), which depletes the endogenous bile salt pool, decreased SHP mRNA levels to 50% of sham-operated controls. Previous experiments from our laboratory have shown that under similar conditions, CYP7A1 activity, protein mass, and mRNA levels are markedly increased [26]. We next tested the ability of squalestatin, a potent and specific inhibitor of squalene synthase, the first committed step in the sterol arm of the cholesterol biosynthetic pathway [27], to regulate SHP. Treatment with squalestatin deprives cells of endogenously synthesized sterols but does not inhibit the biosynthesis of isoprenoid compounds. Squalestatin treatment limits the availability of the substrate (cholesterol) for CYP7A1 thereby leading to a reduced bile acid synthesis. Also, it impedes the formation of oxysterols and prevents LXRα activation. As shown in Fig. 1A, infusion of squalestatin (15 μg/h) in CBD rats resulted in a ~90% decrease in SHP mRNA levels. Previous experiments from our laboratory have shown that continuous squalestatin infusion (15 μg/h) decreased CYP7A1 specific activities to 4% and 12% of paired biliary fistula controls at 24 and 48 h, respectively [28]. Moreover, addition of squalestatin to primary cultures of rat hepatocytes resulted in a rapid decrease in CYP7A1 steady-state mRNA levels [29]. Ness et al. [30] have shown that inhibition of squalene synthesis decreases CYP7A1 mRNA in the intact rat. These observations suggest that cholesterol derived metabolites (oxysterols) mediate the induced expression of CYP7A1. Failure to up-regulate CYP7A1 results in reduced levels of bile acids, which then significantly lower SHP mRNA. Taken together, these experiments strongly support the bile acid dependence of SHP expression in vivo and corroborate the reciprocal relationship between the regulation of SHP and CYP7A1 by bile acids.

Stimulation of CYP7A1 gene expression by high cholesterol diets has been found to vary among species. Rats and mice show marked up-regulation whereas hamsters, monkeys, humans, guinea pigs, and rabbits do not stimulate or even repress Cyp7A1 expression

[31–34]. Rats are therefore able to adapt to large fluctuations in sterol input and are resistant to diet-induced hypercholesterolemia, whereas rabbits, hamsters, and some humans develop hypercholesterolemia on a cholesterol rich diet. Elegant studies by Spady and co-workers [31] have shown that the differences in sensitivity to dietary cholesterol in the rat and hamster are mainly due to differences in the regulation of CYP7A1. A low basal level of CYP7A1 expression coupled with the inability to induce mRNA and enzyme activity renders the hamster more sensitive than the rat to dietary cholesterol. To identify whether differences in CYP7A1 expression and regulation in the two species also manifest themselves in the regulation of SHP mRNA levels, we performed experiments in hamsters fed a high cholesterol (2%) diet for 14 days. As shown in Fig. 2A, SHP mRNA levels decreased to about 60–70% of control animals. As expected, CYP7A1 mRNA levels were also suppressed by ~40% in hamsters fed a high cholesterol diet (Fig. 2B). Together, these data provide direct in vivo evidence that lower basal levels of SHP expression parallel a reduction in bile acid synthesis. Our results are consistent with the findings of Spady and co-workers [31] who reported that high levels of cholesterol intake in hamsters in amounts that raise liver cholesterol levels by >30-fold lead to a trend towards lower CYP7A1 mRNA levels. Although the LXR regulatory elements in the hamster and rat CYP7A1 gene promoters are identical in their hexanucleotide motifs and would therefore be expected to be up-regulated similarly by LXRα [35], in vitro experiments have shown that the LXRα/RXRα binding to a hamster probe was much weaker than the rat probe [36]. Moreover, LXRα was shown to strongly stimulate the rat CYP7A1 promoter activity in HepG2 cells, but has little or no effect on hamster CYP7A1. This suggests that the differential response seen in the hamster may be due to the absence of a positive factor required for LXRα-mediated activation of CYP7A1 or the presence of an inhibitor that suppresses LXRα activity. In addition, the sequences flanking the LXRα regulatory elements may be important for either binding or functional activity of LXRα.

In summary, our studies with in vivo rat and hamster models with cholesterol feeding or an interrupted enterohepatic circulation demonstrated that: (a) in rats fed a 2% cholesterol diet, both SHP a CYP7A1 mRNA levels were elevated; (b) elimination of the hepatic bile acid flux by using cholestyramine or bile fistula resulted in a marked repression of SHP mRNA levels; and (c) inability to induce CYP7A1 mRNA levels in hamsters fed a high cholesterol diet also led to a decline in SHP mRNA levels. These results support the notion that SHP transcription is regulated by bile acids. Also, under conditions when both SHP (via FXR stimulation) and LXRα are activated, the stimulatory effect of LXRα overrides the inhibitory effect of FXR and results in induction of rat CYP7A1 mRNA levels.

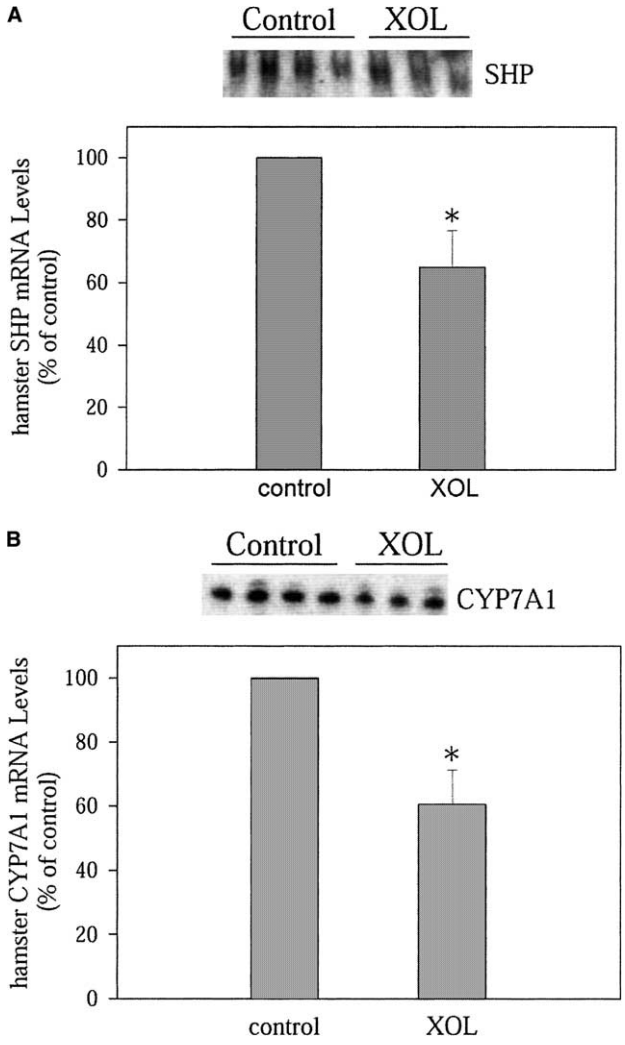

Fig. 2. SHP and CYP7A1 mRNA levels are down-regulated in hamsters. (A) Total RNA was isolated from livers of hamsters fed a 2% cholesterol (XOL; $n = 5$) diet for 14 days. Hamster SHP mRNA levels were quantitated by Northern blot analysis using a ^{32}P-labeled rat SHP cDNA probe. Values shown are means ± SE. *Significant ($P < 0.025$) change. Statistical significance was calculated between control and cholesterol fed animals. Top panel: Representative Northern blot depicting SHP mRNA levels in control diet and cholesterol fed hamsters. (B) Top panel: Representative autoradiogram depicting CYP7A1 mRNA levels in control diet and 2% cholesterol fed hamsters. Lower panel: CYP7A1 mRNA was quantitated by RNase protection assay. Values depicted are means ± SE. *Significant ($P < 0.025$) change. Statistical significance was calculated between control and cholesterol fed animals.

Acknowledgments

We thank Pat Bohdan, Emily Gurley, and Kaye Redford for their invaluable technical help. This work was funded in part by National Institute of Health Grant P01-DK38030 (Hylemon) and a National Research Service Award (Gupta) 1 F32 DK59770-01 from the National Institutes of Health.

References

[1] D.W. Russell, K.D. Setchell, Bile acid biosynthesis, Biochemistry 31 (20) (1992) 4737–4749.

[2] J.Y.L. Chiang, Regulation of bile acid synthesis, Front. Biosci. 3 (1998) 176–193.

[3] T.T. Lu, J.J. Repa, D.J. Mangelsdorf, Orphan nuclear receptors as eLiXiRs and FiXeRs of sterol metabolism, J. Biol. Chem. 276 (41) (2001) 37735–37738.

[4] J.M. Lehmann, S.A. Kliewer, L.B. Moore, T.A. Smith-Oliver, B.B. Oliver, J.L. Su, S.S. Sundseth, D.A. Winegar, D.E. Blanchard, T.A. Spencer, T.M. Willson, Activation of the nuclear receptor LXR by oxysterols defines a new hormone response pathway, J. Biol. Chem. 272 (6) (1997) 3137–3140.

[5] D.J. Peet, S.D. Turley, W. Ma, B.A. Janowski, J.M. Lobaccaro, R.E. Hammer, D.J. Mangelsdorf, Cholesterol and bile acid metabolism are impaired in mice lacking the nuclear oxysterol receptor LXR alpha, Cell 93 (1998) 693–704.

[6] M. Makishima, A.Y. Okamoto, J.J. Repa, H. Tu, R.M. Learned, A. Luk, M.V. Hull, K.D. Lustig, D.J. Mangelsdorf, B. Shan, Identification of a nuclear receptor for bile acids, Science 284 (1999) 1362–1365.

[7] D.J. Parks, S.G. Blanchard, R.K. Bledsoe, G. Chandra, T.G. Consler, S.A. Kliewer, J.B. Stimmel, T.M. Willson, A.M. Zavacki, D.D. Moore, J.M. Lehmann, Bile acids: natural ligands for an orphan nuclear receptor, Science 284 (1999) 1365–1368.

[8] H. Wang, J. Chen, K. Hollister, L.C. Sowers, B.M. Forman, Endogenous bile acids are ligands for the nuclear receptor FXR/BAR, Mol. Cell 3 (1999) 543–553.

[9] B.M. Forman, E. Goode, J. Chen, A.E. Oro, D.J. Bradley, T. Perlmann, D.J. Noonan, L.T. Burka, T. McMorris, W.W. Lamph, et al., Identification of a nuclear receptor that is activated by farnesol metabolites, Cell 81 (1995) 687–693.

[10] J.Y. Chiang, R. Kimmel, C. Weinberger, D. Stroup, Farnesoid X receptor responds to bile acids and represses cholesterol 7alpha-hydroxylase gene (CYP7A1) transcription, J. Biol. Chem. 275 (15) (2000) 10918–10924.

[11] A. del Castillo-Olivares, G. Gil, Role of FXR and FTF in bile acid-mediated suppression of cholesterol 7alpha-hydroxylase transcription, Nucleic Acids Res. 28 (18) (2000) 3587–3593.

[12] T.T. Lu, M. Makishima, J.J. Repa, K. Schoonjans, T.A. Kerr, J. Auwerx, D.J. Mangelsdorf, Molecular basis for feedback regulation of bile acid synthesis by nuclear receptors, Mol. Cell 6 (3) (2000) 507–515.

[13] B. Goodwin, S.A. Jones, R.R. Price, M.A. Watson, D.D. McKee, L.B. Moore, C. Galardi, J.G. Wilson, M.C. Lewis, M.E. Roth, P.R. Maloney, T.M. Willson, S.A. Kliewer, A regulatory cascade of the nuclear receptors FXR, SHP-1, and LRH-1 represses bile acid biosynthesis, Mol. Cell 6 (3) (2000) 517–526.

[14] M. Nitta, S. Ku, C. Brown, A.Y. Okamoto, B. Shan, CPF: an orphan nuclear receptor that regulates liver-specific expression of the human cholesterol 7alpha-hydroxylase gene, Proc. Natl. Acad. Sci. USA 96 (1999) 6660–6665.

[15] W. Seol, H.S. Choi, D.D. Moore, An orphan nuclear hormone receptor that lacks a DNA binding domain and heterodimerizes with other receptors, Science 272 (1996) 1336–1339.

[16] N. Masuda, H. Yasumo, T. Tamura, N. Hashiguchi, T. Furusawa, T. Tsukamoto, H. Sadano, T. Osumi, An orphan nuclear receptor lacking a zinc-finger DNA-binding domain: interaction with several nuclear receptors, Biochim. Biophys. Acta 1350 (1) (1997) 27.

[17] L. Johansson, J.S. Thomsen, A.E. Damdimopoulos, G. Spyrou, J.A. Gustafsson, E. Treuter, The orphan nuclear receptor SHP inhibits agonist-dependent transcriptional activity of estrogen receptors ERalpha and ERbeta, J. Biol. Chem. 274 (1) (1999) 345–353.

[18] Y.K. Lee, H. Dell, D.H. Dowhan, M. Hadzopoulou-Cladaras, D.D. Moore, The orphan nuclear receptor SHP inhibits hepatocyte nuclear factor 4 and retinoid X receptor transactivation: two mechanisms for repression, Mol. Cell. Biol. 20 (1) (2000) 187–195.

[19] C.J. Sinal, M. Tohkin, M. Miyata, J.M. Ward, G. Lambert, F.J. Gonzalez, Targeted disruption of the nuclear receptor FXR/BAR impairs bile acid and lipid homeostasis, Cell 102 (6) (2000) 731–744.

[20] S. Gupta, R.T. Stravitz, P. Dent, P.B. Hylemon, Down-regulation of cholesterol 7alpha-hydroxylase (CYP7A1) gene expression by bile acids in primary rat hepatocytes is mediated by the c-Jun N-terminal kinase pathway, J. Biol. Chem. 276 (19) (2001) 15816–15822.

[21] D.M. Heuman, C.R. Hernandez, P.B. Hylemon, W.M. Kubaska, C. Hartman, Z.R. Vlahcevic, Regulation of bile acid synthesis. I. Effects of conjugated ursodeoxycholate and cholate on bile acid synthesis in chronic bile fistula rat, Hepatology 8 (2) (1988) 358–365.

[22] P.B. Hylemon, E.C. Gurley, R.T. Stravitz, J.S. Litz, W.M. Pandak, J.Y. Chiang, Z.R. Vlahcevic, Hormonal regulation of cholesterol 7 alpha-hydroxylase mRNA levels and transcriptional activity in primary rat hepatocyte cultures, J. Biol. Chem. 267 (24) (1992) 16866–16871.

[23] P.S. Thomas, Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose, Proc. Natl. Acad. Sci. USA 77 (9) (1980) 5201–5205.

[24] P.E. Danielson, S. Forss-Petter, M.A. Brow, L. Calavetta, J. Douglass, R.J. Milner, J.G. Sutcliffe, p1B15: a cDNA clone of the rat mRNA encoding cyclophilin, DNA 7 (4) (1988) 261–267.

[25] G. Xu, P. Lu-Xing, S.K. Erickson, B.M. Forman, B.L. Shneider, M. Ananthanarayanan, X. Li, S. Shefer, N. Balasubramanian, L. Ma, H. Asaoka, S.R. Lear, L.B. Nguyen, I. Dussault, F.J. Suchy, G.S. Tint, G. Salen, Removal of the bile acid pool upregulates cholesterol 7alpha-hydroxylase by deactivating FXR in rabbits, J. Lipid Res. 43 (1) (2002) 45–50.

[26] W.M. Pandak, Y.C. Li, J.Y. Chiang, E.J. Studer, E.C. Gurley, D.M. Heuman, Z.R. Vlahcevic, P.B. Hylemon, Regulation of cholesterol 7 alpha-hydroxylase mRNA and transcriptional activity by taurocholate and cholesterol in the chronic biliary diverted rat, J. Biol. Chem. 266 (6) (1991) 3416–3421.

[27] A. Baxter, B.J. Fitzgerald, J.L. Hutson, A.D. McCarthy, J.M. Motteram, B.C. Ross, M. Sapra, M.A. Snowden, N.S. Watson, R.J. Williams, C. Wright, J. Biol. Chem. 267 (17) (1992) 11705–11708.

[28] Z.R. Vlahcevic, R.T. Stravitz, D.M. Heuman, P.B. Hylemon, W.M. Pandak, Quantitative estimations of the contribution of different bile acid pathways to total bile acid synthesis in the rat, Gastroenterology 113 (6) (1997) 2005–2008.

[29] K.C. Doerner, E.C. Gurley, Z.R. Vlahcevic, P.B. Hylemon, Regulation of cholesterol 7 alpha-hydroxylase expression by sterols in primary rat hepatocyte cultures, J. Lipid Res. 36 (6) (1995) 1168–1177.

[30] G.C. Ness, Z. Zhao, R.K. Keller, Effect of squalene synthase inhibition on the expression of hepatic cholesterol biosynthetic enzymes, LDL receptor, and cholesterol 7 alpha hydroxylase, Arch. Biochem. Biophys. 311 (2) (1994) 277–285.

[31] J.D. Horton, J.A. Cuthbert, D.K. Spady, Regulation of hepatic 7α-hydroxylase expression and response to dietary cholesterol in the rat and hamster, J. Biol. Chem. 270 (10) (1995) 5381–5387.

[32] S. Dueland, J. Drisko, L. Graf, D. Machleder, A.J. Lusis, R.A. Davis, Effect of dietary cholesterol and taurocholate on cholesterol 7α-hydroxylase and hepatic LDL receptors in inbred mice, J. Lipid Res. 34 (1993) 923–931.

[33] L. Rudel, C. Deckelman, M. Wilson, M. Scobey, R. Anderson, Dietary cholesterol and downregulation of cholesterol 7α-hydrox-

ylase and cholesterol absorption in African green monkeys, J. Clin. Invest. 93 (1994) 2463–2472.

[34] L.B. Nguyen, G. Xu, S. Shefer, G.S. Tint, A. Batta, G. Salen, Comparative regulation of hepatic sterol 27-hydroxylase and cholesterol 7alpha-hydroxylase activities in the rat, guinea pig, and rabbit: effects of cholesterol and bile acids, Metabolism 48 (12) (1999) 1542–1548.

[35] J.J. Repa, D.J. Mangelsdorf, The role of orphan nuclear receptors in the regulation of cholesterol homeostasis, Annu. Rev. Cell Dev. Biol. 16 (2000) 459–481.

[36] J.Y. Chiang, R. Kimmel, D. Stroup, Regulation of cholesterol 7alpha-hydroxylase gene (CYP7A1) transcription by the liver orphan receptor (LXRalpha), Gene 262 (2001) 257–265.