Pregnane X Receptor Is a Target of Farnesoid X Receptor*

Received for publication, January 5, 2006, and in revised form, May 5, 2006 Published, JBC Papers in Press, May 8, 2006, DOI 10.1074/jbc.M600116200

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The pregnane X receptor (PXR) is an essential component of the body’s detoxification system. PXR is activated by a broad spectrum of xenobiotics and endobiotics, including bile acids and their precursors. Bile acids in high concentrations are toxic; therefore, their synthesis is tightly regulated by the farnesoid X receptor, and their catabolism involves several enzymes regulated by PXR. Here we demonstrate that the expression of PXR is regulated by farnesoid X receptor. Feeding mice with cholic acid or the synthetic farnesoid X receptor (FXR) agonist GW4064 resulted in a robust PXR induction. This effect was abolished in FXR knock-out mice. Long time bile acid treatment resulted in an increase of PXR target genes in wild type mice. A region containing four FXR binding sites (IR1) was identified in the mouse Pxr gene. This region was able to trigger an 8-fold induction after GW4064 treatment in transactivation studies. Deletion or mutation of single IR1 sites caused a weakened response. The importance of each individual IR1 element was assessed by cloning a triple or a single copy and was tested in transactivation studies. Two elements were able to trigger a strong response, one a moderate response, and one no response to GW4064 treatment. Mobility shift assays demonstrated that the two stronger responding elements were able to bind FXR protein. This result was confirmed by chromatin immunoprecipitation. These results strongly suggest that PXR is regulated by FXR. Bile acids activate FXR, which blocks synthesis of bile acids and also leads to the transcriptional activation of PXR, promoting breakdown of bile acids. The combination of the two mechanisms leads to an efficient protection of the liver against bile acid induced toxicity.

A key function of the liver is the elimination of xenobiotics and endogenous catabolites from the systemic circulation. Although multiple reactions are involved, they generally are divided into three phases, hydroxylation (phase I), conjugation (phase II), and transport (phase III). The expression of many phase I, II, and III genes is regulated by transcription factors belonging to the nuclear receptor family (1–3). Two members of this family, the constitutive androstane receptor (CAR) (4, 5) and the pregnane X receptor (PXR) (6, 7) have been identified as critical transcription factors for the activation of many genes involved in detoxification pathways in the liver and other tissues. Mice deficient in either of these genes have impaired capabilities of detoxifying xenobiotics and endobiotics (8–10). One of the best known examples for the function of PXR and CAR is found within the regulation of the CYP3A family of cytochromes P450 (CYPs). CYPs encode broad specificity heme-containing monoxygenases that catalyze the oxidation of a wide variety of structurally dissimilar compounds. In humans, the CYP3A enzymes are responsible for the oxidation of more than 60% of all prescribed drugs as well as many steroids and bile acids.

CYP3A4 (Cyp3a11 in mice) expression is strongly activated by both CAR and PXR ligands and reduced in PXR−/− and CAR−/− mice (11, 12). PXR is activated by a broad spectrum of lipophilic substrates (7, 10, 13), including xenobiotics like antibiotics, antimycotics or herbal components, and endogenous substrates such as bile acids (14) and their precursors (15). Moreover PXR is critical to the handling of bile acids (14, 16, 17). Bile acids in high concentrations are toxic; therefore, their metabolism and transport is tightly regulated. One major governor here is the nuclear farnesoid X receptor (FXR), representing the physiological sensor for bile acids (18). Bile acids like chenodeoxycholic, lathocholic, or deoxycholic acid as well as their taurine and glycine conjugates directly bind to FXR (19, 20).

FXR plays a critical role in the negative feedback regulation of bile acid synthesis. Bile acids activate FXR, which then induces the expression of another nuclear receptor termed small heterodimer partner (SHP). SHP itself initializes a cascade leading to the down-regulation of cholesterol 7α-hydroxylase (CYP7A1) and sterol 12α-hydroxylase (CYP8B1) (21), thereby providing the repression of bile acid de novo synthesis. Additional roles of FXR in bile acid metabolism have been suggested by the observation that the transcriptional regulation of genes involved in the transport of bile acids also is regulated by FXR, including for example the bile acid export pump (BSEP) (22, 23), the organic anion transporter 1B1 and 1B3, also called organic anion transporting peptide (OATP)-C and OATP8 (24, 25), or the ileal bile acid binding protein (20, 26). Potentially toxic bile acids and bile acid precursors also can activate PXR and thereby induce cytochromes P450 of the CYP3A subfamily,

farnesoid X receptor; IR, inverted repeat; OATP, organic anion transporting peptide; MDR, multidrug resistance protein; PXR, pregnane X receptor; RXR, 9-cis retinoic acid receptor; SHP, small heterodimer partner; TK, thymidine kinase; HEK, human embryonic kidney.
which catalyze metabolism of bile acids. Furthermore PXR has been implicated in the down-regulation of CYP7A1, the mechanism remaining unknown (14, 27–29).

Less is known about how PXR itself is regulated. Several studies demonstrated the down-regulation of PXR expression by inflammatory signals as mediated by lipopolysaccharides (30–32). PXR can be induced by dexamethasone through the glucocorticoid receptor in human hepatocytes, whereas PXR activators such as rifampicin and clotrimazole do not affect its expression (33). Whether bile acids play a role in the regulation of PXR remains unknown (14, 27–29).

EXPERIMENTAL PROCEDURES

Animals and Treatment—Wild type and FXR−/− mice were housed in a temperature-controlled room (22–23 °C) under a 12-h light/12-h dark cycle. The mice were maintained on a low cholesterol (0.02%) chow diet (Purina 5001, Harlan Teklad 75020, Madison, WI) under a 12-h light/12-h dark cycle. The mice were maintained on a low cholesterol (0.02%) chow diet (Purina 5001, Harlan Teklad 75020, Madison, WI) under a 12-h light/12-h dark cycle.

RNA Extraction and Gene Expression Analysis—RNA extraction from mouse liver was performed using the RNA STAT-60 reagent (TEL-TEST B, Inc., Friendswood, TX) for cells using Trizol (Invitrogen). RNA was treated with RNase-free DNase (Roche Applied Science) and reverse-transcribed (Superscript II, Invitrogen) using random hexamers (Roche Applied Science) to a final concentration of 20 ng/μl. Gene-specific primers are shown in Table 1. Gene expression was investigated by real time PCR using syber-green incorporation.

Plasmid Construction—A fragment of the mouse Pxr second intron gene region containing four potential IR1 sites was PCR-amplified using mouse genomic DNA as a template and Pfu Turbo DNA polymerase (Stratagene, La Jolla, CA). The upstream primer (Mo-Int-for) contained an internal SalI site, and the downstream primer (Mo-Int-rev) contained an internal HindIII site. The resulting PCR product was digested and ligated into TK-Luc that had been predigested with HindIII. Chimeric TK-Luc constructs containing a triple or single copy of each individual IR1 element were constructed by ligating dimerized oligonucleotides mutated in the IR1 sites using complementary oligonucleotides mutated in the IR1 sites (Table 1).

Site-direct Mutagenesis—ABCD-TK-derived constructs containing mutated IR1-A, -C, and -D sites were generated by PCR using complementary oligonucleotides mutated in the IR1 sites. The products were digested with DpnI to remove the parental DNA.
template and selected for constructs containing mutations. The mutated plasmids were termed mABCD-TK, AbmCD-TK, ABCmD-TK, and AbmCmD.

Cell Culture and Transfection Studies—HEK293 cells were grown in Dulbecco's modified Eagle's medium (Invitrogen) supplemented with 10% fetal calf serum, 100 units/ml penicillin (Invitrogen), and 100 μg/ml streptomycin (Invitrogen). mHPKT cells were grown in DMEM/F-12 (Invitrogen) supplemented with 5% fetal calf serum, 50 nM dexamethasone (Sigma), 5 mg/ml insulin (Sigma), 100 units/ml penicillin (Invitrogen), and 100 μg/ml streptomycin (Invitrogen). One day before the experiment cells were changed into medium containing 5 or 10% charcoal stripped calf serum, cultivated for 24 h, and then used either for transactivation assays or RNA expression profiling. HEK293 cells were transfected by the CaCl₂ precipitation method using 130 ng of plasmid DNA per well composed of 50 ng of luciferase reporter, 20 ng of β-galactosidase expression plasmid, 15 ng of FXR expression plasmid, and 45 ng of pGEM. Eight hours post-transfection cells were treated with 10⁻⁶ M GW4064 or Me₂SO for 16 h. Transfection experiments were repeated 4–6 times in triplicate. Cells used for real time PCR analysis were treated for 24 h with 10⁻⁶ M GW4064 or Me₂SO.

Electrophoretic Mobility Shift and Chromatin Immunoprecipitation (ChIP) Assays—Mobility shift assays were performed either with in vitro translated proteins or nuclear extracts prepared from livers using the CellLytic NuCLEAR Extraction kit (Sigma) as recently described (34). Oligonucleotides used in these experiments are shown in Table 1. ChIP Assays were performed in mHPDK cells stimulated with 10⁻⁶ M GW4064 for 24 h using a commercial kit (Active Motif Europe, Rixenart, Belgium). Immunoprecipitation was done with 4 mg the antibody FXR C-20 and FXR H130 (Santa Cruz Biotechnology, Heidelberg, Germany).

RESULTS

Activated FXR Induces Mouse PXR Expression—To investigate whether there is a cross-talk between FXR and PXR, we analyzed in a first step if PXR is affected by FXR. Wild type and FXR⁻/⁻ mice were treated with the primary bile acid CA, the synthetic FXR agonist GW4064, or vehicle for 3 days. After sacrifice, mouse liver total RNA was prepared, and expression levels were analyzed by real time PCR. Because Shp is a well described FXR-activated target gene, we first studied changes in Shp gene expression to validate whether our treatment was successful. As expected, an increase in SHP mRNA was observed in wild type mice treated with CA and GW4064 (Fig. 1A). This effect reached significance only in the GW4064-
FIGURE 2. Expression of FXR and PXR target genes in wild type mice and PXR binding to DNA after long term CA treatments. Mice were fed a control diet (con) or a diet supplemented with 1% CA for 7 days. A, total RNA from liver was subjected to real time PCR quantification of nuclear receptors (Pxr and Shp), cytochromes P450 (Cyp3a11 and Cyp2b10), and transporter genes (Bsep, Oatp1a4, Mdr1α, Mdr1β, and Mrp2). Values are normalized relative to cyclophilin and are expressed (means ± S.E., n = 5 animals) relative to control diet mice. *, p ≤ 0.01. B, nuclear extracts were prepared from liver and used for electrophoretic mobility shift assays to analyze binding of PXR to DNA.
treated mice, however. Induction of Shp gene expression was abolished by deletion of the Fxr gene in the FXR−/− mice; no changes in Shp expression after CA or GW4064 feeding were observed in these mice (Fig. 1A).

In a next step we investigated whether PXR is affected by FXR activation or if deletion of the Fxr gene affects basal Pxr gene expression. Whereas FXR−/− mice showed a reduced amount of SHP mRNA as compared with wild type mice, PXR expression did not decrease. However, an induction of PXR mRNA expression was seen in wild type mice after CA and GW4064 treatment for 3 days. As for SHP, this effect only reached significance in the GW4064 treated wild type animals. An increase in PXR expression was not observed in FXR−/− mice, indicating that PXR might be a direct target gene of FXR (Fig. 1B).

To exclude that the PXR induction is an indirect effect of long term treatment, mice were additionally treated for 14 h with CA and GW4064. This experiment demonstrated also that short term FXR activation is able to induce PXR in wild type

FIGURE 3. Functional analysis of an intronic region of the mouse Pxr gene containing four IR1 elements. A, structure of the mouse Pxr gene. Within the second intron a region containing four highly conserved IR1 elements (4xIR1) is highlighted. Generated reporter constructs were generated and shown. B, HEK293 cells were transfected with an expression plasmid for FXR and either the indicated constructs containing parts of the intronic 4xIR1 Pxr gene region or the native TK-Luc plasmid. Six hours after transfection cells were treated with GW4064 (1 µM) for 16 h. Luciferase activity of each construct is expressed in relation to values obtained in Me2SO (DMSO)-treated controls and shown as the ratio of luciferase to β-galactosidase activities. Data represent the mean ± S.D. of 4–6 individual experiments performed in triplicate.
mice, whereas no effect was observed in FXR−/− mice (Fig. 1C). The induction of PXR mRNA due to FXR activation also was observed in the ileum of wild type animals (data not shown).

Long Term Exposure to Bile Acids Induces PXR and PXR Target Genes—Because genes important for the detoxification and elimination of bile acids showed only a trend to be increased after feeding CA for 14 h or 3 days (data not shown), we performed long term bile acid treatment. For this propose wild type mice were fed for 7 days with a control diet or a diet supplemented with 1% cholic acid. Again, PXR as well as the FXR target genes Shp and Bsep showed a significant increase in RNA expression (Fig. 2A). Furthermore, PXR target genes known to be involved in bile acid breakdown showed a significant increase in expression when challenged with CA. In particular, an increased expression was detected for the Cyp3a11 and Cyp2b10 as well as for Oatp1a4, Mrp2, and Mdr1α. In contrast, Mdr1β did not show any changes in gene expression (Fig. 2A).

In view of the predominant role of PXR as transcriptional activator, we examined its binding to DNA. Therefore, nuclear liver extracts were prepared from mice fed a control diet with (1% CA) or without (con) bile acids and subjected to mobility

![Diagram A](image1)

![Diagram B](image2)
HEK235 cells cotransfected with FXR were transiently transfected with TK-ABCD and either treated with Me2SO as control or GW4064 as FXR ligand. Activation of FXR by GW4064 treatment resulted in a 7.6-fold increased luciferase activity (Fig. 3B) in TK-ABCD expression compared with Me2SO-treated cells, in contrast to the empty TK-Luc reporter construct which did not confer any response. These data indicate that indeed functional FXR binding sites are located within the proposed region.

To evaluate the role of individual IR1 sites, several deletion constructs were generated by restriction digestion as shown in Fig. 2A. Deletion of the elements A and B (TK-CD) reduced activation by GW4064 by 37%, indicating that at least one of the elements (A or B) is able to respond to bile acids. Deletion of element D (TK-ABC) resulted in a massive 69% loss of activation, indicating that this element has a more prominent role than A or B. TK-C, a construct only containing element C (TK-C), also had a strongly reduced activation (65%). These data suggest that elements C and D are the functionally important bile acid response elements within the identified region.

The IR1 Binding Sites A, C, and D of the Pxr Gene Confer Activation by GW4064—To assess the importance of each individual IR1 element for the bile acid-dependent activation of PXR, a triple copy of each IR1 was cloned in front of the thymidine kinase promoter of the luciferase gene vector TK-Luc. In transfected HEK235 cells all TK-Luc constructs had similar basal luciferase activities attributable to the TK promoter. Treatment with the synthetic FXR agonist GW4064 resulted in a 16.2-fold increase of TK-3A, a 76.7-fold increase of TK-3C, and a 78.3-fold increase of TK-3D luciferase activity. TK-3B, like the native TK-Luc, did not show any response. A control containing three copies of a perfect IR1 showed a 97-fold induction of luciferase activity (Fig. 4A).

To further confirm the role of each individual IR1 element in mediating PXR induction by FXR activation, IR1-A, -C, and -D were cloned as a single copy in front of the thymidine kinase promoter of the vector TK-Luc. Additionally two new constructs were generated; TK-per IR1, containing a single perfect IR1 element, and TK-mutIR1, in which the IR1 site was mutated (Fig. 4B). All constructs were transfected into HEK293 cells and treated with Me2SO or GW4064. All three elements of the Pxr gene were able to confer a response after FXR activation. In particular, the single copy IR1-A showed the lowest induction, resulting in a 2.1-fold increased luciferase activity after GW4064 treatment. Similar to this, IR1-D showed a 3.3-fold increase. The highest induction (10-fold) was obtained with element IR1-C. Of note, a perfect single IR1 element (TK-
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| Probe | per IR1 |
|-------|---------|
|       | RXRα/FXRα |
|       | RXRα |
| Competition | per IR1 A, mA | mB | C, mC | D, mD |

perIR1) was able to produce an 18.9-fold induction of luciferase activity after FXR activation, whereas neither the mutated IR1 (TK-mutIR1) nor the native vector (TK-Luc) showed any response. These data clearly show that elements A, C, and D of the mouse Pxr gene are able to confer activation by GW4064.

**Mutagenesis of the IR1 Sites Result in a Loss in Induction**—To assess the importance of IR1-A, -C, and -D for the bile acid-dependent activation of PXR, mutations were introduced in each individual response element as illustrated in Fig. 5A. Mutation of the IR1-A binding site (TK-mABCD) resulted in a 20% reduction in response to GW4064 treatment as compared with the native construct TK-ABCD (Fig. 5A); the remaining 5.9-fold induction of luciferase activity was similar to the one with construct TK-CD where IR1-A and IR1-B were deleted (Fig. 3B). Mutations of the IR1-C or -D resulted in a complete loss of induction after treatment.

**IR1-C and -D of the Pxr Gene Bind FXR**—To determine whether the IR1 elements indeed are able to bind FXR, electrophoretic mobility shift assays were performed. A perfect IR1 element (perIR1, Table 1) was 32P-labeled and incubated with in vitro translated FXR/RXR proteins, resulting in two visible DNA protein complexes (arrows). Competition experiments were performed using a 50- or 100-fold excess of unlabeled perIR1, IR1-A, IR1-B, IR1-C, or IR1-D, or a 100-fold excess of mutated binding sites (mA, mB, mC, mD).

**FIGURE 6. FXR/RXR bind to IR1-C and IR-D of the Pxr gene.** Electrophoretic mobility shift assays were performed to analyze binding of FXR/RXR to the putative IR1 sequences in the intronic region of the Pxr gene. An oligonucleotide containing a perfect IR1 binding site (perIR1) was 32P-labeled and incubated with in vitro translated FXR/RXR proteins, resulting in two visible DNA protein complexes (arrows). Competition experiments were performed using a 50- or 100-fold excess of unlabeled perIR1, IR1-A, IR1-B, IR1-C, IR1-D, or a 100-fold excess of mutated binding sites (mA, mB, mC, mD).

*Competition experiments were also performed with each of the individual IR1 sites (A, B, C, D) of the Pxr gene. IR1-A showed a faint inhibition of complex formation using the higher excess (100-fold) of unlabeled oligonucleotide. IR1-B was not able to inhibit the DNA-protein complex. A marked reduction of the complex was found by the addition of IR1-C in a 50- or 100-fold excess. An inhibition of complex formation also was found for IR1-D; however, only the 100-fold excess resulted in a clearly visible reduction of the DNA-protein complex. Oligo-dimers with a mutation in the IR1 sequence (mA, mB, mC, and mD) used in the 100-fold excess were not able to inhibit any DNA-protein complex formation (Fig. 6). These DNA binding properties support the interpretation of the already discussed transfection studies (Figs. 3–5) and confirm that IR1-C has the highest potential for interaction with FXR, followed by IR1-D. By contrast, IR1-A has a limited capacity, and IR-B has no capacity to interact with FXR.

To study the DNA-protein interaction within the context of intact cells, ChIP was performed. In a first step, cells were treated with either Me2SO (DMSO) or GW4064 for FXR activation. A robust induction of SHP and PXR mRNA by GW4064 was observed (Fig. 7).

**FIGURE 7. PXR is induced in mouse mhPKT cells after treatment with GW4064.** MhPKT cells were cultivated and treated with either Me2SO (DMSO, black bars) or GW4064 (1 μM, gray bars) for 24 h. Total RNA was isolated and analyzed by real-time PCR quantification. Values are normalized relative to cyclophilin and are expressed (means ± S.E., n = 4 experiments) relative to vehicle-treated cells. *, p = 0.01.
association of FXR with the Pxr gene can be detected. Therefore, cells were treated for 24 h with GW4064, fixed, sonicated, and used for immunoprecipitation with a FXR-specific or an unspecific Ig antibody. As shown in Fig. 8, binding of FXR was found for IR1-C and -D, confirming the results generated by mobility shift assay. Thus, functionality of these IR1 sites also is confirmed in the context of intact chromatin structures.

**DISCUSSION**

This study shows for the first time that the expression of PXR is regulated by bile acids by means of an FXR-mediated mechanism. This conclusion is based on the following results. Treatment of animals with the primary bile acids CA or the synthetic FXR ligand GW4064 induced PXR expression in wild type but not in FXR\(^{-/-}\) mice, establishing a direct effect of FXR on PXR. The molecular mechanism of PXR activation by FXR was revealed by identifying a region in the second intron of the mouse Pxr gene containing four potential IR1 binding sites and finding evidence in reporter gene, electromobility shift, and ChIP assays for functionality of two of these IR1 sites.

Bile acids are a major product of cholesterol metabolism. In addition to their physiological role in fat absorption, bile acids regulate a number of genes involved in bile acid synthesis, breakdown, and transport. However, when bile acids and their products, as for example the secondary bile acid lithocholic acid, accumulate, they can cause cholestasis, leading to liver damage and other pathological changes (40–43). A number of protective mechanisms are operative in liver cells to prevent this accumulation, but mechanism of bile acid elimination and detoxification are still incompletely understood.

**FIGURE 9.** Proposed model for the role of PXR in bile acid elimination and detoxification. High bile acid concentrations activate FXR. This leads to increased expression of SHP, thereby inhibiting CYP7A1 and sterol CYP8B1 and the de novo synthesis of bile acids. Activated FXR also increases the expression of PXR. PXR itself can be activated by bile acids precursors and toxic derivates such as lithocholic acid, resulting in the expression of cytochromes P450 and transporter genes, involved in the breakdown and elimination of bile acids. PXR also contributes to the repression of CYP7A1. The combination of these FXR-induced mechanisms leads to an efficient protection of the liver against bile acid-induced toxicity.
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Bile acids (44) and their precursors (15) are ligands of PXR leading to increased expression of PXR target genes. PXR regulates an entire program of genes involved in the detoxification and elimination of toxic substrates from the body (10). Among the genes that are regulated by PXR in the liver and intestine are those coding for CYPs of the 3a (35, 45–47) or 2b (48–50) subfamily, genes encoding phase II enzymes including members of the glutathione-S-transferase (51), sulfotransferase (52, 53), UDP-glucuronosyltransferase (54) and carboxylesterase families (55), and genes encoding proteins involved in transport such as multidrug resistance proteins Mdr1 (49, 56) and Mrp2 (57, 58) and the organic anion transport protein 1a4 (Oatp1a4 formerly Oatp2). Members of the OATP family account for Na⁺-independent bile acid transport into the hepatocyte and also remain candidates for bile acid efflux at the basolateral membrane since Oatp1a1 and -1a4 are able to operate as bidirectional exchangers (14, 57).

Enzymes of the CYP P450 family have been shown to metabolize bile acids to their hydroxylated and excretable metabolites. CYPs can also be induced by drugs such as rifampicin or phenobarbital (7, 29, 59). Indeed rifampicin and phenobarbital improve cholestasis by lowering toxic bile acids. CAR and PXR agonists stimulate hepatic bile acid and bilirubin detoxification and elimination pathways in mice (16) and man (60). Moreover, clinical studies suggest that rifampicin is an effective second line therapy for controlling pruritus in patients with chronic cholestatic liver disease (61), presumably by stimulating hydroxylation of bile acids (62). Thus, PXR targets contribute to bile acid detoxification and elimination. Here we report an additional mechanism by which bile acids regulate their own breakdown, namely the activation of PXR by the bile acid receptor FXR.

In the present study mice fed CA for 14 h or 3 days only showed a trend of increased Cyp3a11 expression that did not reach significance. However, long term challenge to bile acids led to a significant increase of PXR as well as of the PXR target genes Cyp3a11, Cpy2b10, and Mdr1 was observed. Therefore, the protective effect of PXR activation via FXR may develop only after a relatively long term exposure to high bile acid levels. In cholestasis exposure to high bile acids concentrations is prolonged, and the bile acid composition between primary and secondary products is altered. Accumulation of lithocholic acid would clearly result in PXR activation, thereby enhancing the breakdown of bile acids by inducing the CYP3A4 expression. This system would be further enhanced as PXR itself is induced by activated FXR.

In addition to being an important regulator of the catabolism of bile acids, PXR has also been implicated in the negative feedback regulation of bile acid synthesis. CYP7A1 is the rate-limiting enzyme in the conversion of cholesterol to bile acids. CYP7A1 expression is tightly regulated by FXR, as high bile acid-dependent activation of FXR leads to the induction of SHP, repressing CYP7A1 expression (19, 21). However, the loss of the nuclear receptor SHP impairs but does not eliminate negative feedback regulation of bile acid synthesis. Redundant pathways, based on the activation of PXR (10, 28) and the c-Jun terminal kinase, are proposed in this context (63, 64). For instance, pregnenolone-16α-carbonitrile (PCN)-activated PXR has been shown to repress the expression of the Cyp7a1 gene and to lead to a significant decrease in bile acid excretion (14, 29). Again, induction of PXR by FXR would feed forward this system.

In conclusion, our results strongly suggest that PXR is regulated by FXR. Bile acids activate the nuclear receptor FXR, which stops synthesis of bile acids and also leads to the transcriptional activation of PXR. FXR promotes the breakdown of bile acids and represses their de novo synthesis. The combination of these mechanisms leads to an efficient protection of the liver against bile acid induced toxicity (Fig. 9).

Acknowledgments—We thank Antonio Moschetta and Amy Liverman from the Mango laboratory, Takeshi Inagaki from the Kliever laboratory, and Carmela Gnerre from the Meyer laboratory for contributing to the in vivo studies. D. Jung specially thanks the Walter and Gertrud Siegenthaler foundation for support.

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