III. Bile acids and nuclear receptors

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Bile Acid Regulation of Hepatic Physiology III. Bile acids and nuclear receptors

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Chiang, John Y. L. Bile acid regulation of hepatic physiology. III. Bile acids and nuclear receptors. *Am J Physiol Gastrointest Liver Physiol* 284: G349–G356, 2003; 10.1152/ajpgi.00417.2002.—Bile acids are physiological detergents that facilitate excretion, absorption, and transport of fats and sterols in the intestine and liver. Recent studies reveal that bile acids also are signaling molecules that activate several nuclear receptors and regulate many physiological pathways and processes to maintain bile acid and cholesterol homeostasis. Mutations of the principal regulatory genes in bile acid biosynthetic pathways have recently been identified in human patients with hepatobiliary and cardiovascular diseases. Genetic manipulation of key regulatory genes and bile acid receptor genes in mice have been obtained. These advances have greatly improved our understanding of the molecular mechanisms underlying complex liver physiology but also raise many questions and controversies to be resolved. These developments will lead to early diagnosis and discovery of drugs for treatment of liver and cardiovascular diseases.

liver orphan receptor; farnesoid X receptor; cholesterol 7α-hydroxylase cytochrome *P*-450; cholesterol homeostasis; gene regulation

BILE ACIDS ARE THE END PRODUCTS of cholesterol catabolism. Bile acid synthesis generates bile flow from the liver to the intestinal tract and back to the liver. This process of enterohepatic circulation of bile is extremely efficient and plays important roles in liver function, metabolic regulation, and liver physiology. Bile acids are amphipathic molecules that function as powerful detergents to facilitate absorption of lipids and nutrients and excretion of cholesterol and toxic metabolites. When accumulated in high concentrations, hydrophobic bile acids damage cell membranes, impair liver function, and cause cholestasis and cirrhosis. The liver plays a central role in maintaining cholesterol homeostasis by balancing de novo cholesterol and bile acid synthesis, dietary cholesterol uptake, biliary cholesterol excretion, lipoprotein synthesis and secretion, and reverse cholesterol transport from peripheral tissues to the liver for catabolism to bile acids (Fig. 1). However, molecular mechanisms underlying this complex metabolic regulation are not completely understood. Cloning of the genes encoding cholesterol 7α-hydroxylase (cytochrome *P*-450 7A1; CYP7A1) and other key regulatory enzymes in the bile acid biosynthetic pathway has provided molecular tools for elucidation of regulatory mechanisms. Discovery of human mutations in bile acid biosynthetic genes in patients with liver and cardiovascular diseases has provided evidence that bile acid synthesis is linked to cholesterol metabolism and that a deficiency of bile acid synthesis leads to dyslipidemia, liver cirrhosis, gallstone disease, and cardiovascular diseases in humans. Recent studies have uncovered that bile acids are ligands of several nuclear hormone receptors involved in regulating bile acid synthesis, transport, and cholesterol metabolism. These recent developments have generated great interest in bile acid research and will lead to the early diagnosis of diseases and discovery of new therapeutic strategies for treating human disorders in bile acid and cholesterol metabolisms. This review will summarize the roles of bile acids and nuclear receptors in regulation of bile acid and cholesterol homeostasis.

BILE ACID SYNTHESIS AND REGULATION

Bile acid synthesis. In the liver, cholesterol is converted to bile acids by a pathway consisting of a cascade of 15 reactions (4). In the classic bile acid biosynthetic pathway (Fig. 1), CYP7A1, a microsomal cytochrome *P*-450, is the first and rate-limiting enzyme of the pathway to synthesize two primary bile acids, cholic acid (CA) and chenodeoxycholic acid (CDCA) in humans. Another microsomal cytochrome *P*-450, sterol 12α-hydroxylase (CYP8B1) is involved in the synthesis of CA and controls the ratio of CA to CDCA. After modifying the steroid ring, sterol 27-hydroxylase (CYP27A1), a mitochondrial cytochrome *P*-450, catalyzes the steroid side-chain oxidation and cleavage. In peripheral tissues, an alternative (or acidic) pathway is

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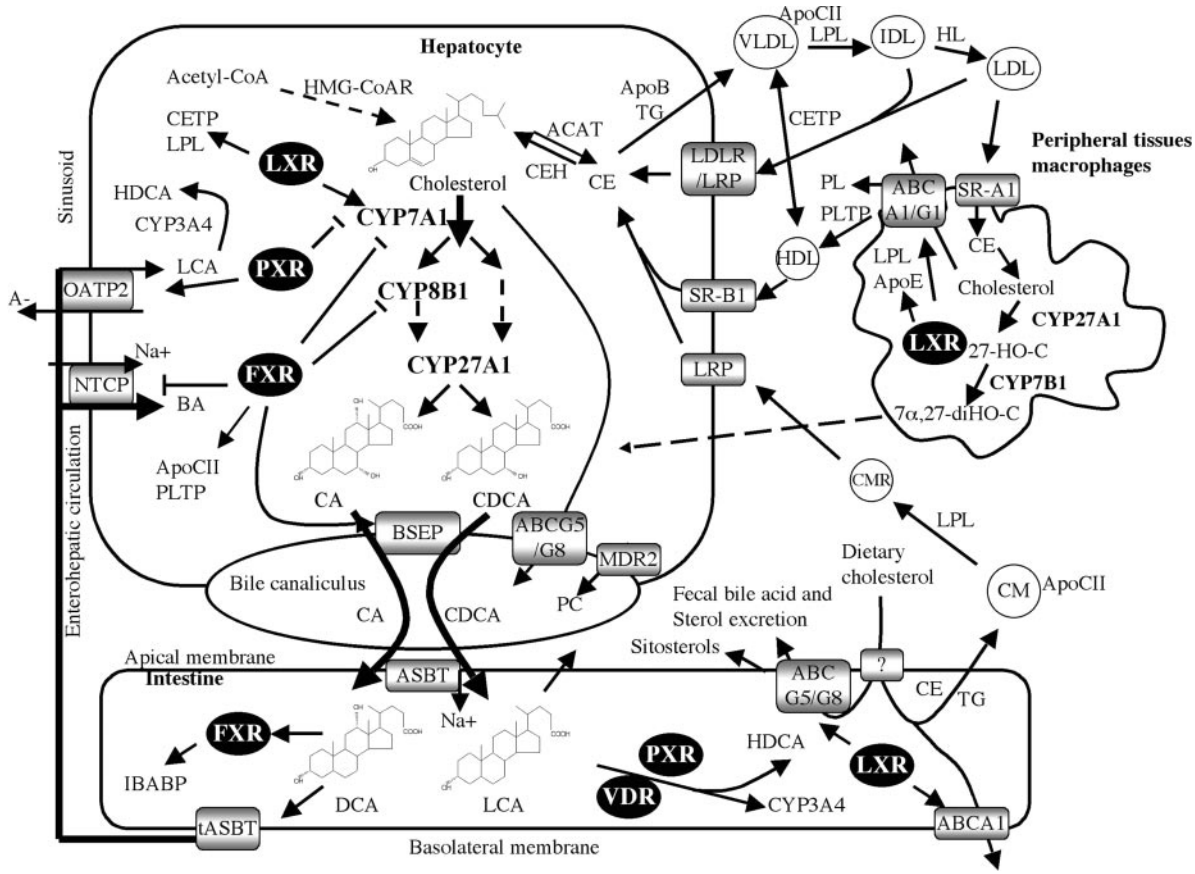

Fig. 1. Bile acid and cholesterol synthesis and transport and nuclear receptor regulation of bile acid and cholesterol homeostasis in liver, intestine, and peripheral tissues. Balance of input and output of cholesterol in the liver maintains cholesterol homeostasis. Four output mechanisms are *1*) conversion of cholesterol to bile acids, *2*) biliary cholesterol excretion, *3*) transport of cholesteryl esters (CE) as very low-density lipoprotein (VLDL) from liver to peripheral tissues (normal cholesterol transport), and *4*) synthesis of steroid hormones in steroidogenic tissues (not shown). Four input mechanisms include *1*) de novo cholesterol synthesis, *2*) LDL receptor-mediated uptake of cholesterol esters, *3*) dietary uptake of CE transported by chylomicron (CM) via LDL receptor-related protein (apoE receptor), and *4*) reverse cholesterol transport of HDL from peripheral tissues to hepatocytes by HDL receptor SR-B1. In hepatocytes, cholesterol 7α-hydroxylase cytochrome *P*-450 (CYP7A1), sterol 12α-hydroxylase (CYP8B1), and sterol 27-hydroxylase (CYP27A1) are key enzymes catalyzing the synthesis of two primary bile acids, cholic acid (CA) and chenodeoxycholic acid (CDCA). In peripheral tissues, CYP27A1 and CYP7B1 catalyze the hydroxylation of cholesterol. Bile acids are excreted to bile by bile salt export pump (BSEP). In the intestine, bile acids are reabsorbed via apical bile salt transporter (ASBT). CA and CDCA are converted to deoxycholic acid (DCA) and lithocholic acid (LCA), respectively. Most LCA is excreted into feces, whereas CDCA, DCA, and CA are transported by portal blood to hepatocytes to inhibit bile acid synthesis. A trace amount of LCA transported to the liver is conjugated with glucuronate and excreted into bile. LCA activates vitamin D_3 receptor (VDR) and pregnone X receptor (PXR) in intestine to induce CYP3A4, which hydroxylates LCA to hyodeoxcholate (HDCA). Key enzymes, transporters, and proteins regulated by farnesoid X receptor (FXR), liver orphan receptor-α (LXRα), PXR, and VDR in liver, intestine, and peripheral tissues are shown. Details are described in text. ACAT, acyl-coenzyme A cholesterol acetyltransferase; Apo, apolipoprotein; ABCA1, ABCG1, ABCG5, and ABCG8, ATP binding cassette transporters; BA, bile acid; CEH, cholesteryl ester hydrolase; CETP, cholesteryl ester transfer protein; CMR, CM remanet; CYP3A4, cytochrome *P*-450 3A4; CYP7A1, cholesterol 7α-hydroxylase; CYP7B1, oxysterol 7α-hydroxylase; 7α,27-diHO-C, 7α,27-dihydroxycholesterol; HDCA, hyodeoxycholic acid; HL, hepatic lipase; HMG-CoA reductase, 3-hydroxy-3-methylglutaryl coenzyme A reductase; 27-HO-C, 27-hydroxycholesterol; IBABP, ileum bile acid binding protein; LPL, lipoprotein lipase; LRP, LDL receptor-related protein; MDR2, multidrug-resistant protein 2; NTCP, Na^+ taurocholate cotransport peptide; OATP2, organic anion transport protein 2; PC, phosphatidylcholine; PL, phospholipid; PLTP, phospholipid transfer protein; SR, scavenger receptor; tASBT, truncated ASBT; TG, triglyceride.

initiated by CYP27A1 to convert cholesterol to 27-hydroxycholesterol and cholestenoic acid. These metabolites are further hydroxylated by oxysterol 7α-hydroxylase (CYP7B1). The acidic pathway may be considered as a reverse cholesterol transport process for removing excess oxidized cholesterol from the peripheral tissues to the liver for conversion to bile acids and thus may protect humans from developing atherosclerosis. Most bile acids are conjugated with taurine or glycine, excreted into bile and stored in the gallbladder as mixed micelles with phosphatidylcholine and cholesterol. After each meal, the gallbladder contracts

to secrete bile into the intestine. A fraction of CA and CDCA are converted to the secondary bile acids, deoxycholic acid (DCA) and lithocholic acid (LCA), respectively, by bacteria 7α-dehydroxylase in the colon. Most bile acids (95%) except LCA are quantitatively reabsorbed and transported back to the liver via portal blood circulation. In healthy humans ~0.2 to 0.5% LCA is reabsorbed, which is sulfated and amidated and rapidly secreted.

Bile acid feedback regulation. Early studies in animal models revealed that hydrophobic bile acids are more potent feedback inhibitors of bile acid synthesis than hydrophilic bile acids and that the hydrophobicity of bile regulates bile acid synthesis (4). The hydrophobicity of a bile acid depends on the number of hydroxyl groups, their positions, and stereochemical structure. Hydrophobic bile acids, such as CDCA, DCA, and LCA, are highly toxic; their concentrations in hepatocytes must be maintained at low levels. The structure, concentration, hydrophobicity, and bile acid pool size vary considerably among different species. Therefore, the rate of bile acid synthesis, the composition of bile, and its regulation differs in different species and individuals and is dependent on the genetic and environmental factors.

Regulation of CYP7A1 gene. The *CYP7A1* gene is predominantly regulated at the gene transcriptional level by cholesterol and bile acids (4). Promoter analyses have identified nucleotide sequences important for basal level transcription and regulation by bile acids, which we named the bile acid response elements (BARE) (7, 30). These elements are highly conserved among different species and contain hexameric repeats of AGGTCA sequence with 1-, 4-, or 5-nucleotide spacing. These direct-repeat sequences (DR1, DR4, and DR5) are binding sites for nuclear hormone receptors. Liver orphan receptor-α (LXRα; NR1H3), an oxysterol sensor, binds to the DR4 of the BARE-I in the mouse *CYP7A1* gene and stimulates *Cyp7a1* gene expression when fed a high cholesterol diet, whereas the *Lxr* null mice fail to stimulate *Cyp7a1* gene expression and accumulate excess cholesteryl esters in the livers (23). However, dietary cholesterol does not induce the human *CYP7A1* transgene in mice (1). This is apparently due to lack of an LXR response element in the human *CYP7A1* gene (3). This DR4 also binds an orphan receptor, chicken ovalbumin upstream transcription factor II (COUP-TFII), and stimulates *CYP7A1* gene expression.

The human α-fetoprotein transcription factor (FTF, or LRH; NR5A2) binds to the BARE-II in the *CYP7A1* gene (11, 17). FTF has to interact with LXRα to induce the *Cyp7a1* gene in mouse liver and is considered a competent factor for LXRα induction of the *Cyp7a1* and cholesteryl ester transfer protein (CETP) genes by cholesterol. Bile acid-activated FXR induces a negative nuclear receptor, small heterodimer partner (SHP; NR0B2), which then interacts with FTF and suppresses *CYP7A1* gene transcription (11, 17). Orphan receptor, hepatocyte nuclear factor-4α (HNF-4α; NR2A1) binds to the DR1 motif in the BARE-II and stimulates *CYP7A1* gene transcription. DR1 is also a binding site for peroxisome proliferator-activated receptor α (PPARα). However, PPARα does not bind to BARE-II but indirectly inhibits *CYP7A1* by reducing HNF-4α expression (2). A DR5 sequence in BARE-II binds retinoic acid receptor/retinoid X receptor, which stimulates rat *CYP7A1* gene transcription. Factors bound to BARE-I and BARE-II (COUP-TFII and HNF-4α, respectively) interact and synergistically stimulate *CYP7A1* transcription (31).

Regulation of the CYP8B1 gene. CYP8B1 regulates the ratio of CA to CDCA, which may determine the hydrophobicity of bile. Bile acids strongly inhibit CYP8B1 activity and mRNA expression in rats. The BARE identified in rat and human *CYP8B1* promoters have overlapping FTF and HNF-4α sites (8, 37). These two receptors may differentially regulate *CYP8B1* genes by competing for binding to the nucleotide sequences in the BAREs (36). SHP may interact with either FTF or HNF-4α to confer bile acid suppression of *CYP8B1* transcription.

In contrast to stimulation of *CYP7A1* gene expression, cholesterol feeding decreases CYP8B1 expression in the rat. The rat *CYP8B1* promoter contains several sterol response elements (SRE), which bind SRE binding proteins (SREBP) and stimulate *CYP8B1* expression (9). SREBP transcription factors regulate genes involved in cholesterol and fatty acid synthesis. LXRα stimulates SREBP-1c gene transcription to induce fatty acid and triglyceride synthesis.

BILE ACID-ACTIVATED NUCLEAR RECEPTORS

Nuclear receptors are ligand-activated transcription factors, which are known to regulate genes involved in liver development and differentiation. Many nuclear receptors have no identified ligands and are referred to as orphan receptors. Typical nuclear receptor ligands are small hydrophobic molecules that bind specifically to the ligand-binding domain of nuclear receptors at physiological concentrations. The unique structure of hydrophobic bile acids makes them excellent candidates as the endogenous ligands for orphan receptors.

FXR is a bile acid receptor. Analysis of orphan receptor expression patterns in enterohepatic tissues and ligand-binding assays identified bile acids as ligands that activate farnesoid X receptor (FXR; NR1H4) (19, 22, 34). Bile acid-activated FXR inhibits *CYP7A1* transcription without binding to the gene, indicating an indirect mechanism involving other liver-specific factors (5). These factors are FTF and SHP (11, 17). FXR also inhibits Na^+-dependent taurocholate cotransport peptide (NTCP), the principal bile acid transporter in the basolateral membrane of hepatocytes (Fig. 1). On the other hand, FXR markedly induces bile salt export pump (BSEP) expression in the canalicular membrane, an ATP-binding cassette (ABC) transporter and the principal bile acid efflux transporter in the liver. In enterocytes, FXR markedly induces the expression of ileum bile acid binding protein (IBABP). FXR induces phospholipid transport protein (PLTP) and apolipopro-

tein CII (ApoCII) is involved in reverse cholesterol transport and triglyceride metabolism (Fig. 1).

Pregnane X receptor is a promiscuous bile acid receptor. Two laboratories have reported that bile acids activate pregnane X receptor (PXR; NR1I2) (28, 35). PXR is a promiscuous xenobiotic receptor activated by structurally unrelated steroids, xenobiotics, and drugs in rodents. PXR induces expression of the CYP3A4 subfamily of the drug-metabolizing cytochrome *P*-450's expression in rat liver and intestine. PXR ligands, dexamethasone, and pregnenolone 16α-carbonitrile, strongly inhibit CYP7A1 expression in rat liver (6, 15). LCA and 3-keto-LCA bind to and activate PXR. These investigators hypothesize that PXR inhibits bile acid synthesis and stimulates CYP3A4 to detoxify LCA, and may be the secondary defense protecting the liver against the accumulation of highly toxic bile acids (28). This mechanism only occurs in rodents, not in humans.

Vitamin D_3 receptor (VDR; NR1I1) is a LCA receptor. VDR is activated by 1α,25-dihydroxyvitamin D_3 to regulate Ca^{2+} and phosphate homeostasis. Mangesldorf's laboratory (18) recently reported that VDR is a highly specific and effective LCA-activated receptor. Among bile acids tested, only LCA and 3-keto-LCA bind and activate VDR. LCA- or 1α,25-dihydroxyvitamin D_3-activated VDR induces the *CYP3A4* gene in the intestine (Fig. 1). These investigators suggest that vitamin D_3 may protect the colon against LCA-induced colorectal cancer in humans (18). It should be noted that most LCA is sulfated and conjugated with glucuronate and rapidly excreted. It is intriguing that a known colon cancer promoter, DCA, is present in large quantity in the colon and is not a VDR ligand.

FXR AND LXR REGULATE BILE ACID AND CHOLESTEROL HOMEOSTASIS

It is now well established that FXR and LXRα coordinately regulate bile acid synthesis and cholesterol homeostasis in liver, intestine, peripheral tissues, and macrophages (Fig. 1). When bile acid levels increase in hepatocytes, FXR inhibits *CYP7A1* and *CYP8B1* to reduce bile acid synthesis and inhibits NTCP to reduce bile acid absorption into hepatocytes. On the other hand, FXR induces BSEP to excrete bile acids into bile. Hence, FXR functions to maintain low bile acid concentrations in hepatocytes. In the intestine, FXR induces IBABP to facilitate bile acid absorption in the apical membrane and excretion in the basolateral membrane into the portal circulation. FXR regulates reverse cholesterol transport and triglyceride metabolism by inducing PLTP and ApoCII in hepatocytes. When cholesterol levels increase in hepatocytes, oxysterols activate LXRα to induce *Cyp7a1* to convert excess cholesterol to bile acids in mice. LXRα also induces CETP. In the intestine, LXRα induces ABCG5/G8 to efflux cholesterol and plant sterols (sitosterols) into the intestinal lumen. Mutations of ABCG5/G8 have been linked to sitosterolemia, a genetic disorder of massive accumulation of plant sterols. In peripheral tissues and macrophages, LXRα induces ABCA1/G1 to efflux cholesterol and phospholipid for synthesis of HDL. LXRα also induces lipoprotein lipase and ApoE in macrophages. Mutations of ABCA1 have been linked to Tangier disease, a disorder of HDL synthesis. Thus LXRα plays a central role in regulating cholesterol transport from peripheral tissues and macrophages to the liver to be converted to bile acids.

MUTATIONS OF BILE ACID SYNTHETIC GENES CAUSE HUMAN LIVER AND CARDIOVASCULAR DISEASES

Because the conversion of cholesterol to bile acids is the predominant pathway for catabolism of cholesterol, it is thought that bile acid synthesis plays a central role in the regulation of whole body cholesterol homeostasis. It is conceivable that a deficiency of bile acid synthesis would disrupt cholesterol homeostasis and lead to hypercholesterolemia and cardiovascular diseases. Several inborn errors of bile acid biosynthesis have been reported in infants and children with phenotypes including neonatal hepatitis, progressive cholestasis, and biliary atresia. Defects in bile acid synthesis may decrease bile formation and cause malnutrition and accumulation of toxic abnormal metabolites. Mutations of the key regulatory genes, *CYP7A1*, *CYP27A1*, and *CYP7B1* and genetic knockout of these genes in mice have been reported recently. These mutations are rare, but observed phenotypes provide important insights into the molecular mechanisms of regulation of bile acid and cholesterol homeostasis.

CYP7A1 mutation. A family of *CYP7A1*-deficient patients has been identified recently. The proband has hypercholesterolemia, hypertriglyceridemia, premature gallstone disease, and peripheral vascular disease (24). These patients have a double deletion of thymidine in codon 1303, which resulted in a frameshift and early termination. Homozygous patients have markedly reduced bile acid synthesis and excretion and upregulation of the alternative bile acid synthesis pathway and are resistant to serum LDL cholesterol lowering by statin (3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor) treatment. These phenotypes are different from *Cyp7a1* null mice, which do not survive well and have vitamin deficiency phenotypes but do not have hypercholesterolemia (12).

CYP27A1 mutations. Mutations of the *CYP27A1* gene have been linked to a rare sterol storage disease, cerebrotendinous xanthomatosis (CTX) in humans (14). These patients do not have hypercholesterolemia but have a severe deficiency of bile acid synthesis, xanthoma in tendons, premature atherosclerosis, and progressive neurological disorders. However, although *Cyp27a1* null mice have a deficiency in bile acid synthesis and hypertriglyceridemia, they do not have hypercholesterolemia, xanthoma, or the neurological disorders found in CTX patients (25).

CYP7B1 mutation. A mutation in the *CYP7B1* gene causes severe neonatal cholestasis in a child (27). This patient has extremely high levels of 27-hydroxycholesterol and cholenoic acids, which are toxic and may inhibit bile acid excretion and cause severe cholestasis.

However, *Cyp7b1* knockout mice are perfectly normal (16).

CYP8B1 mutation. A *CYP8B1* mutation has not been reported in human patients. *Cyp8b1* knockout mice have increased CDCA, muricholic acids, bile acid synthesis and pool size, and cholesterol synthesis. Muricholic acids are predominant bile acids in mouse bile and are not FXR ligands. The highly hydrophilic bile in these mice may derepress the *Cyp7a1* gene. Lack of CA may decrease intestinal cholesterol absorption and stimulates de novo cholesterol synthesis in the liver of *Cyp8b1* null mice. One would predict that a *CYP8B1* mutation in humans might also have different phenotypes from the *Cyp8b1* knockout mice.

These genetic knockout mouse models do not share phenotypes found in human patients with mutations in the *CYP7A*1, *CYP8B1*, and *CYP27A1* genes. It is likely that differences in the synthesis and regulation of bile acid and cholesterol metabolism between mice and humans may explain the different phenotypes observed. It is necessary to be cautious in extrapolating the results from the mouse knockout models to human mutations. Identification of more human patients deficient of bile acid biosynthetic genes would confirm these phenotypes.

MOLECULAR MECHANISMS OF BILE ACID FEEDBACK INHIBITION OF GENE TRANSCRIPTION

Bile acid feedback regulation of bile acid synthesis has been studied for more than three decades. However, the molecular mechanism of bile acid feedback remains to be elucidated. Several mechanisms have been proposed for bile acid feedback inhibition of *CYP7A1* transcription. Chiang and coworkers (4, 7) proposed a receptor-mediated mechanism in 1994 on the basis of the finding that the BARE in the *CYP7A1* gene binds several nuclear receptors. This mechanism is supported by the finding that bile acid-activated FXR induces SHP, which inhibits the *CYP7A1* gene. This mechanism is referred to as a SHP-dependent mechanism in this review. Bile acids have been shown to activate the PKC signaling pathway and inflammatory cytokines (21, 29). These mechanisms are referred to as SHP-independent mechanisms. A receptor-mediated mechanism might inhibit bile acid synthesis when bile acid concentrations increase by enterohepatic circulation, whereas SHP-independent signaling mechanisms provide rapid responses to stress and injury, such as exposure to cholestatic bile acids and inflammatory cytokines.

SHP-dependent mechanism. Increase of Shp mRNA levels by the feeding of bile acids or FXR agonist GW4064 is inversely related to Cyp7a1 and Cyp8b1 mRNA levels in mouse livers (11, 17). CA feeding to *Fxr* null mice neither reduces Cyp7a1 and Cyp8b1 mRNA levels nor increases Shp and Bsep mRNA levels. These findings suggest a cascade mechanism that indirectly suppresses *Cyp7a1* and *Cyp8b1* gene transcription by bile acids through induction of Shp (26). Figure 2 (*top*) illustrates a SHP-dependent mechanism. CDCA-activated FXR binds to the *SHP* promoter and induces *SHP* transcription in the liver. SHP then interacts with FTF or HNF-4α to repress *CYP7A1* and *CYP8B1* transcription (2). It should be noted that SHP inhibits its own expression by interacting with FTF. Therefore, SHP expression has to be tightly regulated by both positive and negative factors. Only pharmacological doses of CDCA or a potent FXR agonist could significantly induce its transcription. This tight regulation is necessary, because SHP is a nonspecific receptor that inhibits many nuclear receptors. This raises an intriguing question as to how a SHP-dependent pathway specifically inhibits bile acid synthetic genes. Two laboratories recently reported a genetic knockout of the *Shp* gene in mice (13, 33). The *Shp* null mice appear normal except for mild defects in bile acid and cholesterol homeostasis. A potent FXR agonist, GW4064, fails to inhibit *Cyp7a1* expression in *Shp* null mice. This is consistent with the SHP-dependent mechanism. Surprisingly, CA feeding still inhibits *Cyp7a1* expression. Redundant mechanisms must exist to inhibit bile acid synthesis in *Shp* null mice. It has been reported that SHP mRNA expression levels are not induced in rats fed CDCA (36). It is likely that *SHP* gene expression is regulated by many factors that may be different in different species.

SHP-independent mechanisms. Figure 2 also illustrates several SHP-independent mechanisms for bile acid inhibition of gene transcription. Stravitz et al. (29) first proposed that bile acids activated the PKC signaling pathway, leading to inhibition of the *CYP7A1* gene by activation and phosphorylation of c-Jun NH_2-terminal kinases 1 and 2 (JNK1/2). JNK phosphorylates c-Jun to form a repressive complex with an unknown factor. Davis and colleagues (21) reported that bile acids induced the inflammatory cytokines TNF-α and IL-1 in Kupffer cells (hepatic macrophages), which then inhibited the *CYP7A1* expression in hepatocytes. Crestani and colleagues (10) reported that bile acid-induced cytokines activated a MAPK signaling pathway, leading to activation of JNK1/2 and reduction of HNF-4α transactivation activity. The MAPK signaling pathway would allow a rapid response to sudden increases in bile acids during inflammation and cholestasis by inhibiting bile acid synthesis. Involvement of the JNK pathway in bile acid inhibition of the *Cyp7a1* gene has been demonstrated in *Shp* null mice (33). Bile acids may inhibit *HNF-4α* gene transcription and *HNF-4α* binding to DNA (36, 37). Bile acid may induce FTF, which competes with *HNF-4α* and suppresses *CYP7A1* and *CYP8B1* gene transcription (36). LCA-activated PXR or VDR inhibits *CYP7A1* gene expression; however, the mechanism by which these two receptors repress *CYP7A1* is unknown at present. PXR activation by bile acids may contribute to bile acid repression of the *Cyp7a1* gene in *Shp* −/− mice. This result suggests that the PXR pathway is independent of SHP (33). Details of these mechanisms are subject to further study.

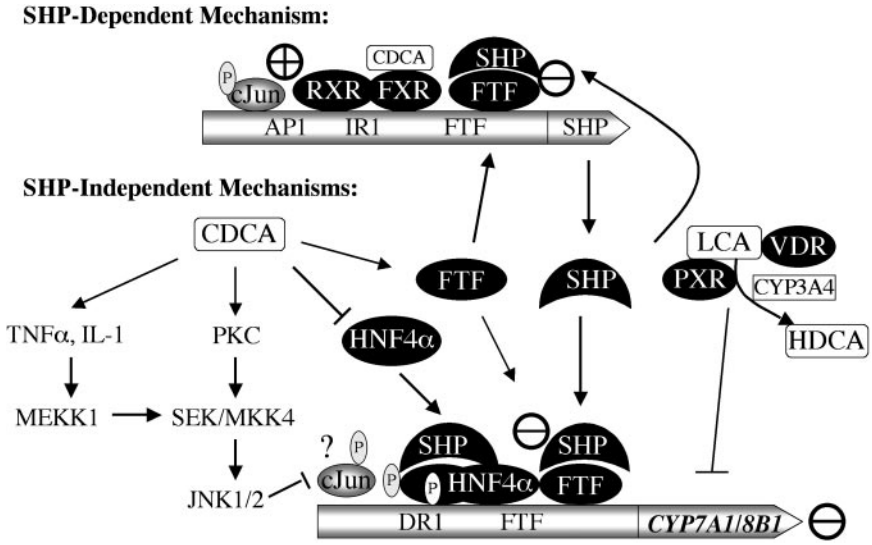

Fig. 2. Small heterodimer partner (SHP)-dependent and SHP-independent mechanisms of bile acid feedback regulation of *CYP7A1* and *CYP8B1* gene transcription. In the SHP-dependent mechanism, bile acid feedback inhibits *CYP7A1* and *CYP8B1* gene transcription by an indirect mechanism. CDCA induces FXR, which activates negative receptor SHP. SHP subsequently interacts with either α-fetoprotein transcription factor (FTF) or hepatocyte nuclear factor-4α (HNF-4α) and suppresses the *CYP7A1* and *CYP8B1* genes. It should be noted that SHP expression is induced by FTF; thus SHP may inhibit its own expression by interacting with FTF. Several SHP-independent mechanisms have been reported. Bile acids induce protein kinase C to activate stress-activated protein kinase kinase (SEK), leading to activation of c-Jun NH_2-terminal kinase (JNK)1/2. Bile acids induce inflammatory cytokines, TNF-α and IL-1 in Kupffer cells (hepatic macrophages), which activate MAPK signaling pathway, including MAPK kinase kinase-1 (MEKK1), MAPK kinase-4 (MKK4), and JNK1/2. JNK phosphorylates c-Jun and inhibits gene transcription directly or through inactivation of HNF-4α; CDCA may induce FTF, which directly inhibits the gene by interfering with HNF-4α activation of the *CYP7A1* and *CYP8B1* genes; LCA activates VDR and PXR and inhibits *CYP7A1* gene transcription. DR1, direct repeat with 1 base spacing; IR1, inverted repeat with 1 base spacing; P, phosphate.

SUMMARY AND PERSPECTIVES

Basic research into the molecular biology of bile acid synthesis in the past decade has led to recent identification of bile acid receptors and mutations of key bile acid biosynthetic genes in human patients with liver and cardiovascular diseases. These studies have advanced our understanding of liver metabolism and physiology. These exciting advances in bile acid research have also raised many unanswered questions to be addressed. The *Shp* knockout results do not provide unequivocal support for a SHP-dependent mechanism of bile acid feedback. Other questions remaining are the specificity of SHP as a bile acid regulator of gene transcription, the identity of the endogenous metabolites as physiological ligands of bile acid receptors, and the roles of these nuclear receptors in the regulation of lipid metabolism under normal physiological conditions. The SHP-independent mechanisms of bile acid feedback are highly feasible, but details of the complex mechanisms remain to be elucidated. Identification of more human patients with mutations in the key regulatory genes in bile acid synthesis and transport would confirm the phenotypes of deficiency.

These discoveries have provided new therapeutic targets for developing drugs for treating human metabolic diseases. Transgenic expression of human CYP7A1 in inbred mice susceptible to diet-induced atherosclerosis has been shown to reduce serum LDL cholesterol and prevent atherosclerosis (20). The overexpression of the human CYP7A1 activity and blocking of the BARE may have potential as antiatherosclerosis therapies in the future. Nuclear receptors are ideal targets of drug screening for agonists and antagonists. Guggulsterone, a natural product that lowers cholesterol, has been shown to be an FXR antagonist; however, its effect on CYP7A1, CYP8B1, and other FXR target genes has not been studied (32). It is puzzling that the hyperlipidemic phenotypes observed in *Fxr* null mice are in contrast to the hypocholesterolemic effect of guggulsterone. FXR agonists may be useful for protection from cholestatic liver diseases. LXRα agonists may reduce hypercholesterolemia, but there is a concern that they may cause hypertriglyceridemia. Many pharmaceutical companies are actively screening for lead compounds targeted to nuclear receptors involved in lipid metabolism and to the *CYP7A1* gene for treating human liver and cardiovascular diseases. Much insight on regulation of bile acid synthesis has been obtained, from animal physiology study in the 1960s to molecular biology research in the past decade. It is anticipated that more exciting results from bile acid research will be forthcoming.

For wisdom and guidance in researching in bile acid synthesis in the past decade, the author is deeply in debt to Alan Hofmann.

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