ENDOCRINE REVIEWS

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Endocr. Rev. 2002 23: 443-463, doi: 10.1210/er.2000-0035

0163-769X/02/$20.00/0
Printed in U.S.A.

Endocrine Reviews 23(4):443–463

doi: 10.1210/er.2000-0035

Bile Acid Regulation of Gene Expression: Roles of Nuclear Hormone Receptors

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Bile acids derived from cholesterol and oxysterols derived from cholesterol and bile acid synthesis pathways are signaling molecules that regulate cholesterol homeostasis in mammals. Many nuclear receptors play pivotal roles in the regulation of bile acid and cholesterol metabolism. Bile acids activate the farnesoid X receptor (FXR) to inhibit transcription of the gene for cholesterol 7α-hydroxylase, and stimulate excretion and transport of bile acids. Therefore, FXR is a bile acid sensor that protects liver from accumulation of toxic bile acids and xenobiotics. Oxysterols activate the liver orphan receptors (LXR) to induce cholesterol 7α-hydroxylase and ATP-binding cassette family of transporters and thus promote reverse cholesterol transport from the peripheral tissues to the liver for degradation to bile acids. LXR also induces the sterol response element binding protein-1c that regulates lipogenesis. Therefore, FXR and LXR play critical roles in coordinate control of bile acid, cholesterol, and triglyceride metabolism to maintain lipid homeostasis. Nuclear receptors and bile acid/oxysterol-regulated genes are potential targets for developing drug therapies for lowering serum cholesterol and triglycerides and treating cardiovascular and liver diseases. (*Endocrine Reviews* 23: 443–463, 2002)

Abbreviations: ABCA1, ATP-binding cassette protein A1; ACAT, acyl-CoA-cholesterol acyltransferase; apo, apolipoprotein; ASBT, apical sodium-dependent bile acid transporter; BARE, bile acid response element; BSEP, bile salt export pump; CA, cholic acid; CAR, constitutive androgen receptor; CDCA, chenodeoxycholic acid; CETP, cholesterol ester transfer protein; CM, chylomicron; CoA, coenzyme A; CPF, CYP7A1 promoter factor; CTX, cerebrotendinous xanthomatosis; CYP, cytochrome P450; CYP7A1, cholesterol 7α-hydroxylase; CYP7B1, oxysterol 7α-hydroxylase; CYP8B1, sterol 12α-hydroxylase; CYP27A1, sterol 27-hydroxylase; DCA, deoxycholic acid; DR, direct repeat; FTF, α-fetoprotein transcription factor; Ftz-F1, Fushi-tarazu factor 1; FXR, farnesoid X receptor; hB1F, hepatitis B virus enhancer 1 factor; HDL, high density lipoprotein; hFTF, human FTF; HMG-CoA, 3-hydroxy-3-methylglutaryl CoA; HNF4α, hepatocyte nuclear factor 4α; 3β-HSD, 3β-hydroxysteroid dehydrogenase; IBABP, ileum bile acid binding protein; IDL, intermediate-density lipoprotein; IR, inverted repeat; JNK, Jun N-terminal kinase; LCA, lithocholic acid; LDL, low density lipoprotein; LPL, lipoprotein lipase; LRH, mouse liver-related homolog; LXR, liver X receptor; MDR1, multidrug-resistant protein 1; NR1, nuclear receptor 1; MODY, maturity onset diabetes of the young; MRP3, multidrug-resistant protein-3; NTCP, sodium taurocholate cotransport peptide; OATP2, organic anion transport peptide 2; PCN, pregnenolone 16α-carbonitrile; PLTP, phospholipid transfer protein; PPAR, peroxisome proliferator activated receptor; PXR, pregnane X receptor; RAR, retinoic acid receptor; RXR, retinoid X receptor; SF-1, steroidogenic factor 1; SHP, small heterodimer partner; SR-B1, scavenger receptor subclass B1; SREBP, sterol response element binding protein; tASBT, terminal apical sodium-dependent bile acid transporter; VLDL, very low density lipoprotein.

I. Introduction

CONVERSION OF CHOLESTEROL to bile acids in the liver and biliary excretion of cholesterol for eventual disposal in stool are two major routes for removing excess cholesterol from the body. Recent studies have shown that bile acids not only serve as the physiological detergents that facilitate the absorption, transport, and distribution of lipid-soluble vitamins and dietary fats, but also are the signaling molecules that activate nuclear receptors and regulate bile acid and cholesterol metabolism. In addition, bile acids induce the cytochrome P450 3A (CYP3A) family of cytochrome P450 enzymes that detoxify bile acids, drugs, and xenobiotics in the liver and intestine, and also induce hepatocyte apoptosis. Bile acids are synthesized in the liver, excreted into the bile, reabsorbed in the ileum, and transported back to the liver via portal circulation to inhibit bile acid synthesis by suppressing the gene encoding the rate-limiting enzyme, cholesterol 7α-hydroxylase (CYP7A1) (1). The mechanisms of bile acid feedback regulation have been studied in animal and tissue culture models for more than three decades. Re-

cent studies suggest that bile acids are able to activate a bile acid receptor, farnesoid X receptor (FXR), which regulates the target genes in bile acid synthesis, transport, and cholesterol metabolism (2–6). Oxysterols are derived from cholesterol and bile acid biosynthetic pathways and are potent ligands that activate oxysterol receptor, liver X receptor (LXR), which induces genes involved in reverse cholesterol transport (7–9). FXR and LXR may coordinately regulate bile acid synthesis and cholesterol homeostasis (10–13). This review will focus on the molecular mechanisms of nuclear receptor regulation of bile acid and cholesterol homeostasis. Diseases caused by bile acid synthesis defects and the potential drug therapies targeted to nuclear receptors for lowering serum cholesterol levels will also be discussed.

II. Bile Acid Synthesis and Regulation

A. Bile acid biosynthetic pathways

The conversion of cholesterol to bile acids occurs exclusively in hepatocytes by a cascade of 12 reactions catalyzed by enzymes located in the endoplasmic reticulum, mitochondria, cytosol, and peroxisomes. Detailed descriptions of the reactions and enzymes involved in bile acid biosynthetic pathways can be found in recent reviews (1, 14–17). Figure 1 shows several of the intermediates and important regulatory enzymes in two major bile acid biosynthetic pathways. The main bile acid biosynthetic (classic or neutral) pathway is initiated by CYP7A1, which is only expressed in the liver, whereas the alternative (or acidic) pathway is initiated by sterol 27-hydroxylase (CYP27A1), which is expressed in many tissues. In the classic pathway, modifications of the steroid nucleus, including hydroxylation at 7α- and 12α-positions, epimerization of the 3β-hydroxyl group, and saturation of the steroid nucleus, precede the oxidative cleavage of a three-carbon side chain. In the alternative pathway, oxidative cleavage of the side chain precedes the modifications of the steroid nucleus. Cholic acid (CA) and chenodeoxycholic acid (CDCA) are two major primary bile acids found in human bile.

1. The classic or neutral pathway. The classic pathway is also known as the neutral pathway because it was identified first, and most intermediates in the pathway are neutral sterols (18). In humans, this pathway produces CA and CDCA in roughly equal amounts. CYP7A1, a microsomal cytochrome P450 isozyme, catalyzes the first and rate-limiting step of the pathway (19). Next, microsomal 3β-hydroxy-C27-steroid dehydrogenase/isomerase (3β-HSD) converts 7α-hydroxycholesterol to 7α-hydroxy-4-cholestene-3-one, the common precursor for both CA and CDCA. Microsomal sterol 12α-hydroxylase (CYP8B1) converts 7α-hydroxy-4-cholestene-3-one to 7α,12α-dihydroxy-4-cholesten-3-one (1, 18). Subsequently, Δ^4-3-oxosteroid-5β–reductase and 3α-hydroxysteroid dehydrogenase (HSD) convert these intermediates to 5β-cholestane-3α, 7α-diol for synthesis of CDCA, and 5β-cholestane-3α, 7α,12α triol for CA. The steroid side chain of these diols and triols is subsequently converted to a carboxyl group by mitochondrial CYP27A1 and leads to the synthesis of CDCA and CA, respectively (20). These two primary bile acids are then conjugated with taurine or glycine before excretion into bile. Under physiological pH, bile acids are present as sodium salts, referred to as bile salts. The term "bile acids" will be used throughout this article.

2. The alternative or acidic pathway. The alternative pathway was originally suggested by the identification of many acidic intermediates, which were not intermediates of the classic pathway (21, 22). The alternative pathway produces mainly CDCA (23, 24). In this pathway, CYP27A1 converts cholesterol to both 27-hydroxycholesterol and 3β-hydroxy-5-cholestenoic acid (25). Oxysterol 7α-hydroxylase (CYP7B1) then converts these two intermediates to 7α,27-dihydroxycholesterol and 3β,7α-dihydroxy-5-cholestenoic acid, respectively. It is believed that the same enzymes of the classic pathway catalyze subsequent modifications of the sterol nucleus (26). Recent studies suggest that the acidic pathway also produces CA (27–29).

The relative contribution of the acidic pathway to overall bile acid synthesis is not certain. Metabolites of the acidic pathway are accumulated in patients with chronic liver diseases and are an indication of a larger contribution of this pathway to bile acid synthesis (21). The acidic pathway may contribute as much as 50% of total bile acid synthesis in primary cultures of rat and human hepatocytes (30). However, the alternative pathway contributes only less than 18% of total bile acid synthesis in humans (31). The neutral pathway is highly regulated and is stimulated by bile fistula or by feeding cholestyramine, a bile acid-binding resin, whereas the acidic pathway is not induced as much (32–34). In *Cyp7a1*$-/-$ mice, bile acid synthesis is markedly reduced and the acidic pathway may be activated after weaning to provide 7α-hydroxylated bile acids (35, 36). In contrast, bile acid synthesis, pool size, and composition are not altered in *Cyp7b1*$-/-$ mice (37), and *Cyp7a1* expression is increased to maintain bile acid homeostasis. These genetic knockout experiments support the suggestion that the neutral pathway involving CYP7A1 is the major regulated pathway, whereas the acidic pathway involving CYP7B1 is a constitutive pathway (37).

B. Regulation of bile acid synthesis and cholesterol homeostasis

The rate of bile acid synthesis parallels the activity of CYP7A1, which is the only rate-limiting enzyme of the bile acid biosynthetic pathway (19). Interruption of enterohepatic circulation of bile acids by biliary diversion or treatment with bile acid sequestrants increases the rate of bile acid synthesis and the activity of CYP7A1 by about 3- to 4-fold. Intraduodenal infusion of bile acids inhibits the rate of bile acid synthesis to the normal level (19). Enterohepatic circulation of bile acids is the most important physiological mechanism for controlling the overall rate of bile acid biosynthesis (38).

1. Bile acid feedback regulation of bile acid synthesis. Bile acids excreted from the liver are reabsorbed in the intestine and transported back to the liver by a process called enterohepatic circulation of bile (39, 40). Conjugated bile acids synthesized in the liver are excreted into the bile canaliculi and stored in the gallbladder. After each meal, gallbladder contraction releases bile acids into the intestine for digestion of fats. Portions of CA and CDCA are converted to the secondary bile acids, deoxy-

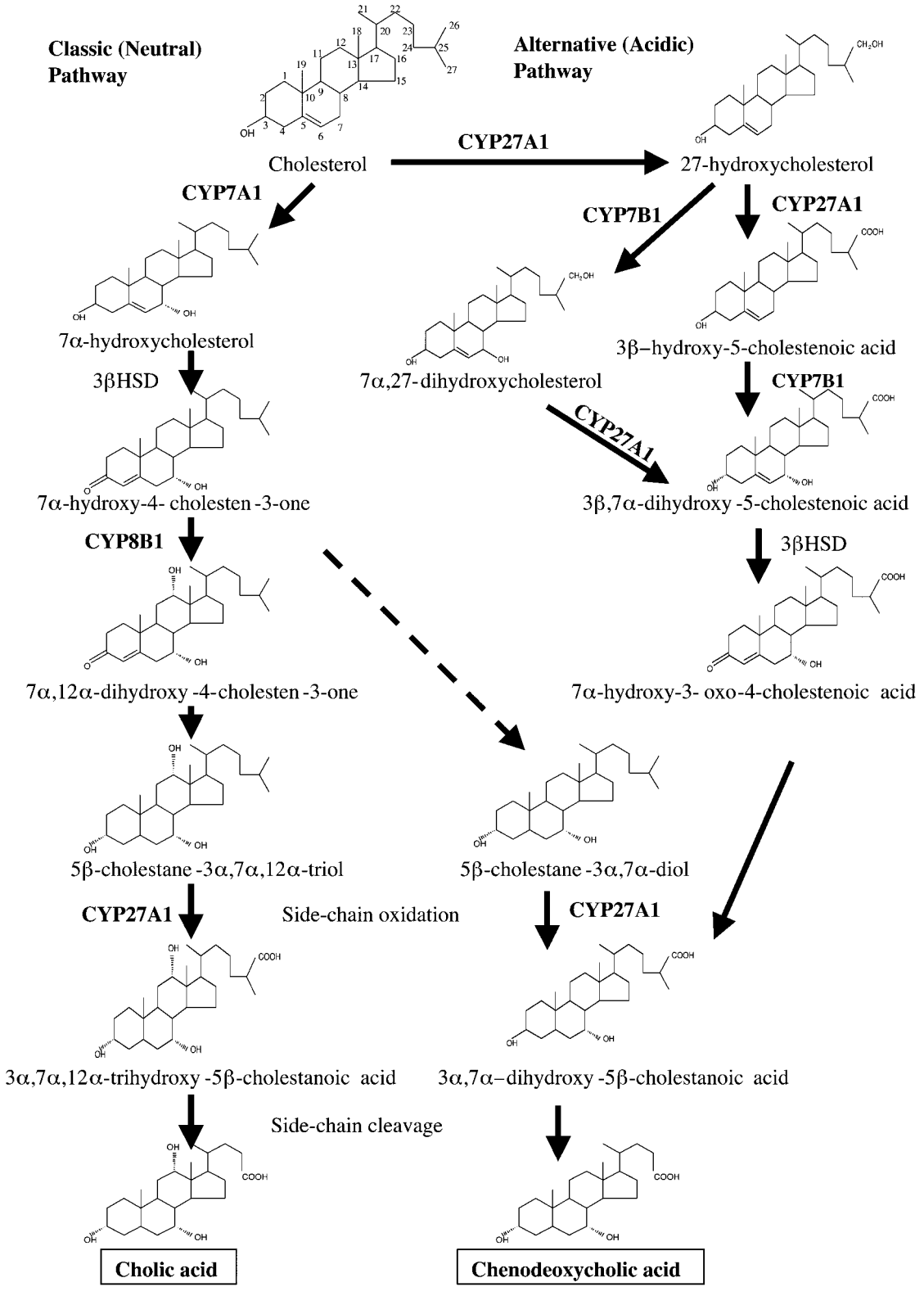

FIG. 1. Bile acid biosynthetic pathways in the liver. Two major bile acid biosynthetic pathways are shown. Only major regulatory enzymes, CYP7A1, CYP8B1, CYP27A1, CYP7B1, and 3β-HSD, and their substrates and products are shown.

cholic acid (DCA) and lithocholic acid (LCA), respectively, by 7α-dehydroxylase in the bacterial flora. These bile acids, with the exception of LCA, are efficiently reabsorbed in the ileum (41) and transported back to hepatocytes via portal venous circulation (42–44). Bile acids bind to hepatic bile acid-binding proteins and are transported to canalicular membrane for se-

cretion into bile (45). This process is repeated several times after each meal and reabsorbs about 95% of bile acids in humans. The remaining 5% lost in feces is replenished by *de novo* bile acid synthesis. The details of bile acid transport systems in hepatocytes and intestine can be found in recent reviews (40, 46–49).

2. Cholesterol homeostasis in the liver. Figure 2 illustrates the central role that the liver plays in maintaining cholesterol homeostasis. Major pathways for input and output of cholesterol are shown. Four major cholesterol input mechanisms in the liver are 1) uptake serum cholesterol esters by a low-density lipoprotein (LDL) receptor-mediated endocytosis; 2) reverse cholesterol transport from peripheral tissues to the liver by the selective uptake of high-density lipoprotein (HDL) by the scavenger receptor subtype B1 (SR-B1) (50, 51); 3) absorption of dietary cholesterol in intestine and transport to the liver as chylomicrons (CM) by LDL receptors-mediated mechanism; and 4) *de novo* synthesis of cholesterol from acetyl-coenzyme A (CoA).

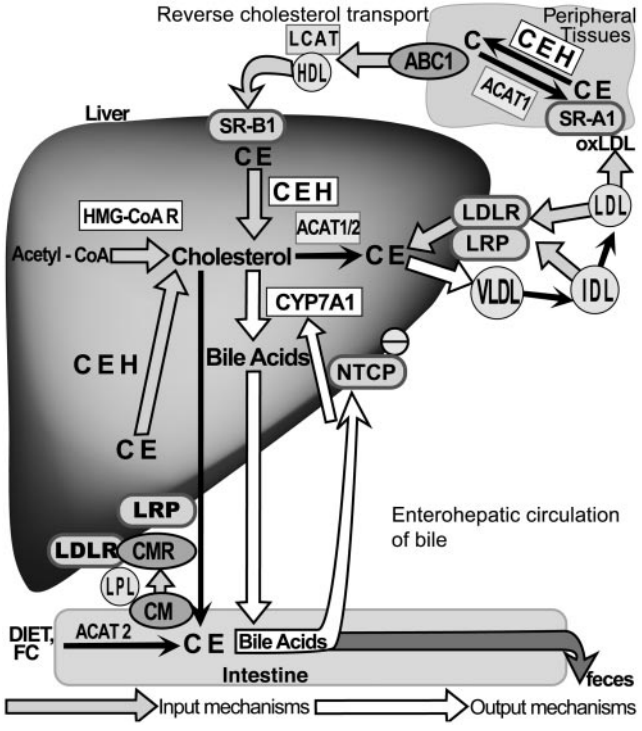

FIG. 2. Bile acid synthesis and cholesterol homeostasis. Liver synthesizes cholesterol from acetyl-CoA, and HMG-CoA reductase is the rate-limiting enzyme of the pathway. The cholesterol pool in the liver is contributed by four input mechanisms. Serum cholesterol esters (CE) carried by LDL and IDL are taken up into the liver by LDL receptor (LDLR) or LDL receptor-related protein (LRP)-mediated endocytosis. Oxidized LDL is taken up into peripheral tissues by scavenger receptors SR-A1 and CD34. CEs are hydrolyzed to free cholesterol by cholesterol ester hydrolase (CEH) in peripheral tissues. Excess cholesterol is effluxed from peripheral tissues by ABCA1 transporter to form HDL. Lecithin-cholesterol acyltransferase (LCAT) converts cholesterol to CE, which is selectively taken up into the liver by HDL receptor, SR-B1. Dietary free cholesterol (FC) is absorbed into intestine and is reesterified to CEs by acyl-CoA-cholesterol acyltransferase 2 (ACAT2). CEs, ApoB48, and triglycerides are assembled to form chylomicron (CM). Triglycerides in CM are hydrolyzed by lipoprotein lipase (LPL) in the capillary of the adipose and muscle tissues to form free fatty acids, and CM is converted to chylomicron remnants (CMR). CEs in CMRs are taken up into liver by LDL receptor and LRP (apoE receptors). Two mechanisms are involved in output of cholesterol. Of the daily cholesterol catabolized, about 50% is converted to bile acids, which facilitate the excretion of 40% cholesterol into bile. The canalicular transport system is illustrated in detail in Fig. 4. Bile acids, cholesterol, and phospholipids form mixed micelles in the gallbladder (not shown), and are secreted into the intestine after each meal. About 95% of the bile acids are reabsorbed in the ileum, excreted into portal circulation, and up-taken into hepatocytes by sodium-dependent taurocholate cotransport peptide (NTCP). CEs, triglycerides, and ApoB100 are assembled to form VLDL in the liver. VLDL excreted in the serum is subsequently converted to IDL, LDL, and oxidized LDL. They are taken up by scavenger receptors, SR-A1 or SR-B1, into macrophages for disposal or into liver or adrenal for synthesis of bile acids or sex hormones.

For cholesterol output, cholesterol esters are assembled into very low-density lipoproteins (VLDLs) and excreted into circulation. VLDLs are converted to intermediary density lipoprotein (IDL) and LDL, and taken up into liver and peripheral tissues by LDL receptors. Of the cholesterol catabolized, about 50% is converted to bile acids and 10% is used for synthesis of steroid hormones. The remaining 40% is excreted together with bile acids and phospholipids into bile for disposal in feces. Bile acids are reabsorbed by enterohepatic circulation of bile described above.

Hydrophobic bile acids are toxic if accumulated in large quantities in hepatocytes. Therefore, bile acid synthesis and transport must be tightly regulated. Cholesterol is important for synthesis of bile acids, biological membranes, and steroid hormones, and its homeostasis needs to be maintained in tissues. The liver plays a central role in maintaining bile acid and cholesterol homeostasis. Interruption of the enterohepatic circulation of bile acids leads to an increase in bile acid synthesis and a reduction of plasma LDL cholesterol concentration (52, 53). Increased input of cholesterol and decreased output of bile acids may cause hypercholesterolemia, atherosclerosis, cholestasis, and cholelithiasis in humans (38, 46, 54).

3. Oxysterol regulation of cholesterol homeostasis. Oxysterols are potent regulators of cholesterol synthesis and lipid metabolism (55). Oxysterols are derived from cholesterol, and the intermediates of the cholesterol and bile acid synthesis pathways by either enzymatic or nonenzymatic oxidations (7, 56). The most abundant oxysterols in human plasma are 27-hydroxycholesterol, 24(S)-hydroxycholesterol, and 7α-hydroxycholesterol, which are generated predominately by CYP27A1 in the lung (57), sterol 24-hydroxylase in the brain (58, 59), and CYP7A1 in the liver, respectively. Another abundant oxysterol, 25-hydroxycholesterol, is synthesized by microsomal sterol 25-hydroxylase, a noncytochrome P450 enzyme (60). Oxysterols regulate cholesterol and fatty acid syntheses through a mechanism involving sterol response element-binding proteins (SREBPs) (61–63). When oxysterol levels are low in cells, SREBP is translocated from endoplasmic reticulum to the Golgi where the N-terminal 58-kDa fragment is cleaved by sterol-sensitive proteases, which are regulated by SREBP cleavage-activating protein. The matured SREBP enters the nucleus and binds to the sterol response elements of LDL receptor, 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase, and other genes in cholesterol and fatty acid synthesis (63–67). In liver and peripheral tissues, CYP7B1 hydroxylates 27- or 25-hydroxycholesterol, whereas another 7α-hydroxylase, CYP39A1, hydroxylates 24-hydroxycholesterol (68). It has been reported that the recombinant human CYP7A1 can function as

an CYP7B1 of 20S-, 24-, 25-, or 27-hydroxycholesterol (69, 70). Conversion of monohydroxycholesterols to dihydroxycholesterols reduces the toxicity of monohydroxyl oxysterols. In atherosclerotic plaques, 27-hydroxycholesterol, 7-ketocholesterol, and 7β-, and 7α-hydroxycholesterol are the most abundant oxysterols, which cause foam cell formation from macrophages and lead to atherosclerosis in humans (56). Some oxysterols in peripheral tissues are excreted to circulation, transported to the liver, and converted to bile acids. This is a process analogous to reverse cholesterol transport and has been suggested as a defense against atherosclerosis in humans (71, 72).

C. Bile acid synthesis deficiency

1. Inborn errors of bile acid biosynthesis. Several inborn errors of bile acid synthesis have been described in infants and children with various clinical presentations including advanced liver diseases, neonatal hepatitis, progressive cholestasis, and biliary atresia (73–75). Primary defects in bile acid synthesis may result in decreased bile formation, malabsorption of fat-soluble vitamins and fats, and accumulation of toxic, abnormal steroid intermediates in the liver, which may interfere with bile acid transport processes and lead to cholestasis and cirrhosis (76). The primary defects in bile acid biosynthesis are the defects in modifications of the sterol nucleus, including 3β-HSD (77–79) and Δ^4-3-oxosteroid-5β-reductase deficiencies (80, 81), and the defects in side-chain oxidation due to CYP27A1 gene mutations (82). Defects in peroxisome biogenesis and enzymes in peroxisomal β-oxidation may manifest as the secondary defect in bile acid synthesis, including Zellweger syndrome and related infantile Refsum disease and neonatal adrenoleukodystrophy (83). A defect in *de novo* cholesterol synthesis also causes the secondary defect in bile acid synthesis, the Smith-Lemli-Opitz syndrome due to a defect in 7-dehydrocholesterol Δ^7-reductase (84, 85). Defects in bile acid transporters or 3β-HSD cause progressive familial intrahepatic cholestasis (40, 46, 74, 77).

2. Deficiency of 7α-hydroxylases. Mice deficient in Cyp7a1 activity have been obtained by knockout of the *Cyp7a1* gene (35). These mice display a complex phenotype including oily coats, hyperkeratosis, vision defects, and behavioral irregularities, which are consistent with malabsorption of vitamins E and D_3. Most *Cyp7a1*−/− mice died within 18 d; 40% of them died between d 1 and 4, and 45% died between d 11 and 18. Vitamin supplement to nursing mothers prevented deaths in the early period, and bile acid supplement prevented deaths in the later period. However, several 7α-hydroxylated bile acids were detected in the bile and stool of adult *Cyp7a1*−/− mice. This was explained by the expression of hepatic CYP7B1 after weaning and accounted for the synthesis of abnormal 7α-hydroxylated bile acids in these mice. The newborn *Cyp7a1*−/− mice developed neonatal cholestasis, which may be due to accumulation of monohydroxylated bile acids, 3α-hydroxy-5-cholenoate and 3α-hydroxy-5β-cholanoate, and 27-hydroxycholesterol (86). An inherited deficiency of CYP7A1 has not been described in the literature (see *Note Added in Proof*, no. 1).

The *CYP7A1* is a candidate gene for familial hypertriglyceridemia (87), gallstone disease (88–90), and hypercholesterolemia (52, 91). Several single-stranded conformation polymorphisms of the *CYP7A1* have been identified (92). Polymorphisms in the 5′-flanking region and coding region were reported (93, 94). Genetic linkage analysis indicates a significant linkage between *CYP7A1* and high plasma LDL-cholesterol concentrations (95). Two polymorphisms in the 5′-flanking region (−278C→A and −554C→T) may contribute to heritable variation in plasma LDL-cholesterol concentrations. The −278C alleles are associated with increased plasma LDL cholesterol concentration.

Setchell *et al.* (96) reported an inborn error of bile acid metabolism due to a defect of CYP7B1 in a child with severe neonatal cholestasis and cirrhosis. The absence of primary bile acid conjugates and accumulation of 3β-hydroxy-Δ^5-cholenoic acids, the products of the acidic pathway, in serum and urine indicated a defect in 7α-hydroxylation. In addition, the 27-hydroxycholesterol levels were 4500-fold higher than normal; however, there were no 7α-hydroxylated bile acids. Neither CYP7A1 nor CYP7B1 activities were detectable. Analysis of the *CYP7B1* identified a C-to-T mutation in exon 5, which converts Arg388 to a premature termination codon. The mechanism of liver injury in this patient is likely due to the accumulation of high levels of hepatotoxic monohydroxylated bile acids. These monohydroxylated bile acids may inhibit bile acid transport across canalicular membranes and reduce bile flow. There is no mutation in the coding exons of the *CYP7A1* gene in this patient. It is possible that CYP7A1 may not be expressed in the neonatal human liver, and bile acid synthesis in early human development may proceed mainly via the acidic pathway (96).

3. Deficiency of CYP27A1. Mutations of the *CYP27A1* have been found in patients with cerebrotendinous xanthomatosis (CTX), a rare autosomal recessive defect of cholesterol metabolism manifested by tendon xanthomatosis, progressive neurological dysfunction, accumulation of cholesterol in the tissues, premature atherosclerosis, osteoporosis, and cholesterol gallstones (18, 82, 97). The defect leads to excessive accumulation of 7α-hydroxycholesterol, 7α-hydroxy-4-cholesten 3-one, 5β-cholestane-3α, 7α, 12α-triol, cholesterol, and cholestanol. The synthesis of bile acids, particularly CDCA, is reduced and leads to up-regulation of CYP7A1 and the accumulation of both 7α-hydroxycholesterol and 7α-hydroxy-4-cholesten-3-one. The precursor 7α-hydroxy-4-cholesten-3-one is converted to cholestanol. Despite the normal circulating cholesterol levels in CTX patients, they develop xanthoma and premature atherosclerosis. This may be due to the reduced elimination of cholesterol from macrophages by CYP27A1. Mutations in the *CYP27A1* of CTX patients have been identified (82, 98). CDCA therapy has been used to prevent or reverse neurological symptoms associated with this disease. Despite the link of *CYP27A1* mutations to CTX, the etiology of this disease is still not known. Disruption of the *Cyp27a1* gene in mice markedly reduced bile acid synthesis and fecal bile acid excretion by 80% (99). However, *Cyp27a1*−/− mice do not accumulate cholestanol and do not exhibit the progressive neurological defects observed in human CTX patients. The *Cyp27a1*−/− mice have enlarged livers and kidneys and have increased triglyceride levels, fatty acid synthesis, cholesterol absorption, and cholesterol synthesis (100). SREBP expression in livers of

Cyp27a1−/− mice is elevated. Feeding CA reverses hepatomegaly and hypertriglyceridemia. It is concluded that *CYP27A1* plays an important role in triglyceride metabolism.

III. Nuclear Hormone Receptor Regulation of Bile Acid Synthesis

Bile acid synthesis is highly regulated by many factors, including diets, nutrients, bile acids, and hormones, mainly by regulating *CYP7A1* gene transcription (1). Many liver-specific transcription factors, mostly nuclear receptors, have been found to bind to and play important roles in regulating *CYP7A1* transcription (1, 101–108). Analysis of nucleotide sequences of two bile acid response elements (BAREs) identified in the rat *CYP7A1*, BARE-I (109) and BARE-II (110), revealed many AGGTCA-like repeating sequences. Chiang and co-workers (108) first reported that these hormone response elements in the BAREs bound retinoic acid receptor (RARα), chicken ovalbumin upstream promoter-transcription factor II (111, 112), hepatocyte nuclear factor 4α (HNF4α) (108, 112), and peroxisome proliferator-activated receptor α (PPARα) (113). They suggested that nuclear receptors might be involved in regulation of basal transcription as well as bile acid feedback regulation of the *CYP7A1* gene (1, 109, 110). Subsequently, the rat *CYP7A1* was identified as the first target gene of oxysterol receptor, LXR (9), and bile acid receptor, FXR (4–6). Further studies also identified pregnane X receptor (PXR), α-fetoprotein transcription factor (FTF), and small heterodimer partner (SHP) as the bile acid-regulated nuclear receptors. Nuclear receptors involved in bile acid and cholesterol metabolism are described below.

A. Structure and function of nuclear hormone receptors

Nuclear receptors have a typical modular structure (Fig. 3), which contains a highly conserved DNA-binding domain in the N-terminal region and a moderately conserved ligand-binding domain in the C-terminal region. Ligand-independent activation function-1 and ligand-dependent activation function-2 are located in the N-terminal and C-terminal regions, respectively. Two cysteine-coordinated Zn^{2+} finger motifs located in the DNA-binding domain are directly involved in DNA binding and dimerization. The E region is also involved in dimerization and coregulator interaction. Nuclear receptors bind to the consensus hormone response elements located in genes. Upon ligand binding, nuclear receptors undergo conformational changes to release corepressors and recruit coactivators to bind to the activation function-2 helix (114, 115).

Classic steroid hormone receptors, *i.e.*, glucocorticoid receptor, mineralocorticoid receptor, androgen receptor, and progesterone receptor, bind palindromic AGAACAN$_3$TGTTCT sequence (116), whereas estrogen receptors and nonsteroid hormone receptors bind to the AG(G/T)TCA-like repeats. The binding specificity of the dimeric receptor is determined by nucleotide spacing between two half-sites, which are arranged as a direct repeat (DR), inverted repeat (IR), or everted repeat (ER). LXR, FXR, PPARs, RARs, and PXR bind to their response elements as heterodimers with retinoid X receptor (RXR). The HNF4α homodimer binds to the DR1 sequence, whereas the NR5A2 family monomeric receptors, *i.e.*, human FTF (hFTF), CYP7A1 promoter factor (CPF), and mouse liver-related homolog (LRH) bind to an extended monomeric site, *i.e.*, TCAAAGGTCA. The SHP, a negative nuclear receptor, does not bind to DNA because it lacks a DNA-binding domain.

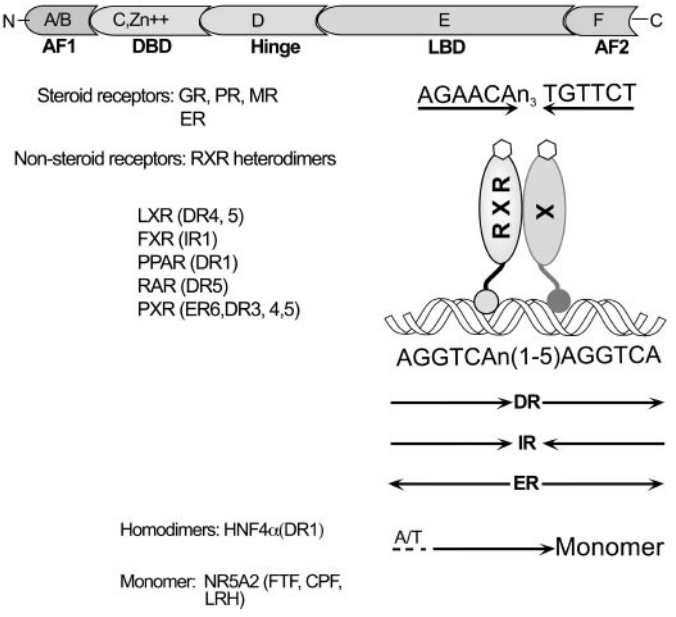

FIG. 3. The general structures of nuclear hormone receptors. *Upper figure* shows the domain structure of a nuclear receptor. It contains activation function domain 1 (AF1), DNA binding domain (DBD), hinge region (D), ligand binding domain (LBD), and activation function domain 2 (AF2). With the exception of estrogen receptors (ERs), all classical steroid hormones receptors, *i.e.*, glucocorticoid receptor (GR), mineralocorticoid receptor (MR), progesterone receptor (PR), androgen receptor (AR), bind to the palindromic repeating sequence, AGAACAN$_3$TGTTCT. ERs bind to a direct repeat (DR) of the AGGTCA motif. Nonsteroid receptors bind to the DR, inverted repeat (IR), or everted repeat (ER) spacing by one to five nucleotides. LXRs, FXR, PPARs, retinoic acid receptors (RARs), and PXR form heterodimers with RXRs and bind to DR, ER or IR sequences as indicated. HNF4α binds to DNA as homodimers. NR5A2 monomeric receptors, α-FTF, CPF, and LRH bind to an extended half-site preceded by a A/T-rich sequence.

B. Nuclear receptors involved in regulation of genes in bile acid synthesis

Nuclear receptors that have been identified to regulate genes in bile acid synthesis pathways and cholesterol metabolism are listed in Table 1 and described in detail below. These nuclear receptors are selectively expressed in the enterohepatic and peripheral tissues involved in bile acid synthesis, absorption, and transport, as well as cholesterol and lipoprotein transport (13). The NR1 family of nuclear receptors, including PXR, PPAR, LXR, and FXR, are activated by micromolar concentrations of bile acids, lipids, or steroids, which are 1000-fold higher than that for activation of the classic steroid hormone receptors, but are within the physiological or pathological concentrations.

1. Retinoic acid receptor (RAR) (NR1B1) and RXR (NR2B1). Retinoids play an important role in regulation of cell growth,

TABLE 1. Nuclear receptors and target genes involved in bile acid and cholesterol homeostasis

Receptor	Target genes	Functions	Ref.
1. RAR/RXR	↑ CYP7A1 (rat)	Bile acid synthesis	108
2. LXR	↑ CYP7A1 (rat, mouse)	Bile acid synthesis	9
	↑ SREBP-1c	Lipogenesis	135
	↑ ABCA1, ABCG1	Cholesterol efflux	139
	↑ CETP	Reverse cholesterol transport	150
	↑ ApoE	Lipoprotein metabolism	149
	↑ LPL	Lipoprotein metabolism	151
	↑ LXR	Cholesterol sensor	257
3. FXR	↓ CYP7A1, CYP8B1, CYP27A1	Bile acid synthesis	4–6
	↑ SHP	Nuclear receptor inhibitor	2, 3
	↑ BSEP	Liver bile acid transport	160
	↑ IBABP	Intestine bile acid binding	6, 159
	↑ PLTP	Reverse cholesterol transport	161
	↑ ApoCII	LPL activator	162
4. PPARα	↓ CYP7A1	Bile acid synthesis	113
	↑ CYP8B1 (rat)	Bile acid synthesis	172
	↑ LXR	Oxysterol sensor	174
5. HNF4α	↑ CYP7A1	Bile acid synthesis	108
	↑ CYP8B1	Bile acid synthesis	157
	↑ CYP27A1	Bile acid synthesis	Chen and Chiang[a]
	↑ FTF	Liver gene expression	202
6. FTF	↑ CYP7A1 (mouse)	Bile acid synthesis	2, 3
	↓ CYP7A1 (human)	Bile acid synthesis	155
	↑ CYP8B1 (rat)	Bile acid synthesis	258
	↓ CYP8B1 (rat)	Bile acid synthesis	184
	↑ SHP	Nuclear receptor inhibitor	205
	↑ HNF4α	Lipid metabolism	202
7. SHP	↓ CYP7A1	Bile acid synthesis	2, 3, 155
	↓ CYP8B1 (rat)	Bile acid synthesis	157, 258
	↓ CYP27A1	Bile acid synthesis	Chen and Chiang[a]
8. PXR	↓ CYP7A1	Bile acid synthesis	223
	↑ CYP3A	Sterol and bile acid hydroxylation	223, 225

[a] Chen and Chiang, unpublished results.

morphogenesis, differentiation, and homeostasis through activation of RARs (RARα, RARβ, RARγ) and RXRs (RXRα, RXRβ, RXRγ) (117, 118). RARs are activated by all-*trans*-retinoic acid and 9-*cis*-retinoic acid, whereas RXRs are activated by 9-*cis*-retinoic acid (119). RXR is a common heterodimer partner of a subgroup of nuclear receptors, including LXRs, FXR, PPARs, and PXR (116). RXR-selective ligands (rexinoids) activate RXR heterodimers, which can be further activated by respective ligands of its heterodimer partners.

RARα/RXRα has been shown to bind to a DR5 motif and stimulates rat *Cyp7a1* transcription (108, 120). Liver-specific disruption of *Rxr*α in mice alters the expression of *Cyp7a1, Apoal,* and *ApocIII* genes in liver (121). Cyp7a1 mRNA levels increase more than 8-fold in *Rxr*α−/− compared with wild-type mice. This suggests that *Cyp7a1* expression in the liver is under negative control mediated predominately by RXRα and its partners FXR and PPARα. These two nuclear receptors negatively regulate *CYP7A1* transcription. When fed a diet high in cholesterol, Cyp7a1 mRNA expression levels increase less than 2-fold in *Rxr*α−/− mice, much less than the 4- to 5-fold increase in wild-type mice. This implies that the inhibitory effect, presumably by FXR and PPARα, must dominate over the stimulatory effect by LXRα (122). This study reveals that RXRα is involved in diverse physiological pathways regulating cholesterol, bile acids, and fatty acids, as well as steroid metabolism and homeostasis.

2. LXR (NR1H3). NR1H3 subfamily receptors are activated by oxysterols (8, 123, 124). LXR has two isoforms, LXRα (or RLD-1) (123, 125) and LXRβ (UR, NER, RIP15, and OR-1) (126–129). LXRα is expressed in liver, spleen, adipose tissue, lung, and pituitary, whereas LXRβ is expressed ubiquitously. Many oxysterols have been identified as the ligands of LXRα (130). Among naturally occurring oxysterols, 22 (R)-hydroxycholesterol, 24 (S)-hydroxycholesterol, and 24 (S), 25-epoxycholesterol are the most potent LXR ligands. However, the physiological relevance of these oxysterols as the LXR ligands is not certain. The most abundant oxysterol in circulation, 27-hydroxycholesterol, has been shown to be a LXR ligand and may be the more relevant natural LXR ligand (131). In addition, 6α-hydroxy bile acid analogs and cholestenoic acid have been identified as the selective ligands of LXRα (132, 133).

LXR can act as either a positive or a negative regulator by binding different metabolites of the mevalonate pathway (134). LXR binds to a DR4 and stimulates rat *CYP7A1* transcription (9). In *Lxr*α−/− mice, the Cyp7a1 mRNA level is expressed normally in the liver, but is not stimulated by a high-cholesterol diet as in the wild-type mice (122), which leads to massive accumulation of cholesterol in the liver. *Lxr*β apparently is unable to compensate for *Lxr*α deficiency in *Lxr*α−/− mice. It was concluded that LXR might function as a cholesterol sensor, which stimulates *Cyp7a1* expression to convert excess cholesterol to bile acids in response to high cholesterol (8, 9). However, LXRα has much less effect on hamster and human *CYP7A1*, which lacks a DR4 motif (109). Therefore, the rat and mouse are unique in that they have the ability to efficiently convert excess

cholesterol to bile acids by LXRα-mediated stimulation of *Cyp7a1* transcription. The role of LXR in regulation of CYP7A1 in humans remains elusive. In *Lxrα−/−* mice, Srebp-1 and stearyl-CoA desaturase mRNA are reduced, suggesting that LXR plays a role in regulating triglyceride synthesis (122). This is confirmed by the identification of an LXR response element in SREBP-1c gene (135). Endogenous oxysterols derived from mevalonate pathway, most likely 24(S), 25 epoxycholesterol, activates LXRα and induces SREBP-1c, which stimulates lipogenesis and leads to hypertriglyceridemia (136). This may explain why LXR-selective ligands induce SREBP-1c and hypertriglyceridemia in mice and hamsters (137). Disruption of *Lxrβ* does not result in accumulation of cholesterol esters on a high-cholesterol diet as was observed in *Lxrα−/−* mice (138).

Wild-type mice treated with a rexinoid, LG268, exhibit marked changes in cholesterol homeostasis including inhibition of intestinal cholesterol absorption and repression of bile acid synthesis (139). The observation that LG268 reduces Cyp7a1 mRNA levels is consistent with the report that *CYP7A1* gene transcription is repressed by LG268 in transfection assays in HepG2 cells (140), and that the negative effect of RXRα/FXR must dominate over the positive effect of RXRα/LXRα (121). Interestingly, the levels of mRNA hybridized with Abca1 cDNA probe in the intestine are increased. These authors suggest that rexinoids prevent the accumulation of cholesterol in liver and serum by both depleting bile acids, thus reducing intestinal reabsorption of cholesterol, and by Lxr induction of Abca1 transporter that efflux cholesterol from enterocytes. ATP-binding cassette transporter type A1 (ABCA1) functions as a cholesterol and phospholipid efflux regulator involved in HDL synthesis. Mutations of the *ABCA1* gene have been identified in Tangier disease patients (141). However, the identity of ABCA1 transporter as a cholesterol efflux regulator in the intestine has not been firmly established. It has been reported that knockout of *Abca1* gene in mice reduces intestinal cholesterol absorption (142). In complete contradiction, another laboratory reported increase of cholesterol absorption by ablation of the *Abca1* gene (143). Recently, studies of sitosterolemia, an autosomal recessive disorder characterized by an increased intestinal absorption and decreased biliary excretion of dietary sterols, hypercholesterolemia, and premature coronary atherosclerosis, have identified mutations in the genes coding for ABCG5 and ABCG8 half-transporters. These transporters function as biliary sterol efflux regulators that limit intestinal absorption and promote biliary excretion of plant sterols (sitosterols) (144, 145). It is not known whether the same ABC transporters also regulate intestinal cholesterol absorption.

Several genes involved in reverse cholesterol transport are regulated by LXRs. Both Lxrα and Lxrβ regulate mouse *Abca1* gene involved in cholesterol efflux in peripheral tissues (146, 147), human macrophage White protein (ABCG1), and the murine homolog *Abc8* (148). LXR also controls lipid-induced expression of the apolipoprotein E (*ApoE*) gene in macrophages and adipocytes (149), the human cholesterol ester transfer protein (CETP) that mediates the exchange of cholesterol esters for triglycerides between HDL and triglyceride-rich lipoproteins (150), and the lipoprotein lipase (*LPL*) gene involved in hydrolysis of triglycerides carried by VLDL and CM (151). Interestingly, LXRα regulates its own synthesis in macrophages, but not in adipocytes, hepatocytes, and other cell types (152).

3. FXR (NR1H4). FXR (also named RIP14 and HRP1) was isolated by low-stringency screening of a liver cDNA library using oligonucleotides directed to the conserved DNA-binding domain of nuclear receptors (153) and by its ability to heterodimerize with RXR using yeast two-hybrid screening (128). FXR is closely related to the *Drosophila* ecdysone receptor and preferentially binds to an IR1 motif (128, 153). FXR and LXR are closely related and belong to the same NR1H subfamily of nuclear receptors. FXR is highly expressed in the liver, intestine, adrenal, and kidney (128, 153). Farnesol, juvenile hormone III, all-*trans*-retinoic acid, and TTNPB activate FXR at high concentrations (154). Recently, bile acids have been identified as the endogenous ligands for FXR (4–6). The hydrophobic bile acid, CDCA, is the most effective activator of FXR, with an EC_{50} of about 10–20 μM, tested in kidney CV1 cells (6). The secondary bile acids, LCA and DCA, are less effective, and hydrophilic bile acids, ursodeoxycholic acid, and muricholic acids, are inactive.

When assayed in liver-derived cell lines, bile acid/FXR repressed *CYP7A1* (4, 6, 140, 155), *CYP27A1* (156), *CYP8B1* (157), and *NTCP* gene transcription (158). In human embryonic kidney 293 cells, however, bile acids and cotransfection of FXR had no effect on *CYP7A1* transcription. The FXR binding sequence IR1 is not present in the *CYP7A1* gene and FXR does not bind to the BARE-II of *CYP7A1* gene. Chiang *et al.* (140) suggested that FXR suppressed *CYP7A1* transcription by an indirect mechanism involving other liver-specific factors (*Section IV.A*). It has been reported that FXR activates target genes by binding to the IR1 motifs in genes encoding ileum bile acid binding protein (IBABP) (6, 159), canalicular bile salt export pump (BSEP) (160), phospholipid transport protein (PLTP) (161), and ApoCII (162). These findings are consistent with elevated serum bile acids, cholesterol and triglycerides, reduced bile acid pool and fecal bile acid secretion, and lack of bile acid inhibition of Cyp7a1 expression in *fxr−/−* mice (163). These observations suggest that FXR plays a key role in lipid metabolism.

4. PPARα (NR1C1). Three forms of PPAR, α, γ, and δ (or β), have been identified (164). PPARα is expressed in the liver, heart, and adipose tissues (164, 165), all of which have an active fatty acid β-oxidation pathway. Fatty acids, eicosanoids, and hypolipidemic agents are ligands of PPARs (166, 167). PPARγ is highly expressed in adipose tissues. PPARδ (or β) is expressed in most tissues. Fibrates are hypolipidemic drugs that affect many genes in lipid metabolism by activation of PPAR (164, 168).

Bile acid synthesis and pool sizes are reduced in gallstone and hypercholesterolemia patients treated with certain fibrates (169, 170). The PPARα ligand, Wy14,643, suppresses CYP7A1 mRNA levels and *CYP7A1* luciferase reporter activity in HepG2 cells (113). A functional PPARα-responsive element has been mapped to the DR1 in BARE-II, which is also a HNF4α binding site. However, PPARα/RXRα does not bind to this DR1 motif. It appears that PPARα interferes with HNF4α activation of the *CYP7A1* by reducing the amount of HNF4α expressed (113,

171). Fibrate treatment changes bile acid composition by increasing CA and decreasing CDCA synthesis. This may be because PPARα stimulates CYP8B1 activity and increases CA synthesis in the rat (172). However, PPARα binds to the rat *CYP8B1* gene rather weakly. In *Pparα−/−* mice, inhibitory effects of fibrates on bile acid synthesis and Cyp7a1 and Cyp27a1 expression were abolished (173).

PPARα appears to mediate fatty acid stimulation of LXRα expression by binding to several PPAR response elements located in the 5′-upstream sequence of the LXRα gene (174). PPARγ has been shown to induce LXRα, which then induces ABCA1 and ABCG1 expression in macrophages (149, 175). These findings suggest that the PPAR-LXR-ABC1 cascade is involved in cholesterol efflux in macrophages. Bile acids have been shown to antagonize PPARα activity; however, the physiological role of bile acids on PPAR regulation is not clear (176).

5. HNF4α (NR2A1). HNF4α is the most abundant orphan nuclear receptor expressed in the liver. HNF4α homodimer binds to the DR1 motif and regulates the liver-specific expression of many genes involved in lipoprotein metabolism, including ApoAI, ApoB, and ApoCIII (177, 178), and glucose metabolism (179, 180). HNF4α has constitutive activity and is able to transactivate genes without ligand binding (181). Fatty acyl-CoA thioesters have been shown to activate HNF4α; however, the physiological relevance of these ligands has been questioned (182). HNF4α binds to a DR1 sequence in the BARE-II and stimulates rat *CYP7A1* promoter/reporter activity (108, 112). Mutation of the HNF4α binding site markedly reduced *CYP7A1* promoter activity, indicating that HNF4α is crucial for basal level transcription (108, 112, 183). HNF4α binding sites have also been identified in the *CYP8B1* (157, 184) and *CYP27A1* genes. HNF4α has been shown to mediate bile acid repression of *CYP8B1* transcription (157, 184). HNF4α activity is also regulated by posttranscriptional mechanisms, *i.e.*, phosphorylation of the DNA-binding domain of HNF4α by protein kinase A reduced HNF4α transactivation activity (185). Bile acids or TNFα has been shown to inhibit the transactivation potential of HNF4α via MAPK cascade (186).

Mutations of the HNF4α gene have been linked to maturity onset diabetes of the young (MODY1) (187, 188). HNF4α is an upstream regulator of *HNF1α* gene, the mutation of which has been linked to MODY3 (189, 190). Disruption of *Hnf4α* in mice is embryonic lethal, because HNF4α is critical for early liver development and differentiation. Liver-specific conditional disruption of the *Hnf4α* gene results in marked accumulation of lipids in the liver, reduction of serum cholesterol and triglycerides, and accumulation of bile acids in serum. These phenotypes may be explained by reduction of mRNA levels for Cyp7a1, Hnf4α, ApoaII, ApocIII, Apob100, Ntcp (SLC10A1), Oatp1 (SLC21A1), and microsomal triglyceride transport protein (191). This is consistent with the important role that HNF4α plays in basal transcription of *CYP7A1* and underscores the importance of this nuclear receptor in regulation of lipoprotein metabolism.

6. FTF (NR5A2). The Fushi-tarazu factor-1 (Ftz-F1) family of monomeric nuclear receptors plays important roles in steroidogenesis, liver growth, endocrine development, and differentiation (192, 193). Two Ftz-F1 genes have been identified. Ftz-F1α (NR5A1) was first identified in *Drosophila* as a factor that activates the homeobox gene, *fushi tarazu* (194). A mouse homolog, steroidogenic factor 1 (SF-1), was first cloned from an adrenal gland cDNA library (195). The Ftz-F1β (NR5A2) gene encodes α-FTF and its homologs, rat FTF (196), human CPF (197), hepatitis B virus enhancer 1 factor (hB1F) (198), human FTF (hFTF) (199), mouse LRH (200), *Xenopus laevis xFF1* (200), and the zebra fish (zFF1) (201). These NR5A2 variants differ in their N-terminal amino acid sequences and C-terminal truncation due to differential promoter usage and alternative mRNA splicing (201). All hFTFs lack a sequence corresponding to exon 2 of mouse FTF. hFTF is similar to CPF variant 1 and hB1F2 (541 amino acid residues) (202). CPF (495 amino acids) is identical with hB1F (198); they lack a sequence corresponding to both exons 2 and 3 of mouse FTF. CPF variant 2 is a truncated form (323 amino acid residues) lacking C-terminal 172 amino acid residues of ligand-binding domain and AF2 domains and is similar to hFTFs (199). FTF is the name recommended by the Genome Database Nomenclature Committee, and it is used here unless specified otherwise.

FTF is expressed in liver, intestine, and pancreas, and is most related to SF-1 expressed in steroidogenic tissues (203). FTF has intrinsic transcriptional activity and its ligand has not been identified. FTF binding sites have been identified in *SF-1* (204), *SHP* (205), *HBV* (206), *HNF3β, HNF4α,* and *HNF1α* genes (202). The binding site for FTF in human *CYP7A1* has been mapped to $-_{134}$TCAAGGCCA-$_{126}$ (197), which overlaps with the HNF4α-binding site (-$_{144}$TGGACTtAGGTCA-$_{132}$) by three nucleotides (*underlined*). In rat *CYP8B1*, two FTF binding sites are identified (207). Embedded in the FTF site is a HNF4α binding site (184). In human *CYP8B1*, there is an overlapping HNF4α and FTF binding site (157). FTF is a weak transcription factor that, when transfected at high concentration, stimulates *CYP7A1* reporter activity by about 2-fold in nonliver cells (197). It has been suggested that FTF functions as a competence factor for sterol regulation of mouse *Cyp7a1* (2) and human *CETP* gene (208) by LXR. It is interesting that bile acids could induce FTF mRNA expression in rat livers and HepG2 cells (155, 184) and functioned as a repressor that inhibited human *CYP7A1* and rat *CYP8B1* transcription when assayed in HepG2 cells (155, 157, 184). Thus, FTF may directly inhibit rat *CYP7A1* and human *CYP8B1* in response to bile acids. The inhibitory effect of FTF is likely due to competition for HNF4α binding to the overlapping binding sites in the BAREs. Bile acids also induce FTF mRNA expression in the intestine (209). It is interesting that FTF induces the multidrug-resistant protein-3 (MRP3) gene involved in excretion of bile acids across basal lateral membrane into portal blood, and FXR does not regulate MRP3 gene (209). Thus, FTF may play a direct role not only in feedback inhibition of bile acid synthesis but also in stimulation of bile acid transport and absorption in the intestine. *FTF* gene transcription is regulated by GATA and basic helix-loop-helix transcription factors (202). FTF, in turn, regulates *HNF4α* and *HNF1α* gene transcription. Hence, FTF is an upstream regulator of the genes involved in early liver development. FTF may protect liver and intestine from cy-

totoxicity of bile acids during the development of the gastrointestinal tract.

7. SHP (NR0B2). Using the mouse nuclear receptor, constitutive androgen receptor (CAR), as bait, two-hybrid screening identified SHP as an interacting factor (210). SHP is a unique orphan nuclear receptor that lacks a conserved DNA-binding domain but contains a receptor-interacting domain and a repressor domain (211). SHP is known to inhibit transactivation activity of RAR, CAR, HNF4α, estrogen receptor α and β, PPAR, and thyroid hormone receptor (211–215). Thus, SHP is a promiscuous inhibitory heterodimer partner of nuclear receptors. SHP is closely related to DAX-1, a nuclear receptor expressed in steroidogenic tissues. DAX-1 was originally identified in X-linked adrenal hypoplasia congenita patients who have deletions or mutations of *DAX-1* (216–218). Two mechanisms have been suggested for repression of nuclear receptor activity by SHP. First, SHP competes with other nuclear receptors for coactivators such as steroid receptor coactivator families of steroid receptor coactivators. Second, SHP represses nuclear receptors directly by its repressor function located at the C-terminal region (215). *SHP* transcription is stimulated by monomeric nuclear receptors bound to DNA, *i.e.*, SF-1 and FTF (205). Because SHP interacts with FTF, SHP inhibits its own transcription by inhibiting FTF activity (155). Hence, FTF, FXR, and SHP tightly regulate the expression of SHP in the liver, similar to the regulation of *CYP7A1* gene by these receptors. It would be interesting to disrupt the *Shp* gene or overexpress Shp in the mouse liver to verify the genes and pathways regulated by SHP (see *Note Added in Proof,* no. 2). SHP mRNA levels are relatively high in mouse livers but lower in rat livers. This may explain a much higher CYP7A1-specific activity in the rat than in mouse livers.

Recently, Goodwin *et al.* (3) reported that the FXR agonist, GW4064, repressed CYP7A1 mRNA but stimulated SHP mRNA expression in rats. They found an inverse relationship between CYP7A1 and SHP mRNA expression levels. FXR has been shown to bind mouse and human *SHP* promoter and stimulates SHP reporter activity (2, 3). In *Fxr*$-/-$ (163) and in *Cyp7a1*$-/-$ mice (2), SHP expression is reduced. Furthermore, SHP represses *CYP7A1* in a dose-dependent manner by inhibiting the transactivating activity of FTF (LRH) (2, 3, 155). This is analogous to DAX-1 inhibition of SF-1 in steroidogenic tissues. Recently, Chen *et al.* (155) reported that feeding CDCA to rats had no effect on SHP mRNA expression in livers. However, overexpression of FXR in the presence of CDCA stimulated SHP mRNA expression levels in HepG2 cells. Species differences in SHP expression in response to bile acids may explain these descriptions. SHP mutations have been identified in obese Japanese with early onset of diabetes (219). It was suggested that SHP was a MODY gene that might regulate HNF4α activity and energy metabolism in the pancreas. A cascade mechanism of FXR regulation of *CYP7A1* involving SHP will be described in *Section IV.A.*

8. PXR (NR1I2). Mouse PXR or the human ortholog, steroid xenobiotic receptor, is a promiscuous xenobiotic receptor that is activated by structurally unrelated steroids, xenobiotics, and drugs, such as phenobarbital and antibiotics, and expressed predominantly in the liver and intestine (220, 221). PXR is most closely related to CAR, and shares common ligands and function. PXR ligands also induce the CYP3A family of cytochrome P450 enzymes. CYP3A4 is the most abundant cytochrome P450 isozyme expressed in human liver and intestine and metabolizes about 60% of clinical drugs in the liver and intestine (222). Dexamethasone, pregnenolone 16α-carbonitrile (PCN), rifampicin, phenobarbital, and other drugs activate PXR, which forms a heterodimer with RXR and binds to the promiscuous response elements consisting of DR3, DR4, DR5, or ER6 in the *CYP3A* genes. Recently, LCA has been identified as a ligand of PXR (221, 223). It was suggested that PXR might function as a bile acid sensor that induced CYP3A4 to convert LCA to a hydrophilic bile acid, hyodeoxycholic acid (HDCA). PXR also induces the OATP2 (SLC21A6) in sinusoidal membrane. This was consistent with the observation that PCN did not affect bile acid secretion in *Pxr*$-/-$ mice (224) and that *Pxr* null mice developed inflammatory response and liver damage upon LCA treatment. However, *Pxr* null mice were responsive to LCA induction of CYP3A (225). These results suggest that LCA induces OATP2, which transports LCA into hepatocytes to induce PXR to inhibit *CYP7A1* transcription (225). Thus, PXR may play a protective role against hepatotoxicity and cholestasis induced by LCA (225). This is consistent with a previous report by Chiang *et al.* (226) that PCN and dexamethasone strongly inhibit CYP7A1 activity and protein expression in rat livers. Phenobarbital is known to stimulate CYP7A1 activity (226, 227). However, the mechanism by which PXR inhibits and phenobarbital induces *CYP7A1* expression is not known at present. PXR also induces multidrug-resistant protein 1 (MDR1) and MRP2, which transport sulfate-conjugated tauco-CDCA and tauro-LCA to canaliculi (228).

C. Nuclear receptor regulation of cholesterol homeostasis

Figure 4 illustrates the central roles LXR and FXR play in coordinate regulation of bile acid synthesis, transport, and absorption in the liver and intestine, and cholesterol metabolism in the liver and peripheral tissues. When cholesterol levels increase in hepatocytes, oxysterols activate LXRα, which stimulates the conversion of cholesterol to bile acids by inducing *CYP7A1* transcription. LXR induces SREBP-1c to stimulate triglyceride synthesis by inducing genes involved in fatty acid synthesis. LDL receptor, HMG-CoA reductase, and other genes in cholesterol synthesis pathway may be also induced. LXR also induces genes involved in lipoprotein metabolism, including LPL, CETP, and PLTP.

Dietary cholesterol is absorbed into the enterocytes, likely by protein-mediated transporters (229–231). LXR induces ABCA1 in peripheral tissues for efflux of cholesterol and phospholipids. ABCA1 has been implicated in cholesterol efflux in the intestine (139). Thus, LXR plays a critical role in regulating cholesterol homeostasis by 1) stimulating *CYP7A1* transcription to convert cholesterol to bile acids; 2) facilitating the efflux of cholesterol from peripheral tissues and intestine by inducing ABCA1/ABCG1 (141, 232); and 3) regulating lipoprotein metabolism by inducing CETP and LPL.

Increases in bile acid synthesis and pool size stimulate

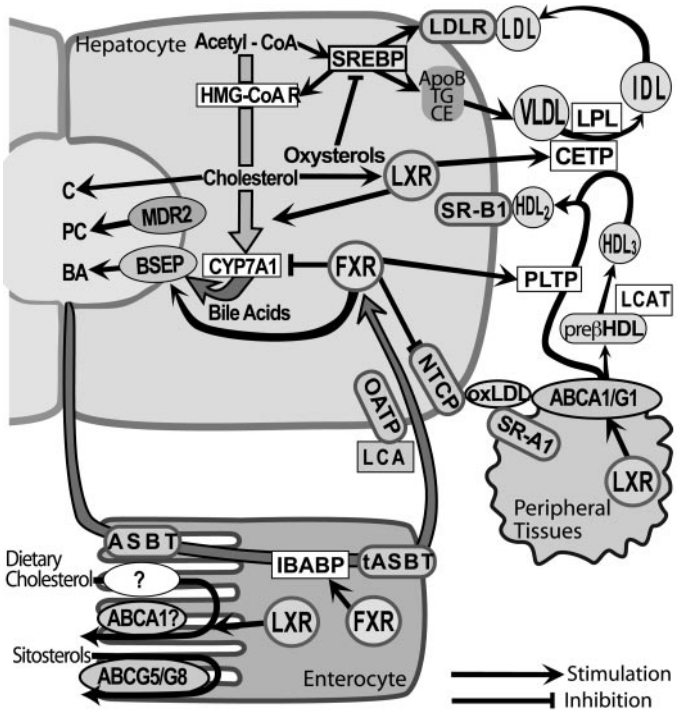

FIG. 4. LXR and FXR regulation of bile acid synthesis, transport, and absorption, as well as cholesterol homeostasis in the liver. In the liver, cholesterol is converted to bile acids (BA) and also oxidized to oxysterols by sterol hydroxylases. Oxysterols activate LXR, which induces transcription of genes for *CYP7A1* and *SREBP-1c*. SREBP induces genes involved in fatty acid synthesis, LDL receptor, HMG-CoA reductase, and other genes in cholesterol synthesis. LXR also induces CETP and LPL involved in lipoprotein metabolism. Bile acids activate FXR and inhibit *CYP7A1* and *NTCP* transcription. On the other hand, FXR induces the expression of bile salt export pump (BSEP), which excretes bile acids into bile. Cholesterol is excreted into bile by an unknown mechanism. Phosphatidylcholine (PC) is excreted by MDR2 to bile canaliculi. Cholesterol, PC, and bile acids form mixed micelles and are stored in the gallbladder. Bile acids secreted from the gallbladder are reabsorbed in the intestine by ASBT located in the brush border membrane. FXR induces ileum bile acid binding protein (IBABP), which binds and facilitates the efflux of bile acids by truncated ASBT (tASBT) located in the basolateral membrane into portal circulation to hepatocytes where bile acids are taken up by NTCP. LCA, a secondary bile acid formed in the intestine, induces OATP2 and transports LCA into hepatocytes for conversion to hyodeoxycholic acid by CYP3A, which is induced by PXR (not shown). Dietary cholesterol is absorbed into the intestine by an unknown mechanism. In the intestine, LXR induces ABCG5/G8 transporters and perhaps also ABCA1, which effluxes sitosterol (plant sterol) and cholesterol, respectively, from enterocytes. In peripheral tissues, oxidized LDL is taken up by SR-A1, and LXR induces ABCA1/G1, which effluxes cholesterol and phospholipids to form pre-HDL with ApoAI/ApoE. FXR induces PLTP, which transfers phospholipids from VLDL and LDL to pre-βHDL and HDL_3 to form HDL_3 and HDL_2, respectively. LXR induces CETP, which exchanges triglycerides for cholesterol between HDLs and other lipoproteins.

FXR, which inhibits *CYP7A1* to decrease bile acid synthesis but stimulates BSEP expression to excrete bile acids into bile. Cholesterol is excreted into bile by an unknown mechanism, possibly involving ABCG5/G8, and MDR2 excretes phosphatidylcholine into bile to form mixed micelles with bile acids. In the intestine, bile acids are reabsorbed into enterocytes by sodium-dependent bile acid transporter (ASBT, SLC10A2) located in the brush border membrane and bind to IBABP, which may facilitate bile acid efflux by the truncated ASBT (tASBT), located in the basolateral membrane, to portal circulation (233). FXR inhibits the expression of NTCP in sinusoidal membrane to reduce reabsorption of bile salts into hepatocytes. Therefore, FXR may play major roles in bile acid metabolism, reverse cholesterol transport, and protect hepatocytes against cholestasis by 1) feedback inhibition of bile acid synthesis by *CYP7A1*; 2) stimulation of bile acid efflux from hepatocytes by BSEP; 3) inhibition of bile acid uptake into hepatocytes by NTCP; and 4) regulation of reverse cholesterol transport by inducing ApoCII and PLTP.

It should be emphasized that cholesterol metabolism in rats and mice is very different from humans and other species. Rats and mice have very little LDL and do not express CETP. Stimulation of CYP7A1 by a high-cholesterol diet is observed only in rats and some inbred strains of mice. The bile acid pools of rats and mice are more hydrophilic, containing mostly muricholic acids, and thus less effective in activation of FXR. Therefore, the positive effect of LXR may dominate over the negative effect of FXR to explain the high efficiency in conversion of cholesterol to bile acids in the rat and mouse. In contrast, a high-cholesterol diet does not stimulate, but represses *CYP7A1* in the monkey, guinea pig, rabbit, and human. In the latter species the inhibitory effect of FXR may dominate over the stimulatory effect of LXR to explain the inhibition of *CYP7A1* gene transcription by cholesterol. Therefore, cholesterol may indirectly activate FXR by stimulating the synthesis of bile acids. Thus, rats are resistant to diet-induced hypercholesterolemia, whereas rabbits, hamsters, and some humans develop hypercholesterolemia on a diet high in cholesterol.

IV. Molecular Mechanisms of Regulation of Bile Acid Metabolism

Bile acid feedback regulation of bile acid synthesis has been studied for more than three decades. Despite that, the molecular mechanism of bile acid feedback is poorly understood. During the last decade, cloning of the *CYP7A1* cDNAs and the genes has contributed significantly to our understanding of the molecular mechanism of bile acid synthesis and regulation (1). Several mechanisms have since been proposed to explain bile acid feedback regulation of *CYP7A1* transcription. The receptor-mediated mechanism originally proposed by Chiang and Stroup (109) is based on the finding that hormone response element-like repeats are present in the BAREs identified in the *CYP7A1*. Bile acids have been shown to activate protein kinase C (PKC) signaling pathway (234) and inflammatory cytokines (235, 236). A receptor-mediated mechanism might regulate bile acid synthesis under physiological conditions, whereas a cell-signaling mechanism possibly provides a rapid response to stress that is induced by bile acid overload (such as in cholestasis). These two mechanisms may converge to down-regulate the genes through the same transcription factors.

A. Nuclear receptor-mediated mechanism

Chiang and associates (1, 109) proposed that bile acids might bind to and activate a nuclear bile acid receptor,

which interacts with a bile acid-responsive protein that transactivates *CYP7A1* gene transcription. Interaction between bile acid receptor and bile acid-responsive protein might prevent a transactivating factor from binding to the BARE, thus inhibiting *CYP7A1* transcription. It was further proposed that bile acid receptors and bile acid responsive proteins might be liver-enriched transcription factors or orphan nuclear receptors (1, 110). Identification of FXR as a bile acid receptor supports this mechanism. The comparison of nucleotide sequences of the BAREs identified in rat and human *CYP7A1* (110, 155), rat and human *CYP8B1* (157, 184), and human *CYP27A1* show similar characteristics: they all contain overlapping binding sites for HNF4α and FTF. These two nuclear receptors may differentially regulate these genes by competing for binding to the BAREs. The relative expression levels of these two nuclear receptors in liver may also regulate these genes under different physiological conditions.

Figure 5 illustrates the receptor-mediated mechanisms of bile acid regulation of gene transcription based on the original mechanism proposed by Chiang and co-workers (1, 109, 110) and modified according to the FXR/SHP cascade mechanism proposed recently (2, 3, 155, 157). CDCA-activated FXR binds to the IR1 sequences and stimulates *IBABP*, *BSEP*, *PLTP*, and *SHP* transcription in the liver. SHP then interacts with FTF to repress *CYP7A1*, or with HNF4α to repress *CYP8B1* and *CYP27A1* transcription. It should be noted that bile acids also induce FTF, which directly inhibits genes in bile acid synthesis in the liver (155, 157, 184) but stimulates MRP3 gene in the intestine (209). Figure 5 also shows an SHP-independent mechanism by which the PXR activated by LCA represses *CYP7A1* transcription by an unknown mechanism. On the other hand, PXR induces OATP2 to facilitate the transport of LCA to hepatocytes to induce CYP3A family enzymes that convert LCA to hyodeoxycholic acid. Vitamin D receptor has recently been identified as a LCA-activated receptor, which may also regulate bile acid synthesis (see *Note Added in Proof*, no. 3). Therefore, bile acids regulate bile acid synthesis, transport, absorption, and detoxification in the liver and intestine. It is intriguing that bile acids activate a very specific receptor, FXR, which induces a nonspecific, negative receptor, SHP. SHP then interacts with other nonspecific receptors (FTF and HNF4α) and specifically inhibits the genes regulated by bile acids. The unique structures of BAREs in bile acid-repressed genes must be critical to provide specificity for bile acid inhibition. Tissue-specific expression of SHP, HNF4α, and FTF may also provide specificity for this cascade mechanism of gene transcription. Further study by knocking out the *shp* gene in mice to study bile acid feedback regulation of genes transcription would provide more convincing evidence for the FXR/SHP-dependent mechanism (see *Note Added in Proof*, no. 2). In addition, bile acids may down-regulate a gene by SHP-independent mechanism, *i.e.*, reducing HNF4α expression level and stimulation of FTF (184). When bile acid pool in the liver is reduced, increasing HNF4α expression and decreasing FTF expression would allow HNF4α to bind to the BARE and stimulate gene transcription. When bile acid pool increases, HNF4α expression is reduced and FTF is increased to allow FTF to bind to the BARE and down-regulate gene transcription.

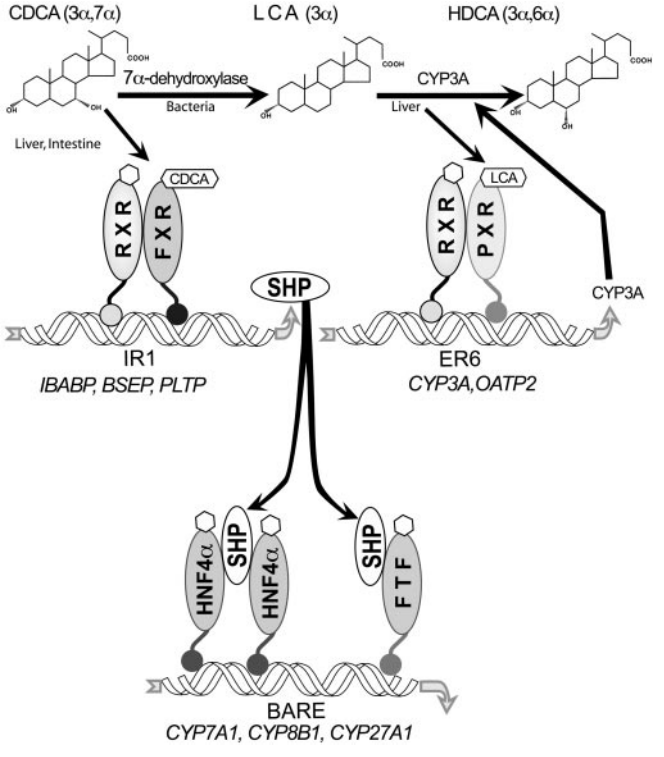

FIG. 5. Nuclear receptor-mediated mechanism. FXR and PXR are bile acid receptors. SHP, HNF4α, and FTF are bile acid-responsive proteins that are regulated by bile acids. FXR/RXRα binds to the IR1 sequence in IBABP, BSEP, PLTP, and SHP gene and activates their gene transcription. FXR indirectly represses gene transcription by induction of a negative receptor, SHP, which interacts with HNF4α or FTF and represses *CYP7A1*, *CYP8B1*, and *CYP27A1* transcription. Bile acids also induce FTF, which interact with SHP or functions as a negative regulator that inhibits human *CYP7A1*, rat *CYP8B1*, and human *CYP27A1* transcription. Bile acids also inhibit HNF4α gene transcription and contribute to the inhibition of HNF4α-activated rat and human *CYP8B1* and human *CYP27A1*. In SHP-independent mechanism, LCA activates PXR, which binds to an ER6 sequence in CYP3A and OATP2 genes. PXR inhibits *CYP7A1* transcription by an unknown mechanism.

B. Cell-signaling mechanism

Figure 6 illustrates a cell-signaling mechanism based on the PKC signaling pathway proposed by Stravitz and colleagues (234, 237), bile acid activation of inflammatory cytokines by Miyake *et al.* (235), and the MAPK signal transduction pathway by De Fabiani *et al.* (186). Bile acids mimic phorbol esters, which activate PKC and lead to activation and phosphorylation of c-Jun N-terminal kinase 1, 2 (JNK1, 2) (238). It has been suggested that phosphorylated c-Jun might form a transcriptional repressor complex with a positive transcription factor and prevent it from activating *CYP7A*1. This repressor complex has not been identified, however. A recent study from the same laboratory showed that c-Jun could induce SHP by binding to an AP1 site in the promoter (239). Therefore, the PKC pathway and the nuclear receptor-mediated mechanism may converge to regulate a common receptor, SHP. Bile acids have been shown to induce inflammatory cytokines

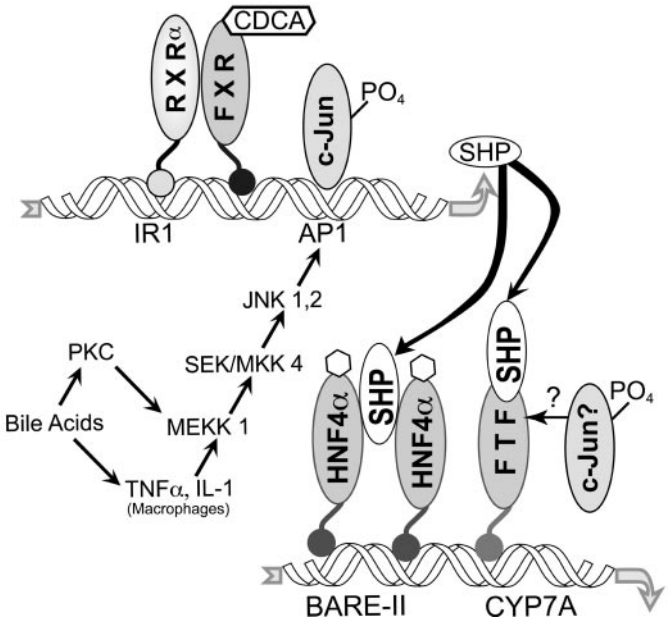

FIG. 6. Cell signaling mechanism. Bile acids activate PKC, which initiates a MAPK signal transduction pathway to phosphate JNK1, 2. Bile acids also induce inflammatory cytokines, TNFα and IL-1, which also activate MAPK cascade involving MEEK1 and SEK/MKK4, and phosphorylate JNK1/2. JNK1/2 phosphorylates c-Jun, which may interact with FTF (or other unknown factors) and repress *CYP7A1*. The phosphorylated c-Jun may induce SHP, which interacts with FTF and represses *CYP7A1* transcription as in the nuclear receptor-mediated mechanism. JNK1/2 may phosphorylate HNF4α and inhibit its transactivation activity, leading to repression of *CYP7A1* transcription. MEKK1, MAPK kinase kinase 1; SEK, stress-activated protein kinase kinase; MKK4, MAPK kinase kinase; JNK, c-Jun N-terminal kinase.

in Kupffer cells (hepatic macrophages) (235). Induction of cytokine expression in macrophages was correlated to bile acid inhibition of CYP7A1 mRNA expression in hepatocytes. Miyake *et al.* (235) suggested that cytokines induced by bile acids in hepatic macrophages traverse the sinusoidal surface and enter the parenchyma cells to inhibit *CYP7A1* expression. The downstream transcription factors that are involved in this mechanism have not been identified. More recently, De Fabiani *et al.* (186) reported that bile acids could suppress *CYP7A1* transcription by reducing transactivation activity of HNF4α by a MAPK pathway, including activation of MAPK kinase kinase 1, stress-activated protein kinase kinase/MAPK kinase kinase, and JNK1/2 (240). De Fabiani *et al*. (186) proposed that phosphorylation of HNF4α by JNK might reduce HNF4α transactivation of *CYP7A1*. This pathway allows rapid adoption to sudden increase of bile acids by inhibiting bile acid synthesis. It remains to be verified that the JNK pathway phosphorylates HNF4α and that phosphorylated HNF4α lost its ability to activate gene transcription. Nevertheless, this mechanism is consistent with the critical role that HNF4α plays in mediating bile acid repression of *CYP8B1* and *CYP27A1* gene transcription. Inhibition of *HNF4α* gene transcription and its transactivating activity by phosphorylation reduce transcription of these genes involved in bile acid synthesis.

V. Drug Therapies Targeted to Nuclear Receptors and Genes in Bile Acid Metabolism

Identification of nuclear receptor LXR and FXR as regulator of genes in bile acid and cholesterol metabolism has provided potential new targets for screening cholesterol-lowering drugs by manipulating bile acid synthesis, transport, and absorption (Table 2) (241). In principle, stimulation of bile acid synthesis, increasing biliary bile acid excretion, and reducing bile acid and cholesterol reabsorption in intestine would lead to cholesterol lowering. In addition, these potential drugs also could be used for the treatment of liver diseases, such as cholestasis, cholelithiasis, and cirrhosis.

A. Bile acid synthesis

Gene transfer techniques have been used to overexpress CYP7A1 activity in the liver. Adenovirus-mediated transfer of CYP7A1 to LDL receptor-deficient mice causes a dose-dependent decrease of plasma LDL (242). Infection of recombinant adenovirus containing human CYP7A1 increases CYP7A1 activity in mice (243). Introducing CYP7A1 by asialoorosomucoid-polylysine conjugate into mouse hepatocytes decreases plasma cholesterol (244). Overexpression of CYP7A1 in primary human hepatocytes and HepG2 cells activates the classic pathway of bile acid synthesis and decreases HMG-CoA reductase and ACAT, but increases LDL receptor and cholesterol ester hydroxylase mRNA and activity (245). It is interesting that over-expression of CYP7A1 in transgenic mice increases VLDL assembly and secretion without inducing hyperlipidemia (246). It was suggested that induction of the LDL receptor by overexpression of CYP7A1 reduced serum cholesterol. Furthermore, overexpression of CYP7A1 blocked lithogenic diet-induced atherosclerosis and gallstone formation in the atherosclerosis and gallstone-susceptible C57BL/6 strain of mice (247). These experiments

TABLE 2. Potential drug therapies targeted to nuclear receptors and bile acid metabolism

A.	Bile acid synthesis
	Gene transfer: CYP7A1 and CYP27A1 reduce serum cholesterol; prevent atherosclerosis and gallstone formation (242–248)
	FXR antagonists: should induce CYP7A1, CYP8B1, and NTCP, and inhibit SHP
	LXR agonists: induce CYP7A1 and CYP8B1 (via SREBP) in rats (139)
	Rexinoids: induce FXR and LXR, but increase serum triglycerides (139)
B.	Bile acid transport
	FXR agonists: induce BSEP and IBABP; repress NTCP (3, 162, 249)
	ASBT inhibitors: inhibit bile acid reabsorption (241, 250, 251)
	Bile acid sequestrants: inhibit bile acid reabsorption (252, 253)
C.	Reverse cholesterol transport
	FXR agonists: induce PLTP and ApocII; reduce triglycerides (162)
	LXR agonists: induce CETP, ABCA1/ABCG1, and LPL; increase triglycerides (12, 135)
D.	Cholesterol absorption
	Cholesterol absorption inhibitors: reduce intestine cholesterol absorption (241, 255, 256)
	Rexinoids: induce intestine ABCA1/ABCG1 (139)
	LXR agonists: induce intestine ABCA1/ABCG1 (139)

demonstrated the principle that increasing CYP7A1 expression would lead to cholesterol lowering and prevention of atherosclerosis. Overexpression of CYP27A1 in HepG2 cells increases bile acid synthesis, HMG-CoA reductase, and ACAT activity (248). However, in Chinese hamster ovary cells, overexpression of Cyp27A1 decreases HMG-CoA reductase activity. It appears that overexpressing Cyp27A1 causes different responses in different cell types. Increasing 27-hydroxycholesterol levels in peripheral cells may down-regulate cholesterol synthesis and induces LXR, which in turn induces ABCA1/ABCG1 expression for cholesterol efflux from peripheral cells (131). Therefore, CYP27A1 may have antiatherogenic activity (248). These results suggest that CYP7A1 and CYP27A1 are potential therapeutic targets for lowering serum cholesterol and preventing atherosclerosis.

Therapies targeted to LXR and FXR would be ideal for drug development because nuclear receptors are activated by natural and synthetic ligands, which could be identified by high-throughput screening. FXR antagonists should dampen bile acid feedback inhibition and stimulate *CYP7A1* transcription and result in increasing conversion of cholesterol to bile acids. However, it may be argued that an increase in bile acid synthesis and pool size would lead to stimulation of FXR, which subsequently reduces bile acid synthesis. This is compensated by FXR stimulation of BSEP for excretion of bile acid from hepatocytes. LXR agonists may stimulate *CYP7A1* transcription in rats and mice (139) but may have much less effect on human *CYP7A1*. Therefore, FXR antagonists may be more effective than LXR agonists in stimulating bile acid synthesis and reducing serum cholesterol levels. Individual differences in response to a high-cholesterol diet may have different responses to FXR antagonists and LXR agonists. Rexinoids may stimulate FXR, LXR, PPARα, and other nuclear receptors heterodimerized with RXR (139). Because the effect of FXR may dominate over the effect of LXR in humans, rexinoids may inhibit bile acid synthesis (140).

B. Bile acid transport

FXR agonists should increase BSEP and reduce NTCP expression (3). However, stimulation of BSEP expression to excrete bile acids may not subsequently stimulate bile acid synthesis. FXR agonists also should stimulate IBABP expression in enterocytes, thus protecting intestine cells from the toxicity of bile acids. Bile acids and FXR agonists have been shown to reduce serum triglyceride level (162, 249). FXR agonists may be ideal drugs for treatment of cholesterol gallstone disease, hypertriglyceridemia, and cholestatic liver diseases. The effectiveness of bile acid sequestrants in interrupting bile acid reabsorption and stimulating bile acid synthesis suggests that the inhibitor of intestinal ASBT would be effective in reducing bile acid reabsorption. Several ileal bile acid transport inhibitors have been developed recently for cholesterol lowering (241, 250, 251). New bile acid sequestrants have been developed recently for improving efficacy and reducing gastrointestinal side effects (252, 253). The possible hypertriglyceridemic effects of these drugs need to be evaluated.

C. Reverse cholesterol transport

FXR agonists may increase HDL levels by inducing PLTP, which facilitates the synthesis of HDLs for reverse cholesterol transport, and by inducing ApocII (162), which activates LPL for hydrolysis of triglycerides in VLDL and CM. This may explain the hypotriglyceridemic effect of FXR agonists (162). LXR agonists may induce reverse cholesterol transport by inducing CETP, LPL, and ABCA1/G1 (12). Thus, LXR agonists may reduce serum cholesterol and intestinal cholesterol absorption by increasing cholesterol efflux from enterocytes. However, most of the cholesterol absorbed in the intestine is retained in the body, and cholesterol efflux from enterocytes does not contribute significantly to whole-body cholesterol homeostasis in humans. A potential problem for using LXR agonists is hypertriglyceridemia induced by induction of SREBP-1c, which stimulates fatty acid and triglyceride synthesis (135). A combination therapy of LXR and FXR agonists and compounds that activate both LXR and FXR, if obtained, may be used as an alternative therapy for treating hypercholesterolemia without causing hypertriglyceridemia.

D. Cholesterol absorption

About 50% of dietary cholesterol is absorbed in the intestine in humans by selective processes, most likely involving protein/receptor-mediated transport (229, 231, 254). In principal, inhibition of cholesterol absorption in the intestine would be an attractive strategy for reducing serum cholesterol. Several cholesterol absorption inhibitors have been developed (241, 255). Dietary supplement of margarine containing sitostanol esters (benetol) may inhibit cholesterol absorption and reduce serum cholesterol in a hypercholesterolemic population (256). Increasing cholesterol efflux in intestine also may reduce net intestinal cholesterol absorption. However, this process varies widely among different species and individuals. Rexinoids have been shown to stimulate cholesterol efflux from the intestine by inducing Abca1 transporter in mouse intestine (139). LXR agonists should have similar effects on cholesterol efflux.

VI. Conclusion and Future Perspectives

The cloning of the major regulatory genes in the bile acid biosynthetic pathways in the last 10 yr has contributed significantly to our understanding of the mechanism of regulation of bile acid synthesis and cholesterol homeostasis. These advances have led to the recent discovery of bile acids and oxysterols as signaling molecules and nuclear receptors LXR and FXR as oxysterol and bile acid receptors, respectively. It is predicted that many bile acid target genes that are involved in lipid metabolism will be identified. Further research on the complex mechanism of gene regulation by bile acids and oxysterols, and identification of endogenous ligands for nuclear receptors involved in regulation of lipid metabolism and homeostasis, will help elucidate the mechanism of pathogenesis of several metabolic diseases (see *Note Added in Proof*, no. 3). New drugs targeted to nuclear receptors and bile acid-regulated genes for treatment of hypercholesterolemia, hypertriglyceridemia, ath-

erosclerosis, cholesterol gallstone disease, and cholestatic liver disease will be developed in the foreseeable future.

Note Added in Proof

Several papers, which are very important for this review, appeared after submission of this manuscript.

1. A family of CYP7A1 deficiency has recently been identified (259). Patients have hyperlipidemia, premature coronary and peripheral vascular disease, and premature gallstone disease. A double deletion (TT) in codon 413 results in a frame shift that converts a Leu to Arg, followed by a premature stop codon. The mutation is located in the putative sterol-binding sites of cytochrome P450 enzymes and results in a truncated protein of 413 amino acid residues devoid of the heme-binding domain. Patients are resistant to stain and have markedly reduced bile acid synthesis, and compensatory increase of CYP27A1 activity of the alternative pathway. However, the severe malnutrition phenotypes observed in *Cyp7a1*-null mice were not present in these patients.

2. Mice deficient of SHP have been obtained (260, 261). These mice appear normal except mild defects in bile acid and cholesterol homeostasis, and increase of bile acid synthesis, which is expected due to lacking SHP inhibition of CYP7A1. Surprisingly, these mice still responded to bile acid feedback inhibition when fed bile acids. Studies confirmed that SHP-independent mechanisms, such as bile acid activation of PXR and JNK pathways, were involved in bile acid feedback regulation of bile acid synthesis.

3. Vitamin D receptor (VDR) has recently been identified as the third bile acid receptor (262). VDR is activated by LCA at much lower concentrations (ED_{50} = 8 μM) than PXR. This receptor may be an intestinal bile acid sensor that activates CYP3A4 in liver and intestine to detoxify LCA and protect against colon cancer.

Acknowledgments

I gratefully acknowledge Lynn Anderson for creating the graphics for this study.

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This work was supported by NIH Grants GM-31584, DK-58379, and DK-44442 and by Aventis Pharma, Frankfurt, Germany.

This work is dedicated to the memory of Professor Z. Reno Vlahcevic, M.D., for his guidance, wisdom, and friendship.

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