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Up to date on cholesterol 7 alpha-hydroxylase (CYP7A1) in bile acid synthesis

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Abstract

Cholesterol 7 alpha-hydroxylase (CYP7A1, EC1.14) is the first and rate-limiting enzyme in the classic bile acid synthesis pathway. Much progress has been made in understanding the transcriptional regulation of *CYP7A1* gene expression and the underlying molecular mechanisms of bile acid feedback regulation of CYP7A1 and bile acid synthesis in the last three decades. Discovery of bile acid-activated receptors and their roles in the regulation of lipid, glucose and energy metabolism have been translated to the development of bile acid-based drug therapies for the treatment of liver-related metabolic diseases such as alcoholic and non-alcoholic fatty liver diseases, liver cirrhosis, diabetes, obesity and hepatocellular carcinoma. This review will provide an update on the advances in our understanding of the molecular biology and mechanistic insights of the regulation of CYP7A1 in bile acid synthesis in the last 40 years.

Keywords

Cholesterol 7 alpha-hydroxylase (CYP7A1); Bile acid metabolism; Farnesoid X receptor (FXR); Takeda G protein-coupled receptor 5 (TGR5); Bile acid receptors; Liver metabolism

1. Introduction

Cholesterol 7 alpha-hydroxylase (CYP7A1, EC1.14) is the first and rate-limiting enzyme in the bile acid synthesis pathway. This enzyme is exclusively expressed in the endoplasmic reticulum of hepatocytes and is regulated by the end products, bile acids, returning to the liver via enterohepatic circulation. In 1977, Myant and Mitropoulos[1] published the first exclusive review on CYP7A1. Since then, tremendous progress has been made regarding the cloning and characterization of the *CYP7A1* gene, the molecular mechanisms of bile acid feedback regulation of *CYP7A1* gene expression, the role of bile acid-activated receptor signaling in the regulation of metabolism, and bile acid based-drug therapies for cholestasis

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Authors' contributions

J. Y. L. Chiang conceived, wrote, and edited the manuscript and figures. J. M. Ferrell wrote and edited the manuscript and figures.

Declaration of competing interest

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and metabolic diseases. This review will provide an in-depth update on CYP7A1 and other sterol hydroxylases in bile acid synthesis pathways.

In early 1990, a breakthrough was achieved in the purification and cloning of the gene coding Cyp7a1.[2–4] Availability of specific antibodies and cDNAs helped elucidate the molecular regulatory mechanisms of *Cyp7a1* gene transcription. Ten years later, a nuclear receptor, farnesoid X receptor (FXR),[5–8] and a membrane G protein-coupled receptor (GPBAR-1, also known as Takeda G protein-coupled receptor 5, TGR5) were identified as bile acid-activated receptors, and the roles of FXR and TGR5 in the regulation of bile acid synthesis, transport and metabolic homeostasis were unveiled.[9–20] Several genetically modified mouse models were generated for the study of bile acid synthesis and the regulation of lipid, glucose and energy metabolism.[21–26] Distinct species differences in bile acid synthesis and composition between mice and humans have been recognized.[20,27] Rodents (rats and mice) synthesize more cholesterol, and they clear and catabolize cholesterol to bile acids more efficiently than humans.[28] Mice have higher serum high density lipoprotein (HDL)-cholesterol and very little low density lipoprotein (LDL)-cholesterol compared to humans. Mice also produce 6-hydroxylated muricholic acids as the major primary bile acid in the liver and they produce a larger, more hydrophilic bile acid pool compared to humans.[29] In contrast, the human bile acid pool and composition are highly hydrophobic. Different bile acid species have different efficacies for biliary cholesterol secretion, intestinal fat absorption and feedback regulation of bile acid synthesis and signaling. Thus, the species differences in bile acid composition and regulation of metabolism and homeostasis have been a challenge to the translation of results from animal studies to human physiology and pathophysiology.[30] Nevertheless, research in bile acid synthesis and signaling using novel mouse models has been crucial for the development of bile acid-based drugs to treat cholestatic liver diseases and metabolic liver diseases such as diabetes, obesity and alcoholic and non-alcoholic fatty liver diseases.[17,19,20,31,32] This review will cover the latest advances regarding CYP7A1 and other sterol hydroxylases in bile acid synthesis in mice and humans, and the roles of these enzymes in human health and disease.

2. CYP7A1 and sterol hydroxylases in bile acid synthesis

There are 17 enzymes involved in the synthesis of bile acids from cholesterol which occurs in two main pathways: the classic (or neutral) pathway, which is developed after weaning and becomes the major pathway for bile acid synthesis in humans, and the alternative (or acidic) pathway, which may be the major bile acid synthesis pathway in the neonate.[33] The classic pathway is more important in humans and the alternative pathway is more active in mice and rabbits compared to humans.[34–36] Bile acid synthesis pathways have been reviewed previously.[16,19,20,37,38] Several cytochrome P450 (CYP) hydroxylase enzymes are involved in bile acid synthesis; here, only the key regulatory enzymes in the classic and alternative pathways are shown in Fig. 1.

The classic pathway is initiated by CYP7A1, a member of the CYP family 7, subfamily A1. Expression of CYP7A1, which is the only rate-limiting enzyme in the classic pathway, is limited to the liver and it determines the rate of bile acid synthesis. CYPs are mixed function

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oxidases (monooxygenases) that hydroxylate drugs, fatty acids, steroids, carcinogens and xenobiotics by adding one atom of O_2 to the substrate while the second oxygen atom is reduced to water. Reduced nicotinamide adenine dinucleotide phosphate (NADPH)-cytochrome P450 reductase mediates the transfer of two electrons from NADPH to the substrate-bound CYPs. CYP7A1 is located in the cholesterol-poor endoplasmic reticulum (microsome) and is substrate-specific; that is, it only metabolizes cholesterol to 7α-hydroxycholesterol. Hydroxysteroid dehydrogenase 3B7 converts 7α-hydroxycholesterol to 7α-hydroxy-4-cholesten-3-one (C4), which is a surrogate serum marker for bile acid synthesis. C4 is the common precursor for synthesis of the two major primary bile acids, cholic acid (CA) and chenodeoxycholic acid (CDCA).[39,40] Sterol 12α-hydroxylase (CYP8B1) is required for the synthesis of CA, which is a tri-hydroxy-bile acid. Without 12α-hydroxylation of C4, CDCA, a di-hydroxy-bile acid, is produced. Mitochondrial sterol 27-hydroxylase (CYP27A1) catalyzes steroid side-chain oxidation to form 3α, 7α, 12α-trihydroxycholestanic acid (THCA) and 3α, 7α-dihydroxycholestanic acid (DHCA). THCA and DHCA are activated by bile acid-CoA synthase (BACS, also known as long-chain acyl-CoA synthase, SLC27A5) and are transported into peroxisomes where peroxisomal β-oxidation reactions cleave a propionyl-CoA to form cholyl-CoA and chenodeoxycholyl-CoA, respectively. These bile acyl-CoAs are subsequently conjugated to the amino acids glycine (G) or taurine (T) by bile acid-CoA: amino acid N-acyltransferase (BAAT) to form T/GCA and T/GCDCA.[19] In mice, most bile acids (~95%) are conjugated to taurine,[41] whereas in humans bile acids are conjugated to glycine more than taurine in a ratio of about 3:1.[42] Conjugation of bile acids increases bile acid solubility under physiological pH to form sodium salts, and bile acids are stored in the gallbladder until food intake stimulates the release of bile.

The alternative bile acid synthesis pathway is initiated by CYP27A1 to form 25(R)-26-hydroxycholesterol, which is 7α-hydroxylated to 3β, 7α-dihydroxy-5-cholestanoic acid by CYP7B1 for synthesis of CDCA and CA.[43] CYP7B1 is widely expressed in most tissues, and oxysterols produced in extrahepatic tissues can be converted to bile acids in the liver. In steroidogenic tissues, such as the adrenal glands, CYP7B1 plays a key role in steroid sex hormone synthesis.[44] Both CYP27A1 and CYP7B1 are highly expressed in macrophages, where CYP27A1 and CYP7B1 are involved in the metabolism of cholesterol to oxidized sterols (oxysterols). Oxysterols, such as 25-hydroxycholesterol and 27-hydroxycholesterol, are highly abundant in serum and liver. Microsomal cholesterol 25-hydroxylase, a non-CYP enzyme, has been implicated in the hydroxylation of cholesterol to 25-hydroxycholestrol.[45] The activity of this enzyme is very low in human and mouse liver and in other tissues. More recent studies demonstrate that human CYP3A4 (mouse Cyp3a11) catalyzes the hydroxylation of cholesterol to 25-hydroxycholesterol in liver.[46] CYP7B1 can also hydroxylate cholesterol to 25-hydroxycholesterol in the liver.[22] CYP3A4 is the predominant CYP expressed in the liver and intestine and metabolizes more than 70% of drugs and xenobiotics.[47] In the brain, CYP46A1 hydroxylates cholesterol to 24-hydroxycholesterol and is the major route for cholesterol catabolism in the brain.[48] 24-hydroxycholesterol is excreted through blood-brain barrier to the liver, where 24-hydroxysterol 7α-hydroxylase (CYP39A1) hydroxylates it to 7α−24-dihydroxycholesterol for bile acid synthesis.[22]

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CA and CDCA are the two major primary bile acids synthesized in human liver, whereas CA and α-muricholic acid (α-MCA) and β-MCA are the major primary bile acids in rodents. CDCA is 6β-hydroxylated to α-MCA (3α, 6β, 7α) and β-MCA (3α, 6β, 7β) (Top, Fig. 2). Cyp2c70 is a muricholic acid 6β-hydroxylase responsible for the conversion of CDCA to α-MCA and ursodeoxycholic acid (UDCA) to β-MCA.[49] Two recent studies reported that Cyp2c70 sequentially converts CDCA to α-MCA and then to β-MCA.[50,51] CDCA is 6α-hydroxylated to hyocholic acid (HCA) by Cyp4a12 (3α, 6α, 7α) in humans and pigs. HCA and MCA are 6α/β-stereoisomers and are highly soluble.

3. Bile acid biotransformation by the gut microbiota

Conjugated bile acids are deconjugated by bacterial bile salt hydrolase (BSH) mainly in the colon. Then, bacterial 7α-dehydroxylase converts CA and CDCA to the secondary bile acids deoxycholic acid (DCA) and lithocholic acid (LCA), respectively. BSH activity is restricted to the genera *Clostridium*, *Enterococcus*, *Bifidobacterium*, *Bacteroides*, and *Lactobacillus*, while 7α-dehydroxylase activity mainly originates from *Clostridium* XIVa clusters.[52,53] LCA is sulfate-conjugated and excreted into feces and urine, while some DCA is passively reabsorbed in the colon. In mice, gut bacteria convert β-MCA to ω-MCA (3α, 6α, 7β), a highly soluble bile acid, for fecal and urine excretion. CDCA (3α, 7α) is epimerized by 7β-hydroxysteroid dehydrogenase to UDCA (3α, 7β), a secondary bile acid synthesized in humans. Conversion of the 7α -HO group in CDCA to a 7β -HO group in UDCA changes a hydrophobic bile acid to a hydrophilic bile acid. In general, β-epimers and increasing the number of hydroxy groups increases bile acid solubility and decreases the hydrophobicity of bile acids (Bottom, Fig. 2). Bile acid hydrophobicity increases in the order of ω-MCA<α-MCA<β-MCA < UDCA < CA < CDCA < DCA < LCA.[54]

In rodents, UDCA recirculated to the liver can be converted to β-MCA by Cyp2c70, and DCA and LCA can be 7α-hydroxylated back to CA and CDCA, respectively, by Cyp2a12 (Fig. 2). Also, in rodents, LCA can be converted to hyodeoxycholic acid (HDCA) and murideoxycholic acid (MDCA) by intestinal 6α- and 6β-hydroxylase (Cyp3a11), respectively.[55] In human liver microsomes, LCA is hydroxylated to HDCA by CYP3A4.[56] Therefore, DCA and LCA levels are very low in rodents compared to humans. In mice deficient of Cyp2c70, MCAs are not synthesized and CDCA is increased, while in Cyp2a12 deficient mice DCA and LCA accumulate. In mice deficient of both Cyp2c70 and Cyp2a12, DCA, CDCA and LCA are all increased, and bile acid hydrophobicity is increased, similar to humans.[51]

4. Bile acid homeostasis

Bile acids are detergent molecules that are toxic to cells if accumulated in large amounts. Thus, bile acid synthesis and metabolism must be tightly regulated to maintain homeostasis and prevent cellular toxicity. It should be noted that bile acid composition in serum, liver, gallbladder bile, intestine and feces are different. Serum bile acids contain both conjugated and free bile acids, while bile acids in the liver and gallbladder bile are predominantly conjugated bile acids. In the ileum, most bile acids are conjugated whereas the colon contains more deconjugated secondary bile acids. The total bile acid pool consists of bile

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acids in liver, gallbladder bile and intestine. Serum bile acid content is typically very low (1–2%) and may not be included in measuring the total bile acid pool. With respect to the bile acid pool in mice, about 80% of bile acids are in the intestine, 15% are in the gallbladder, and the remaining bile acids (5%) are in the liver. In humans, the gallbladder and bile duct have the highest bile acid levels and decrease from the upper intestine to the colon and feces.[57]

4.1. Enterohepatic circulation of bile acids

Immediately after synthesis, bile acids are conjugated to glycine and taurine to increase their solubility and bile salt export pump (BSEP) excretes conjugated bile acids from hepatocytes into the biliary system. Here cholangiocytes, stimulated by release of the hormone secretin from duodenal S cells, modifying bile content via the secretion of bicarbonate, water and ions (Fig. 3).[58] A small portion of bile acids are passively reabsorbed by cholangiocytes and return to hepatocytes, referred to as the cholehepatic shunt. Bile acids form mixed micelles with phosphatidylcholine and cholesterol and are stored in the gallbladder. After meal intake, bile acids are released from the gallbladder into the upper intestine where a small amount of bile acids are passively reabsorbed. In the terminal ileum, most bile acids (95%) are reabsorbed into enterocytes via apical sodium-dependent bile acid transporter (ASBT). Bile acids bind to ileum bile acid binding protein (IBABP) and are transported to the sinusoidal membrane, where the organic solute transporter α and β (OSTα and OSTβ) heterodimer effluxes bile acids to portal blood circulation. Bile acids returning to the liver are taken up by hepatic sinusoidal sodium taurocholate co-transport peptide (NTCP) to inhibit bile acid synthesis. The enterohepatic circulation of bile acids is highly efficient and maintains a consistent total bile acid pool size and composition in the gastrointestinal system.

4.2. FXR

FXR is widely expressed in the gastrointestinal tract and plays a central role in regulation of the enterohepatic circulation of bile acids, though the contribution of FXR to the regulation of bile acid synthesis may be highly tissue-specific.[59] FXR was first identified as a nuclear receptor activated by farnesol metabolites.[5–8] Taurochenodeoxycholic acid (TCDCA) is the most efficacious endogenous FXR agonist (EC_{50} = 17 mM), while taurocholic acid (TCA) is a weak FXR agonist (EC_{50} = ~600 μM).[8] *Fxr*$^{-/-}$ mice have increased serum bile acids, cholesterol, and triglycerides, and increased hepatic cholesterol and a proatherogenic serum lipoprotein profile.[25] Bile acid pool and fecal bile acid secretion are reduced in *Fxr*$^{-/-}$ mice due to reduced biliary bile acid secretion. These mice also have reduced expression of BSEP, IBABP, and multi-drug resistant protein 2 (Mdr2),[25] suggesting bile acid transporters and binding proteins are FXR targets and that FXR plays critical roles in regulating bile acid and hepatic lipid homeostasis (Fig. 3). In the liver, FXR induces BSEP to efflux bile acids into bile. FXR inhibits NTCP, whose expression is regulated by the retinoic acid receptor (RAR)/retinoid X receptor (RXR) heterodimer.[60] Thus, FXR maintains very low levels of intrahepatic bile acids. In the ileum, FXR inhibits ASBT and induces IBABP,[59] which transports bile acids from the apical brush border membrane to the sinusoidal membrane, where FXR induces OSTα/OSTβ to efflux bile acids into portal blood. Therefore, FXR

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plays a critical role in the control of the enterohepatic circulation of bile acids to maintain a constant circulating bile acid pool (Fig. 3).

In the ileum, bile acid concentrations are high and activate FXR, which induces the expression and release of the enteroendocrine hormone fibroblast growth factor 19 (FGF19; Fgf15 in mice). FGF19 is secreted into portal circulation and activates hepatic membrane fibroblast growth factor receptor 4 (FGFR4)/β-Klotho complex/extracellular signal-regulated kinase (ERK) and c-Jun N-terminal kinase (JNK) signaling to inhibit bile acid synthesis.[61] This intestinal FXR/FGF19-to-liver FGFR4 signaling pathway may be the primary physiological mechanism for bile acid feedback regulation of bile acid synthesis. When bile acids accumulate in hepatocytes, such as during cholestatic liver injury, bile acids may activate FXR to induce the negative nuclear receptor small heterodimer partner (SHP), which inhibits trans-activation of the *CYP7A1* and *CYP8B1* genes by interfering with the transcriptional activators hepatocyte nuclear factor 4α (HNF4α) and liver-related homologue-1 (LRH-1) (Fig. 3). Thus, FXR plays a critical role in the regulation of bile acid synthesis, transport and enterohepatic circulation of bile acids to inhibit bile synthesis and prevent cholestatic injury. The small amount of bile acids excreted into feces (5%, ~0.5 g/day in humans) are replenished by *de novo* synthesis in hepatocytes.

5. Regulation of *CYP7A1* gene transcription

5.1. CYP7A1 gene

CYP7A1, the critical point of regulation in bile acid synthesis, is tightly regulated by bile acids returning to the liver via the enterohepatic circulation.[1] The underlying molecular mechanism of bile acid-based regulation of CYP7A1 has been difficult to elucidate. Most early studies of bile acid synthesis and CYP7A1 were carried out using rat, rabbit and hamster models. Purification of CYP7A1 from rat liver and reconstitution with NADPH-cytochrome reductase confirmed that CYP7A1 is a cytochrome P450 enzyme expressed in liver microsomes and utilizes cholesterol as a substrate.[3] It was also shown that CYP7A1 specific activity is 2-fold higher in female rats compared to male rats, and its activity is regulated by drugs and hormones: phenobarbital, 3-methylcholanthrene, pregnenolone 16α-carbonitrile and thyroid hormone T_3 induce CYP7A1 activity, and dexamethasone inhibits its activity.[3,62–64]

The *CYP7A1* gene encodes multiple mRNA species, which are translated to a protein of 503 amino acid residues with a heme binding sequence typically present in CYP enzymes.[2,4] In rats, cholesterol or bile acid-binding resin (sequestrant) feeding induced CYP7A1 mRNA, protein and activity in parallel, suggesting bile acids and cholesterol regulate CYP7A1 mainly by transcriptional mechanisms. The full length 3.6 kb mRNA species contains a coding region of 1509 bp and a 2.5 kb 3′-untranslated region (3′-UTR) with multiple polyadenylation signals and AUUU sequence motifs, indicating short half-life mRNAs and post-translational modification may be also involved in the regulation of CYP7A1 expression. The human *CYP7A1* gene spans ~11 kb of the genome.[65] The human and rat CYP7A1 amino acid coding sequences are highly homologous (90%), but the human *CYP7A1* gene promoter sequence diverges substantially from rat and mouse *Cyp7a1* genes, [66] and the human *CYP7A1* gene responds to few hormones. Thyroid hormone T_3 induces rat

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Cyp7a1,[62] but represses human *CYP7A1* gene expression and several thyroid hormone response elements have been identified in the human *CYP7A1* and rat *Cyp7a1* gene promoter.[65,67] Glucocorticoid and T_3 synergistically induce *Cyp7a1* mRNA in rats,[68] while glucocorticoid, glucagon and 3′,5′-cyclic adenosine monophosphate (cAMP) all inhibit human *CYP7A1* gene transcription.[69]

5.2. Mechanisms of bile acid regulation of CYP7A1 gene transcription

The underlying molecular mechanism of bile acid feedback regulation of bile acid synthesis appears to be very complex and not completely understood.[38,70,71] Analysis of the rat *Cyp7a1* gene promoter by DNase I footprinting identified several nuclear hormone response elements (AGGTCA motifs) and two putative bile acid responsive elements (BAREs), BARE-I (direct repeat with 4 nucleotide spacing, DR4, nt−74 to −55) and BARE-II (DR1, nt −149 to −128) (Fig. 4).[72–74] Transcription factors bind to the BARE-I and BARE-II to stimulate RNA polymerase and basal transcription of the *CYP7A1* gene (Fig. 4).

5.2.1. Hepatic FXR-dependent mechanisms—It was first proposed that FXR induced the nuclear receptor SHP to inhibit transactivation of the *CYP7A1* gene by HNF4α and LRH-1 (Mechanism i, Fig. 4).[75,76] However, in *Shp* knockout mice bile acid-feeding still inhibits *Cyp7a1* gene transcription, suggesting that redundant pathways may exist for bile acids to inhibit transcription of the *Cyp7a1* gene.[77] A recent study showed that FXR induced V-maf avian musculoaponeurotic fibrosarcoma oncogene homologue G (MAFG), which was a negative regulator of bile acid synthesis (Fig. 4).[78] Overexpression of MAFG in mouse liver repressed *Cyp7a1*, *Cyp8b1*, *Cyp7b1* and *Cyp27a1* gene transcription. Interestingly, MAFG reduced CA but increased muricholic acids. In *Mafg* knockdown mice, *Cyp7a1* and *Cyp8b1* expression were increased with increased CA levels. These results suggest that MAFG may inhibit *Cyp8b1* gene transcription more than *Cyp7a1* in an FXR-dependent manner. The cause of the differential regulation of *Cyp8b1* by MAFG compared to *Cyp7a1*, *Cyp7b1* and *Cyp27a1*, and the mechanism of MAFG oncogene in the inhibition of these genes, are not clear.

5.2.2. Intestinal FXR/FGF15 to liver FGFR4 pathway—In 1995, Pandak *et al.*[79] first reported that intraduodenal, but not intravenous, infusion of TCA inhibited *Cyp7a1* mRNA expression in biliary fistula rats. These authors suggested that a putative intestinal factor released or reabsorbed in the presence of bile acids in the ileum might be involved in the regulation of bile acid synthesis. Years later, a study showed that *Cyp7a1* mRNA and bile acid pool size were induced in *Fgfr4*$^{-/-}$ mice.[80] This study also suggested that JNK mediated Fgfr4 repression of the *Cyp7a1* gene. Later, Inagaki *et al.*[61] identified that the intestinal FXR-induced endocrine hormone FGF15/FGF19 activated hepatic FGFR4/β-Klotho signaling to inhibit *CYP7A1* via JNK (Mechanism ii, Fig. 4). In mice, Fgf15 is produced in the intestine but not liver.[81] In human primary hepatocytes, FGF19 is expressed and is induced by bile acids to activate ERK1/2 signaling to inhibit *CYP7A1* expression.[82] In obstructive cholestatic patients, FGF19 is highly induced in the liver to inhibit *CYP7A1* expression and bile acid synthesis.[83]

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Another study reports that intestinal FGF15 was down-regulated by Kruppel-like factor 15 (KLF15) in the ileum and the KLF15-FGF15 signaling axis regulated the circadian expression of *Cyp7a1* and bile acid synthesis.[84] Klf15 is expressed in mouse liver but did not regulate *Cyp7a1* mRNA expression. Overexpression of Kfl15 reduced *Fgf15* expression in mouse ileum, which was reversed by Klf15 knockdown. In *Klf15*$^{-/-}$ mice, Fgf15 was strongly induced in the ileum. However, KLF15-FGF15 regulation of bile acid synthesis is independent of bile acid/FXR/FGF15 signaling. Another study reports that Diet1 is co-expressed with Fgf15 in mouse enterocytes.[85] Diet1 encodes a 236 kD protein consisting of LDL receptor meprin-A5-protein phosphatase *mu* domains. Diet1 deficiency in mice increased the bile acid pool size and impaired bile acid feedback regulation of Cyp7a1, while transgenic overexpression of Diet1 restored Cyp7a1 regulation in these mice. Interestingly, mutations in the *Diet1* gene increased FGF19 secretion in bile acid diarrhea (BAD) patients.[86] How Diet1 regulates Fgf15 and the relevance of the Diet1/Fgf15 axis in the regulation of bile acid synthesis is not clear.

5.3. Regulation of CYP7A1 by cell signaling

Studies from Roger Davis' laboratory showed that CDCA activates macrophages to secret cytokines, tumor necrosis factor (TNF) α and interleukin (IL)-1β, which may cross the hepatic sinusoidal membrane to activate protein kinase C and JNK signaling to inhibit *CYP7A1* expression independent of FXR (Mechanism iii, Fig. 4).[28,87,88] In primary human hepatocytes, CDCA and IL-1β markedly induced c-Jun to suppress *CYP7A1* and *CYP8B1* expression.[89,90] Furthermore, CA feeding induced TNFα and IL-1β, and induction of cytokines was blunted in *Jnk*$^{-/-}$ mice, suggesting that bile acids may activate the bile acid receptor TGR5 in macrophages and Kupffer cells to stimulate pro-inflammatory cytokine production via the JNK pathway to inhibit *CYP7A1* and *CYP8B1* gene transcription.[88] The cell signaling mechanism initiated by Kupffer cells may be rapidly activated to reduce bile acid synthesis as an adaptive response to cholestatic liver injury. Concurrently, infiltration and accumulation of neutrophils in the liver mediates cholestatic liver injury in mice,[91] but evidence for this in humans is still controversial.[92]

5.4. TGR5 signaling in bile acid synthesis

TGR5 is widely expressed in the epithelial cells of intestine and gallbladder, liver sinusoid endothelial cells, and Kupffer cells, but is not expressed in hepatocytes.[9,93] The secondary bile acids DCA and LCA bind to TGR5, increasing intracellular cAMP and activating protein kinase A and cAMP response element binding protein (CREB) signaling pathways to regulate gene transcription.[94] *Tgr5*$^{-/-}$ mice have a reduced bile acid pool size and are protected from lithogenic diet-induced cholesterol gallstone formation.[95,96] TGR5 is highly expressed in the gallbladder, and activation of TGR5 causes smooth muscle relaxation and stimulates gallbladder filling.[97] Activation of TGR5 stimulates brown adipose tissue energy metabolism by inducing deiodinase type 2 (DIO2), which converts thyroid hormone T_4 to trihydroxythyronine T_3. White adipose tissue browning and mitochondrial energy metabolism are also stimulated by TGR5.[98] *Tgr5*$^{-/-}$ mice have reduced *Cyp7b1* expression, increased TCA and reduced tauromuricholic acid (TMCA) in bile, and are resistant to fasting-induced hepatic steatosis compared to wild type mice.[99] Fasted *Tgr5*$^{-/-}$ mice have increased activation of hepatic growth hormone-signal transducer and activator of

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transcription 5 (Stat 5) signaling, which may regulate *Cyp7b1* gene transcription. FXR and TGR5 are co-expressed in enteroendocrine L cells and activation of FXR induces *Tgr5* gene transcription,[100] proglucagon synthesis, and glucagon-like peptide-1 (GLP-1) secretion,[101] while activation of TGR5 stimulates glucose-induced GLP-1 secretion from L cells and improves insulin sensitivity (Fig. 3).[100]

5.5. Regulation of CYP7A1 gene transcription by transcription factors

The *Cyp7a1* gene promoter regions BARE-I and BARE-II contain several hormone receptor binding sequence motifs that have been shown to bind liver-enriched transcriptional factors and nuclear receptors (Fig. 4).

5.5.1. Liver-enriched transcription factors—In the rat *Cyp7a1* gene proximal promoter, HNF4α, HNF1 and CAAT/enhancer-binding protein bind BARE-I (C/EBP).[102,103] Nuclear receptors are ligand-activated transcription factors that bind to DNA sequences to activate RNA polymerase II and general transcription factors. A heterodimer comprised of orphan nuclear receptors chicken ovalbumin upstream promoter transcription factor II (COUP-TFII) and RXRα binds to both BARE-I and BARE-II (Fig. 4).[72,104] HNF4α homodimers and COUP-TFII/RXRα may interact to regulate *CYP7A1* gene transcription.[74] At physiological concentrations (10 μM), CDCA and CA activate FXR and repress *CYP7A1* promoter activity via BARE-II, but FXR/RXRα do not bind to this element, suggesting an indirect mechanism for FXR to mediate bile acid repression.[105] Later studies reported that FXR induced SHP to inhibit *CYP7A1* gene transcription activated by HNF4α and LRH-1 (Fig. 4).[75,76] The human *CYP7A1* promoter sequence diverges from the rat *Cyp7a1* gene promoter sequence but the BARE sequences are preserved among different species.[65] HNF4α is a liver-enriched transcription factor that plays a critical role in the basal transcription of *Cyp7a1* and many other liver-specific genes.

The human CYP7A1 transcription factor (CTF), mouse LRH-1, and α-fetal protein transcription factor (FTF) belong to the NR5A2 family of nuclear receptors and control the basal level expression of the *CYP7A1* gene.[106] Several mechanisms have been implicated in the modulation of HNF4α/LRH-1 binding to the human and rat BAREs to inhibit *CYP7A1* promoter activity (Fig. 4). As described previously, FXR induces SHP to inhibit transactivation of the *CYP7A1* gene by HNF4α and LRH-1 (Mechanism i, Fig. 4). FGF19/FGFR4 activates JNK1/2 and ERK1/2 to phosphorylate HNF4α and reduce HNF4α homodimer binding to the BARE (Mechanism ii, Fig. 4),[82,107] while bile acids and cytokines activate ERK1/2 and JNK1/2 pathways to inhibit human *CYP7A1* gene transcription (Mechanism iii, Fig. 4).[90]

In addition, hepatocyte growth factor (HGF) has been shown to inhibit human *CYP7A1* via c-Jun, JNK1/2 and ERK1/2 signaling (Fig. 4).[108] HGF is known to activate the tyrosine protein kinase c-Met to phosphorylate PI3K, Ras and Src kinases mediating cell proliferation and regeneration. Knockdown of *c-Met* by siRNA increased *CYP7A1* mRNA levels and blocked HGF inhibition of *CYP7A1* mRNA in human hepatocytes. Further, HGF recruited c-Jun and SHP but blocked the co-activators peroxisome proliferator activated

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receptor γ-coactivator 1 (PGC-1α) and CREB-binding protein (CBP) from binding to CYP7A1 chromatin, thus repressing *CYP7A1* gene expression (Fig. 4).

Lastly, prospero-related homeodomain protein 1 (Prox1) has been shown to interact with FTF and repress *Cyp7a1* expression (Fig. 4) by interacting with HNF4α and PGC-1α to inhibit *CYP7A1* expression in human hepatocytes.[109] siRNA knockdown of *Prox1* significantly increased *CYP7A1* mRNA expression in human hepatocytes.

5.5.2. Peroxisome proliferator-activated receptor alpha (PPARα)—PPARα is highly expressed in the liver. Activation of PPARa by fibrates stimulates lipolysis of triglycerides in adipose tissue as well as efflux of free fatty acids to the liver and muscle for fatty acid oxidation and energy metabolism. However, activation of the PPARα/RXRα heterodimer by fibrates enhances its binding to DR1 in the BARE-II, thus preventing HNF4α binding to the BARE and ultimately inhibiting human *CYP7A1* gene transcription (Fig. 4).[110]

5.5.3. Pregnane X receptor (PXR)—PXR is a bile acid, steroid and drug-activated nuclear receptor that binds to the BARE-II in the human *CYP7A1* gene promoter (Fig. 4) and is involved in coordinate regulation of drug metabolism and bile acid homeostasis.[111] PXR is activated by LCA and induces CYP3A4 in liver and intestine, leading to induction of phase I drug oxidation CYPs, phase II drug conjugation enzymes and phase III drug transporters to detoxify and secrete drugs and LCA. Rifampicin is a specific human PXR agonist that inhibits *CYP7A1* gene expression via blockade of PGC-1α/HNF4α interaction in human hepatocytes (Fig. 4),[112] but has no effect in mice, while pregnane activates mouse PXR, but not human PXR.[113] In Cyp3a11 deficient mice, Cyp7a1 is activated and bile acid synthesis is increased, which increases *de novo* cholesterol synthesis.[114]

5.5.4. Liver X receptor (LXR)—Humans are resistant to diet-induced hypercholesterolemia due to the presence of the ATP-binding cassette subfamily G member 5/8 (ABCG5/8) heterodimer, which effluxes most dietary cholesterol and plant sterols absorbed in the intestine.[115] Cholesterol homeostasis is maintained by the conversion of cholesterol to bile acids, which facilitates biliary excretion of cholesterol. Cholesterol feeding induces Cyp7a1 via activating LXRα in mice, but not in humans (Fig. 4).[116] In LXRα deficient mice, dietary cholesterol accumulates in liver and fails to upregulate Cyp7a1. LXRα is an oxysterol-activated receptor expressed in hepatocytes and macrophages that is involved in lipogenesis.[117] LXRα binds to the DR4 motif in the rat BARE-I to induce *Cyp7a1* gene transcription, but the human CYP7A1 promoter lacks the DR4 motif, and thus, does not bind LXRα.[118] Cholesterol is a substrate of CYP7A1, but cholesterol feeding inhibits CYP7A1 and bile acid synthesis in human hepatocytes.[119] It is possible that activation of FXR signaling may override the stimulation by LXRα to inhibit CYP7A1.[120]

5.5.5. Vitamin D receptor (VDR)—VDR is activated by 1α,25-dihydroxyvitamin D_3, the active form of vitamin D_3 produced in the kidney. VDR is closely related to PXR and plays a critical role not only in calcium and phosphate homeostasis and bone formation, but also in immunomodulation, cell growth and differentiation.[121] Deficiency of VDR or mutations of the *VDR* gene cause low serum calcium, type II rickets and bone malformation.

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VDR is also a bile acid-activated nuclear receptor in the intestine and that is efficaciously activated by LCA.[122] VDR is abundantly expressed in the kidney, intestine and bone but is expressed in very low levels in other tissues. Activation of VDR induces CYP3A4 and sulfotransferases in intestine and liver to sulfonate and detoxify drugs for biliary and renal excretion. VDR is expressed in mouse stellate cells but not hepatocytes. Activation of VDR inhibits stellate cell activation by Tgfβ1/small mothers against decapentaplegic (Smad) signaling and protects against liver fibrosis in mice.[123] On the contrary, VDR is expressed in human hepatocytes and activation of VDR by LCA inhibits *CYP7A1* expression by interacting with HNF4α and competing with co-activators.[124] VDR also has non-genomic actions at the plasma membrane. Activation of VDR by LCA and 1α, 25 $(OH)_2$-D_3 induces VDR translocation from the cytosol to the plasma membrane to induce tyrosine phosphorylation of c-Src and the downstream c-Raf/MEK1/2/ERK1/2 pathway, which phosphorylates HNF4α to inhibit *Cyp7a1* gene expression.[125] This may be a rapid response of bile acids to protect hepatocytes from cholestatic injury.

5.5.6. Regulation of the CYP8B1 gene—CYP8B1 is also inhibited by bile acids.[126] CYP8B1 determines the ratio of CA to CDCA in humans and the ratio of CA to MCA in mice, and thus, the hydrophobicity of circulating bile acids. The rat and human *CYP8B1* gene promoters contain HNF4α and LRH-1/FTF binding sequences.[126–129] Thyroid hormone suppresses Cyp8b1 activity and mRNA expression.[130] In contrast to *Cyp7a1*, cholesterol feeding suppresses *Cyp8b1* expression in mice.[131] Interestingly, steroid responsive element binding protein (Srebp)-1a and Srebp1-c, but not Srebp-2, stimulates *Cyp8b1* mRNA expression and SREBP response element (SRE) and E boxes were identified in the rat *Cyp8b1* gene promoter. SREBP activation and procession are inhibited by oxysterols, consistent with cholesterol inhibition of *Cyp8b1* via Srebp-1. A recent study reports that the anabolic steroid hormone epistane elevated GCA by upregulation of *CYP8B1* in human hepatocytes.[132] This recreational steroid activates androgen receptor, LXR α, and PXR, leading to increasing expression of *CYP8B1*. Interestingly, an *in vitro* assay showed that CYP8B1 can convert CDCA to CA.[133] Similar to *Cyp7a1*, *Cyp8b1* gene transcription is suppressed by the FXR/SHP mechanism.[134] Furthermore, the liver FXR/SHP pathway preferentially suppresses *Cyp8b1*, while the intestine FXR/FGF15 pathway preferentially inhibits *Cyp7a1*.[107]

5.5.7. Regulation of the CYP7B1 gene—Bile acids modestly reduce *Cyp7b1* expression in mice.[135,136] Inhibition of cholesterol synthesis by squalestatin decreased CYP7B1, whereas cholesterol increased CYP7B1 activity and mRNA expression. The human *CYP7B1* coding sequence shares about 40% sequence identity to *CYP7A1*.[137] CYP7B1 catalyzes the 7α-hydroxylation of cholesterol, 25- and 27-hydroxycholesterol, and dehydroepiandrosterone (DHEA) and CYP7B1 is critical for the metabolism of oxysterols.[44,138] SREBP inhibits LXRα-induced *CYP7B1* gene transcription.[139] Thus, cholesterol activates LXRα to inhibit SREBP and induce CYP7B1 to metabolize oxysterols to bile acids. In *Cyp7b1* deficient mice, bile acid pool size and serum cholesterol and triglycerides are maintained, but 25- and 27-hydroxycholesterol are increased compared to wild type mice.[26] *CYP7B1* expression is upregulated by retinoic acid-related orphan receptor alpha (RORα) and is repressed by LXRα.[140] Cyp7b1 expression is sexually diphormic and male-

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specific, and is regulated by growth hormone/Stat5.[99,141] Estrogen regulates CYP7B1 expression via insulin/phospho-inositol-3 kinase signaling.[142] Interestingly, a recent study reported that cold exposure in mice induces cholesterol synthesis and *Cyp7b1* expression to convert cholesterol to bile acids via reshaping the gut microbiota.[143] This study suggests that bile acids are important metabolic effectors in sustained brown adipose tissue activation during thermogenesis.

5.5.8. Regulation of the CYP27A1 gene—CYP27A1 is located in the inner mitochondrial membrane. CYP27A1 catalyzes oxidation of the steroid side chain of cholesterol for bile acid synthesis. Transport of cholesterol into mitochondria is mediated by steroidogenic acute regulatory protein D1 (StarD1) to activate CYP27A1.[44,144,145] Bile acids inhibit HNF4α-mediated trans-activation of human *CYP27A1* gene transcription.[146] Growth hormone, insulin-like growth factor-1, steroidogenic acute regulatory protein and dexamethasone increase *CYP27A1*, whereas T_4 and PMA reduce *CYP27A1* gene promoter activity.[147] *CYP27A1* is induced by dexamethasone and suppressed by cyclosporin A, while CA reduced *CYP27A1* mRNA stability.[148] Glucocorticoid receptor mediates dexamethasone induction of *CYP27A1* gene transcription.[142] Interestingly, PXR induces Cyp27a1 and the cholesterol efflux transporters ABCA1 and ABCG1 by activating LXRα in the intestine rather than in liver.[149]

6. Post-transcriptional regulation of CYP7A1

Several post-transcriptional mechanisms have been implicated in the regulation of *Cyp7a1* expression. These include regulation of mRNA stability, regulation by microRNAs (miRNAs), and epigenetic regulation.

6.1. Regulation of mRNA stability

The 3′-UTR of *Cyp7a1* mRNA transcripts have multiple AUUUU sequences indicative of short half-life mRNA, which is regulated by bile acids.[150] It has been reported that Apob mRNA editing enzyme catalytic polypeptide 1 (Apobec-1)-deficient mice had significantly lower expression of *Cyp7a1* mRNA and protein, indicating Apobec-1 may stabilize *Cyp7a1* mRNA.[151] Apobec-1 is an AU-rich RNA binding protein and a cytidine deaminase that converts a cytosine in the glutamine (CAA) codon to uracil, creating a stop codon (UAA) in the *Apob100* gene, thus synthesizing a truncated Apob48 in human and mouse intestine and in mouse liver. It was demonstrated that Apobec-1 binds to AU-rich regions of the 3′-UTR of *Cyp7a1* mRNA to stabilize the transcript. Thus, bile acids and lipids coordinately regulate bile acid synthesis and *Apob* expression.[151] On the other hand, a recent study reports that activation of FXR induces the RNA binding protein ZFP36L1 which binds to the 3′-UTR region to destabilize *Cyp7a1* mRNA.[152] ZFP36L1-deficient mice have altered bile acid synthesis, reduced adiposity and are protected from diet-induced obesity and hepatic steatosis. This study uncovered SHP-independent FXR signaling for rapid inhibition of *Cyp7a1* expression and bile acid synthesis by post-transcriptional mechanisms.

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6.2. Regulation by miRNA

MiRNAs are small non-coding RNAs (about 19e25 nucleotides) that bind to complementary sequences in the 3′-UTR of mRNA to inhibit mRNA transcription. The liver-specific miRNA miR-122a is the predominant miRNA expressed in hepatocytes and has been shown to regulate cholesterol synthesis and lipid metabolism.[153,154] MiR-122 expression level is decreased in hepatocellular carcinoma and non-alcoholic steatohepatitis (NASH).[155,156] Screening of a human miRNA microarray identified 5 differentially expressed miRNAs, including miR-122a, in human hepatocytes treated with CDCA, the FXR agonist GW4064 or FGF19. *Cyp7a1* expression is down-regulated by miR-122a mimic and induced by miR-122a antagomir. A miR-122a binding sequence has been identified in the 3′-UTR of human *CYP7A1* mRNA near the Apobec-1 binding site.[157] MiR-122a also regulates many liver-specific transcription factors, including HNF1, HNF3, HNF4 and C/EBP. MiR-422a binding sites were also identified in the 3′-UTR of human *CYP7A1* and *CYP8B1* mRNAs. MiR-33a, which is located in the intron on the SREBP-2 gene, has been shown to regulate CYP7A1 and plays a key role in regulation of cholesterol homeostasis.[158–160] Overexpression of miR-33a in mouse liver increased hepatic cholesterol and reduced the bile acid pool and serum cholesterol.[158]

6.3. Regulation by epigenetic mechanisms

It was first reported that bile acid synthesis is regulated by epigenetic mechanisms through SHP-mediated chromatin remodeling.[161] Acetylation of FXR by histone acetylase p300 is important for ligand-activated FXR to induce SHP.[162] SHP is rapidly degraded through a ubiquitin-proteasomal degradation pathway, and FGF19 increased SHP stability by inhibiting ERK-dependent ubiquitin-proteasomal degradation.[163] Interestingly, SHP stability was increased in leptin-deficient mice and diet-induced obese mice. FXR is also regulated by sirtuin 1 (SIRT1), a histone acetylase that mediates nutrient and hormone regulation of hepatic metabolism.[164] Acetylation of FXR increases its stability but inhibits its interaction with RXRα, as well as its DNA binding and transcriptional activity. In metabolic disease, FXR interaction with SIRT1 and p300 was altered and FXR acetylation was increased. Chromatin immunoprecipitation assay showed that bile acids induced recruitment of the mSin3A and Swi/Snf complex containing Brm ATPase to interact with SHP and repress *CYP7A1* promoter activity. Bile acids or FGF19 induced protein kinase C zeta to phosphorylate and translocate SHP to the nuclei to regulate its target genes in liver metabolism.[165] Phosphorylation of Thr-55 is critical for SHP function and interaction with chromatin modifiers at bile acid responsive genes. Mutation of Thr-55 attenuates SHP-mediated epigenomic and metabolic effects. Another interesting study showed that FXR is acetylated in diet-induced obese mice.[166] Acetylation of FXR blocked interaction with small ubiquitin like modifier (SUMO) ligase, resulting in reduced SUMOylation and activation of inflammatory gene expression. Thus, dysregulation of acetylation and SUMOylation of FXR reduced the anti-inflammatory effect of FXR signaling in metabolism and obesity.[167]

Glucose stimulates *CYP7A1* transcription in human hepatocytes.[168] Activation of AMP-activated protein kinase (AMPK) reduces *CYP7A1* expression by inhibiting HNF4α binding to *CYP7A1* chromatin. Glucose is metabolized to acetyl-CoA, which stimulates histone acetylation and decreases H3K9 di- and tri-methylation of *CYP7A1* chromatin. This study

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suggested that glucose regulates *CYP7A1* and bile acid synthesis by epigenetic mechanisms in diabetes. In streptozocin-induced diabetic mice and genetically obese mice, hyperglycemia increased histone acetylation status of the *Cyp7a1* gene promoter, leading to elevated basal levels of *Cyp7a1* expression and enlarged bile acid pool size with altered bile acid composition.[169] In these mice, CA levels are increased while MCAs are decreased, total bile acid pool size is increased, and *Cyp7a1* chromatin is hyperacetylated. The fasting to refeeding response of *Cyp7a1* is impaired and may exacerbate metabolic disorders in diabetic mice.

G9a, a methyltransferase, inhibits *Cyp8b1* gene transcription via epigenetic modification of its chromatin. G9a is co-localized with SHP and inhibits *CYP7A1* by inducing H3K9 methylation.[170] SHP is associated with lysine-9-methylated histone 3. The naturally occurring SHP mutant R213C interacts less avidly with lysine-9-methylated histone and has lower transcriptional repressor activity.[171] SHP repression involves a multistep mechanism, including histone deacetylation followed by H3K9 methylation by G9a to stabilize the association of SHP with chromatin. Another study reports that Prox-1 inhibits *Cyp7a1* by interacting with the lysinespecific demethylase 1 (LSD1)/nucleosome remodeling and histone deacetylase (NuRD) repressive complex.[172] Prox-1 was shown to recruit LSD1 and histone deacetylase 2 (HDAC2) to the *Cyp7a1* promoter to increase H3K4 demethylation, while bile acid treatment increased Prox-1 and LSD1/NuRD occupation at the *Cyp7a1* promoter to stimulate deacetylation.

7. Circadian regulation of CYP7A1 and bile acid synthesis

Circadian rhythms coordinate transcription and epigenetic regulation of many metabolic pathways linked to energy metabolism and play a critical role in liver function and disease.[173–176] Circadian rhythms are generated by the central biological clock located in the suprachiasmatic nucleus (SCN) in the hypothalamus, which synchronizes physiological and cellular responses with environment cues such as light, and non-photic cues including nutrient availability, behavioral activity, drugs and alcohol.[177] At the molecular level, rhythms are generated by transcription and translation of core clock genes both in the SCN and in the periphery. The core clock protein products Clock and brain and muscle ARNT-like 1 (Bmal1) heterodimerize and induce two negative clock genes, *Cryptochrome 1* and *2* (*Cry1/2*) and *Period 1* and *2* (*Per1/2*), whose proteins inhibit *Clock* and *Bmal1* gene transcription. As Per and Cry proteins accumulate, they are phosphorylated by casein kinase, tagged for degradation, and *Clock* and *Bmal1* gene transcription is disinhibited (Fig. 5). *Clock* mutant mice are obese and hyperphagic, and have increased hepatic cholesterol when fed a high cholesterol and CA diet, suggesting *Clock* and circadian rhythms play an important role in liver and lipid metabolism.[178]

The expression of liver Cyp7a1 mRNA, protein and activity levels in rats and mice, which are nocturnal, exhibit a distinct diurnal rhythm peaking at mid-night and declining to a nadir at midday.[3,177,179] Serum bile acids exhibit two peaks in mice, at the beginning and end of the dark phase. Primary bile acids were increased during the dark phase while secondary bile acids were increased during the light phase.[180] Unconjugated bile acids peaked during the

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day and were lowest at night. Serum Fgf15 peaked at approximately 4 h after the peak in *Cyp7a1*, consistent with Fgf15 inhibition of bile acid synthesis.

The postprandial rise in bile acid synthesis and *Cyp7a1* expression corresponds to the time of feeding.[19] Fasting and refeeding differentially affects clock gene expression, dependent upon the tissue and its metabolic activity.[181,182] Feeding rapidly induced *Cyp7a1* expression and altered its diurnal expression pattern, which declined during the post absorptive and fasting states.[179] Circadian disruption affects liver and bile aid metabolism. Even short-term sleep disruption (6 h/day for 1 week) markedly altered the diurnal rhythm and amplitude of *Cyp7a1* mRNA expression in mice. The increase in *Clock* and *Bmal1* expression parallels the increase in *Cyp7a1* and *HNF4α* mRNA expression levels, which peaks 2 h into the dark phase in mice.[179] The circadian transcription of *Cyp7a1* mRNA also parallels the circadian expression of D-site binding protein (DBP), a liver-enriched b-ZIP protein, suggesting DBP regulates rhythmic expression of *Cyp7a1*.[183] Dbp is a clock-controlled gene that peaks at night and is undetectable in the morning in rat liver.[184,185] *Dbp* mRNA expression peaks 4 h before *Cyp7a1* mRNA peaks and multiple functional DBP binding sites were identified in the rat *Cyp7a1* gene promoter.[186] The nuclear receptors *Rev-erbα*, *Rorα*, *Pparα*, and *Pgc1α* are clock-controlled genes that regulate *Clock* and *Bmal1* expression by binding to the E-box motifs (CANNTG) on the clock gene promoter (Fig. 5).[187] Rev-erbα is a transcriptional repressor and a heme sensor that coordinately regulates the cellular clock, glucose homeostasis and energy metabolism.[188] *Rev-erbα* expression peaks earlier than Dbp and decreases to basal levels during the dark phase when *Cyp7a1* peaks. Dbp and Rev-erbα may be the major positive and negative regulators, respectively, of the *Cyp7a1* diurnal rhythm in mice.[169,179]

Clock/Bmal1-controlled Rev-erbα plays a key role in the regulation of rhythmicity of bile acid synthesis and is the first direct metabolic output of the circadian clock.[173] In mice, Rev-erbα regulates the circadian expression of Srebp, which regulates its target LXRα to induce *Cyp7a1* expression.[189] Upon feeding, Rev-erbα inhibits the cyclic expression of insulin sensitive gene 2, which sequesters Srebp in the Golgi membrane and blocks its translocation to the endoplasmic reticulum. Interestingly, Rev-erbα deficient mice have increased lipogenesis through induction of Srebp1c and Srebp2. Shp and E4bp4, negative regulators of *Cyp7a1*, are increased in *Rev-erbα*$^{-/-}$ mice, indicating they are direct targets of Rev-erbα.[190] Another clock-controlled gene, Dec2, is a basic-helix-loop-helix transcriptional repressor that binds to the E-box and suppresses *Cyp7a1* gene expression induced by Dbp in rat liver.[191] In free-fed mice the circadian rhythm of *Cyp8b1* is opposite to that of *Cyp7a1* (Fig. 5). RORa induces *Cyp8b1* expression while Rev-erbα suppresses *Cyp8b1* expression and Dec2 suppresses *Cyp8b1* expression induced by Dbp.[191,192] Another study reports that restricted feeding shifted the peripheral clock and increased bile acid synthesis, activating PXR and constitutive active receptor (CAR) to increase serum aspartate amino transferase and cholestatic injury.[193] *Per1/Per2* double knockout mice have disrupted rhythms in *Cyp7a1*, *Ntcp* and *Dbp* which altered bile acid homeostasis and caused hepatic cholestasis.[193] Another interesting study reports that in *Klf15*$^{-/-}$ mice, the oscillation of Cyp7a1 and Cyp7b1 mRNA and protein was attenuated.[84] KLF15 plays a role in nutrient flux and utilization during fasting and feeding cycles. Oscillation of KLF15 regulates the circadian expression of Fgf15, which inhibits *Cyp7a1* expression in mouse liver. *Klf1*5 mRNA

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expression shows a strong circadian rhythm that coincides with the peak in *Cyp7a1* mRNA. Klf15 may not directly regulate circadian rhythms in bile acid synthesis but could play a more important role in nutrient supply during starvation.

There are a few studies of circadian rhythms of bile acid synthesis in human patients. One study reports bile acid synthesis (assayed by serum C4 levels) in humans has a rapid diurnal variation over 24 h, with peaks at 1 p.m. and 9 p.m., coinciding with food intake and declining at night.[194] These variations are not synchronized with the cholesterol synthesis marker lathosterol, which peaks at night. Other studies indicate conjugated and unconjugated bile acids have asynchronous rhythms, or that unconjugated bile acids peak late at night. The diurnal change in serum C4 is not synchronized with serum lipids or the postprandial rise of serum bile acids. Limited studies in human patients showed serum FGF19 levels vary greatly among individuals and have a pronounced diurnal variation that precedes the decline of bile acid synthesis during fasting conditions.[195] Bile acid synthesis varies greatly in normal individuals and is typically higher in men than women.[196] Serum bile acid levels are positively correlated to serum triglycerides. Fasting induces FGF21 in serum, which varied 250-fold among individuals, did not display any diurnal variation, and was unrelated to serum bile acids.[197] Postprandial transintestinal bile acid flux increases circulating FGF19 levels and suppresses bile acid synthesis, supporting the role of FGF19 in the regulation of bile acid synthesis in humans.[198]

8. CYP7A1 in human diseases and therapies

Inborne errors of bile acid synthesis have been identified in 13 of the 17 enzymes involved in bile acid synthesis (reviewed in Ref 19 and 199)[199]. Most inborne errors of bile acid synthesis are identified in neonates and newborns. Deficiency of bile acid synthesis genes causes malabsorption of fats, steroids and nutrients, and accumulation of toxic steroid intermediates, which cause liver injury and cholestatic liver diseases.

8.1. Mutations and polymorphisms of CYP7A1 and other sterol hydroxylase genes

8.1.1. CYP7A1 mutations—Only one family of patients with a *CYP7A1* gene mutation has been identified. These patients have dyslipidemia, premature atherosclerosis and gallstone disease, consistent with the role of bile acids in maintaining cholesterol and lipid metabolism.[200] The double mutation in the *CYP7A1* coding sequence results in a frame shift mutation that causes early termination and translation of a truncated peptide. These patients have reduced CA and DCA, which are likely synthesized via the alternative bile acid synthesis pathway. The first single nucleotide polymorphism (SNP) (−203A < C) identified in the human *CYP7A1* gene promoter is linked to increased LDL-cholesterol.[201] Several SNPs in the *CYP7A1* promoter and coding sequences have been shown to affect bile acid synthesis,[202] LDL-cholesterol lowering response to statins,[203,204] cholesterol and serum lipids,[205–208] coronary artery disease,[209] and gallstone disease.[210–212] Interestingly, the −203A < C polymorphism has been shown to alter the diurnal rhythm of CYP7A1 activity, monitored by modeling the dynamics of serum C4 in patients.[213]

As a proof of concept study, *Cyp7a1* transgenic mice (*Cyp7a1-Tg*) were created by cloning a rat *Cyp7a1* coding sequence over-expressing Cyp7a1 to study bile acid and lipid

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metabolism. These mice had increased insulin sensitivity and glucose tolerance and were protected from western high fat diet-induced obesity and hepatic steatosis.[214] In *Cyp7a1-Tg* mice, *Cyp7a1* mRNA levels were increased 10-fold, but Cyp7a1 protein expression, enzyme activity and bile acid pool sized only increased ~2–3 fold. In bile, TCA was absent and TCDCA became the predominant bile acid. It is likely that increased TCDCA activated the FXR/SHP pathway to inhibit *Cyp8b1* expression. Microarray gene profiling showed that the top up-regulated genes and pathways were *de novo* cholesterol synthesis and transport, Ldl receptors and Srebp.[215] These mice had increased biliary secretion of bile acids and cholesterol to maintain cholesterol homeostasis. Interestingly, in *Cyp7a1-Tg* mice acetyl-CoA was preferentially used for cholesterol synthesis rather than fatty acid synthesis, explaining observed reduced lipogenesis and increased cholesterol synthesis. This mouse model demonstrates that increasing Cyp7a1 and the bile acid pool, and decreasing Cyp8b1, has metabolic benefits against fatty liver diseases, diabetes and obesity. The original *Cyp7a1* knockout mice (*Cyp7a1*$^{-/-}$) in a mixed genetic background had a malnutrition phenotype and exhibited reduced survival, as 80% of pups died and surviving mice required vitamin supplementation.[21] Surprising, *Cyp7a1*$^{-/-}$ mice bred into a pure C57BL/6J background survived well and were phenotypically normal.[216] *Cyp8b1*, *Cyp7b1* and *Cyp27a1* were all upregulated, indicating the alternative bile acid synthesis pathway was upregulated to produce a smaller (60%) but more hydrophobic bile acid pool compared to wild type mice. It is interesting that *Cyp8b1* expression was upregulated in *Cyp7a1*$^{-/-}$ mice to produce substantial amount of TCA (32% of the bile acid pool), indicating the alternative pathway also can produce CA. It has been reported that human CYP8B1 can convert CDCA to CA.[133] It is also surprising that *Cyp7a1*$^{-/-}$ mice are insulin sensitive and are protected from high fat/high cholesterol diet-induced metabolic disorder. All these studies suggest that both the classic and alternative bile acid synthesis pathways are important in maintaining bile acid homeostasis and whole-body metabolic homeostasis. Cyp7a1 expression and bile acid pool and composition can be targeted for therapeutic treatment of metabolic disorders.

8.1.2. CYP7B1 mutations—In contrast to the mild metabolic phenotype in mice and humans with *CYP7A1* gene mutations, mutations in the *CYP7B1* gene cause severe neonatal cholestasis, cirrhosis and liver failure in neonates.[217] In these patients, serum levels of 27-hydroxycholesterol are markedly elevated, leading to accumulation of hepatoxic unsaturated monohydroxy-cholenoic acids. These findings indicate that the alternative bile acid synthesis pathway is quantitatively important in the neonate. A functional SNP in the *CYP7B1* gene promoter has been reported.[218] The allele frequency differs in Asians and Caucasians and has been linked to prostate cancer. CYP7B1 is an oxysterol 7α-hydroxylase involved in steroid sex hormone synthesisand is regulated by estrogen and androgens in the prostate in humans.[219–222] Many *CYP7B1* mutations have been identified in hereditary paraplegias and neurodegenerative disorders.[223–228]

8.1.3. CYP8B1 polymorphism—*CYP8B1* gene mutations have not been identified in humans. Only one SNP in the *CYP8B1* gene has been reported and is associated with gallstone disease in the Han Chinese population.[229] *Cyp8b1* expression is increased in obese and diabetic mice and increased serum 12α-hydroxylated bile acids are associated with obesity and insulin resistance in humans.[230–232] *Cyp8b1*$^{-/-}$ mice have increased TMCAs,

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which antagonize FXR and reduce Fgf15 to stimulate bile acid synthesis, increase bile acid pool and improve glucose homeostasis by stimulating GLP-1 secretion, and prevent western diet-induced obesity and hepatic steatosis.[233–237] Overexpression of *Cyp8b1* exacerbated dyslipidemia and diet-induced obesity and diabetes via stimulation the ceramide/mechanistic target of rapamycin complex 1 (mTORC1)/SREBP1 pathway.[230] In hibition of CYP8B1 and reduction of CA protect against NAFLD, diabetes, and obesity, while activation of CYP8B1 causes cholestasis.[132] Several inhibitors of Cyp8b1 have been identified.[132]

8.1.4. CYP27A1 mutations—There are over 200 reported cases of human *CYP27A1* gene mutations, which have been linked to cerebrotendinous xanthomatosis (CTX), a rare autosomal recessive disorder of bile acid synthesis.[238–240] CTX patients have abnormally high levels of cholestanol in the blood and accumulate cholestanol and cholesterol in the brain and tendons, forming tendon xanthomas. However, *Cyp27a1* deficiency in mice does not accumulate cholestanol and does not induce CTX-like symptoms because *Cyp27a1*$^{-/-}$ mice and CTX patients accumulate different bile acid intermediates.[241] In mice, accumulation of 5β-cholestane-3α,7α,12α -triol activates mouse PXR to induce Cyp3a11, which metabolizes triol and reduces accumulation of this toxic metabolite,[242] while human PXR is not activated by triol. CDCA treatment inhibits CYP7A1 to reduce cholestanol and bile acid metabolites and improve clinical and neurophysiological outcomes of CTX.[243] It has been reported that 27-hydroxycholesterol is an endogenous selective estrogen receptor modulator that inhibits cardiovascular effects of estrogen,[244] but activates estrogen receptor-dependent growth and LXR-dependent metastasis of breast cancer in mice.[245] CYP27A1 expression and 27-hydroxycholesterol are associated with breast cancer pathogenesis in human patients.[246]

8.2. Bile acid-based drug therapies

Most studies on bile aid synthesis were performed using animal models. Despite marked differences in bile acid composition and pool size between humans and mice, results obtained from mouse studies have been translated to therapies for metabolic diseases (reviewed in Refs. 17, 19 and 20). For decades, bile acids have been used as supplements to increase the bile acid pool in patients with inborne errors of bile acid synthesis. Bile acid sequestrants have long been used to reduce the bile acid pool and stimulate bile acid synthesis. More recently, bile acid derivatives are used to activate FXR signaling and reduce the bile acid pool in cholestasis patients. Drugs that regulate bile acid synthesis, bile acid pool size and bile acid composition are in various phases of clinical trials for treating liver-related diseases, such as cholestasis and NASH. NASH is a progressive form of non-alcoholic fatty liver disease (NAFLD), which is a significant complication of obesity and type 2 diabetes mellitus (T2DM), and an independent risk factor for cardiovascular disease. NAFLD has become the most common chronic liver disease, affecting about 30% of the US population. About 30% of NAFLD patients progress to NASH cirrhosis, while about 0.5% of patients with NAFLD progress to hepatocellular carcinoma.[247,248] NAFLD patients have increased CA, CDCA and bile acid synthesis, and increased ratio of primary bile acids to secondary bile acids.[249] NASH patients also have increased circulating conjugated primary bile acids, an increased ratio of conjugated CA to CDCA, and decreased secondary bile

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acids.[250] Currently, no Food and Drug Administration-approved drug therapy is available for NASH fibrosis or NAFLD.

8.2.1. Increasing the bile acid pool—CA (Cholbam®) and CDCA (Chenodiol®) have been used to treat inborne errors of bile acid synthesis by increasing the bile acid pool in these patients.[251] CDCA and UDCA (Ursodiol®, Actigall®) are used to dissolve cholesterol gallstones. UDCA is highly soluble and is used to reduce bile acid toxicity in patients with primary biliary cholangitis (PBC). PPARα agonists (fibrates) and selective modulators have been used in combination with UDCA to treat cholestasis in primary biliary cirrhosis patients.[20] Fibrates may induce cholesterol gallstones as a side effect in humans, likely due to repression of *CYP7A1* and bile acid synthesis.

8.2.2. Reducing the bile acid pool size—During cholestasis, the bile acid pool is increased and bile acids accumulate in the liver, causing liver inflammation and injury. Reducing CYP7A1 and bile acid synthesis by bile acid sequestrants and FXR agonists reduces the bile acid pool during cholestasis.

Bile acid sequestrants bind bile acids, reduce the bile acid pool to induce CYP7A1, and stimulate bile acid synthesis and reduce serum LDL cholesterol. Cholestyramine is an established drug used for gallstone dissolution, hypercholesterolemia, and BAD.[252] Second generation sequestrants, such as Colesevelam and Colestimide, stimulate GLP-1 secretion and thermogenesis in brown adipose tissue to improve insulin sensitivity and glycemic control in NASH patients.[253–255]

The FXR agonist obeticholic acid (OCA, OCALIVA®) is a CDCA derivative that activates FXR/FGF19 to inhibit *CYP7A1* and bile acid synthesis. OCA can be used to treat BAD and is approved to treat PBC and is in phase 3 clinical trials for NASH,[256–260] though side effects of OCA treatment may include pruritus, increased LDL-cholesterol and decreased HDL, and gallstone disease.[259,261] The non-steroidal FXR agonist Cilifexor improves cholestasis and liver injury in primary sclerosing cholangitis patients and Tropifexor is in clinical trials for PBC and NASH.[262,263] The FXR antagonists GUDCA and Gly-MCA reduce weight and improve diabetes,[264,265] while the non-tumorigenic FGF19 analogue NGM282 reduces *CYP7A1* expression and improves inflammation and is in clinical trials for NASH fibrosis.[266] However, non-steroidal FXR agonists also have the same side effects as bile acid-based FXR agonists.

9. Conclusions and future perspective

CYP7A1 has attracted increasing attention in the last two decades as an important enzyme in hepatic metabolism and as a regulator of whole body metabolic homeostasis.[38] CYP7A1 and bile acid synthesis are increasingly used as biomarkers for monitoring liver metabolism and disease progression. The molecular mechanisms that regulate CYP7A1 expression in health and disease have been slowly elucidated but require further study. Targeting the bile acid receptor FXR may have therapeutic benefit for treating liver diseases and injury,[20] however, unwanted side effects of bile acid-based drugs have been recognized. The proof-of-concept studies that manipulated bile acid synthesis and bile acid composition in mouse liver by

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transgenic overexpression of *Cyp7a1* demonstrated Cyp7a1 may protect against diet-induced obesity and diabetes.[214,215] The genomic editing of the *CYP7A1* gene, miRNA silencing or epigenetic regulation of CYP7A1 translation and mRNA stability may alter CYP7A1 expression to control the rate of bile acid synthesis, pool size and composition and could lead to effective therapies for treating liver-related diseases.

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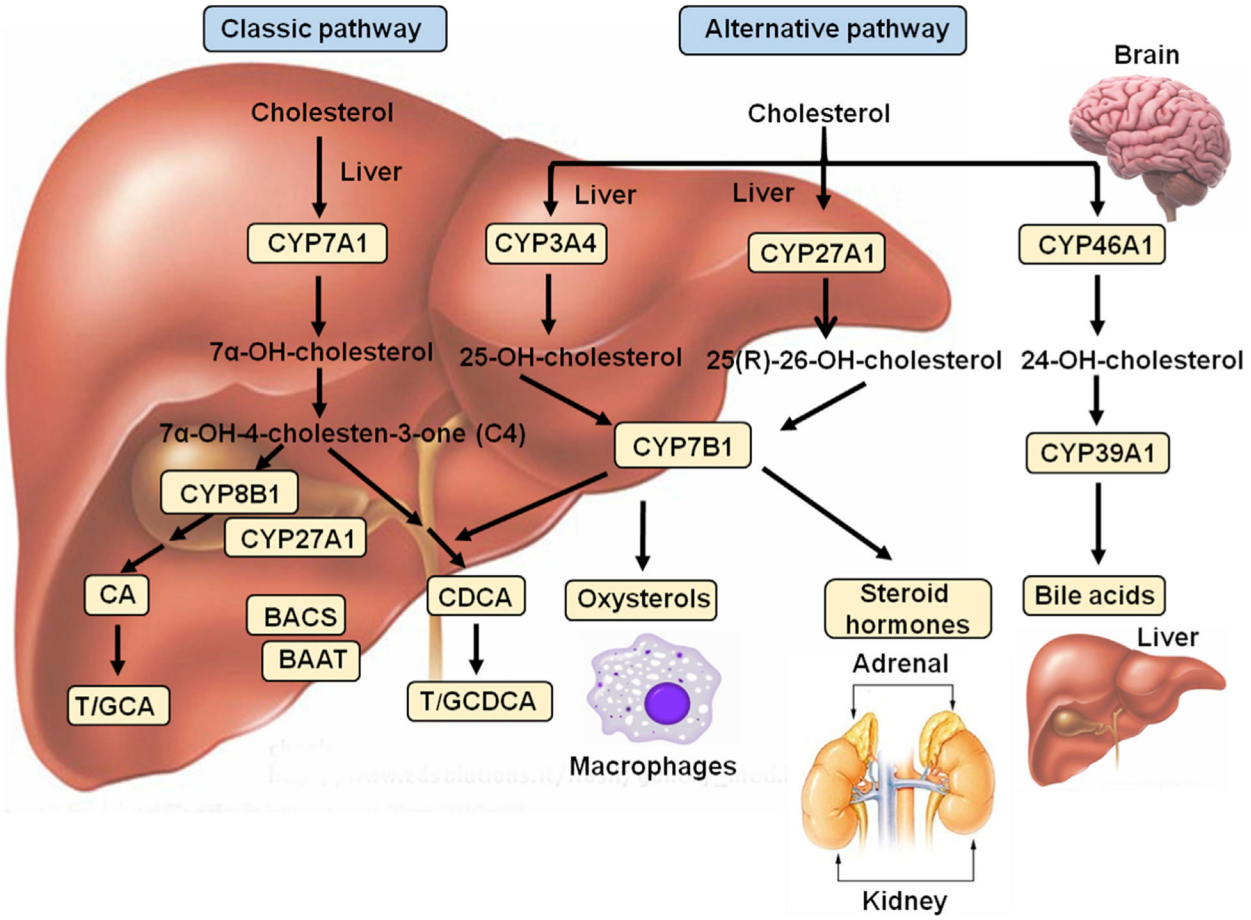

Fig. 1. Bile acid and oxysterol synthesis in the liver and other tissues.

The liver is the only organ that expresses all the enzymes required for bile acid synthesis in the classic bile acid synthesis pathway. The alternative pathways exist in macrophages, adrenal and brain. The classic pathway of bile acid synthesis from cholesterol is catalyzed by cholesterol 7 alpha-hydroxylase (CYP7A1). 7α-hydroxycholesterol is then converted to 7α-hydroxy-4-cholesten-3-one (C4), which is the common precursor for cholic acid (CA) and chenodeoxycholic acid (CDCA) synthesis. C4 is 12α-hydroxylated by sterol 12α-hydroxylase (CYP8B1), producing CA. Without CYP8B1, C4 is converted to CDCA. Mitochondrial sterol 27-hydroxylase (CYP27A1) catalyzes steroid side-chain oxidation to form 3α, 7α, 12α-trihydroxycholestanic acid (THCA) and 3α, 7α-dihydroxycholestanic acid (DHCA). THCA and DHCA are activated by bile acid-CoA synthase (BACS, also known as long-chain acyl-CoA synthase, SLC27A5) for peroxisomal β-oxidation reactions to cleave a propionyl-CoA to form cholyl-CoA and chenodeoxycholyl-CoA, which are conjugated to the amino acids glycine (G) or taurine (T) by bile acid-CoA: amino acid N-acyltransferase (BAAT) to form T/GCA and T/GCDCA, respectively. The alternative bile acid synthesis pathway is initiated by CYP27A1, which converts cholesterol to 25(R)-26-hydroxycholesterol (27-hydroxycholesterol) in the liver. Oxysterol 7α-hydroxylase (CYP7B1) catalyzes 7α-hydroxylation of 27-hydroxycholesterol. In the liver, CYP3A4 catalyzes 25-hydroxylation of cholesterol to 25-hydroxycholesterol, followed by hydroxylation by CYP7B1. CYP27A1 and CYP7B1 are also expressed in macrophages for

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oxysterol metabolism and in adrenal glands for steroid hormone synthesis. In the brain, cholesterol 24-hydroxylase (CYP46A1) hydroxylates cholesterol to 24-hydroxycholesterol, an abundant sterol in the brain, which can be transported to hepatocytes and 7α-hydroxylated by 24-hydroxysterol 7α-hydroxylase (CYP39A1) for bile acid synthesis.

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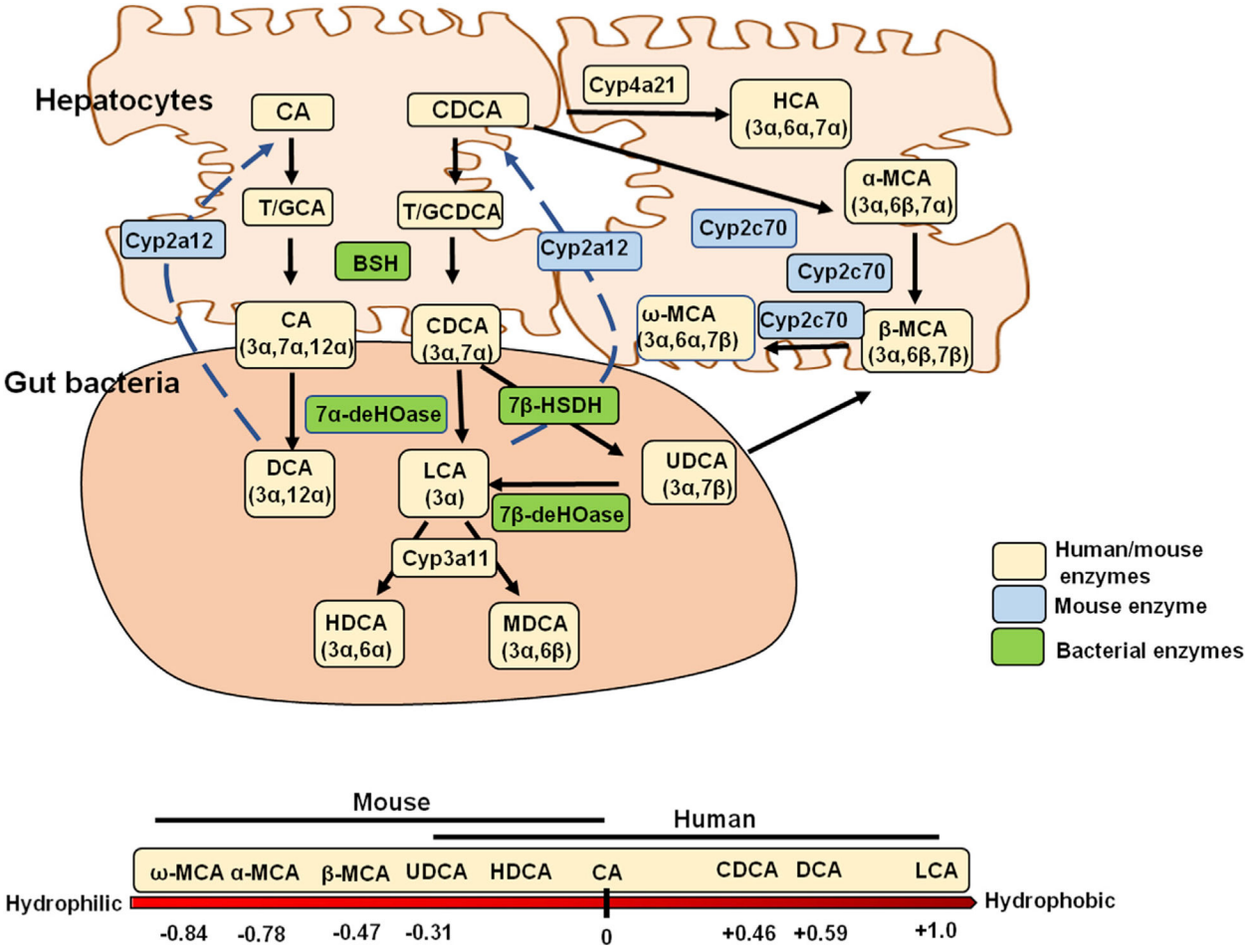

Fig. 2. Primary and secondary bile acid synthesis in humans and mice.

Top: The primary bile acids CA and CDCA are conjugated to taurine (T) or glycine (G) in the liver. In mice, CDCA may be further metabolized to α- and β-muricholic acid (α- and β-MCA) by Cyp2c70. CDCA can be converted to hyocholic acid by Cyp4a12 in humans and pigs. In the intestine, T/GCA and T/GCDCA are deconjugated by bacterial bile salt hydrolase (BSH) and dehydroxylated by bacterial 7α-dehydroxylase, forming deoxycholic acid (DCA) and lithocholic acid (LCA), respectively. In mice, DCA and LCA recycled to the liver can be converted back to CA and CDCA by Cyp2a12. A small amount of CDCA is converted to UDCA in humans, and in mice UDCA can be converted to β-MCA by Cyp2c70. Bottom: Bile acid composition and hydrophobicity in humans and mice. Abbreviations: CA, cholic acid; CDCA, chenodeoxycholic acid; UDCA, ursodeoxycholic acid; HCA, hyocholic acid; HDCA, hyodeoxycholic acid.

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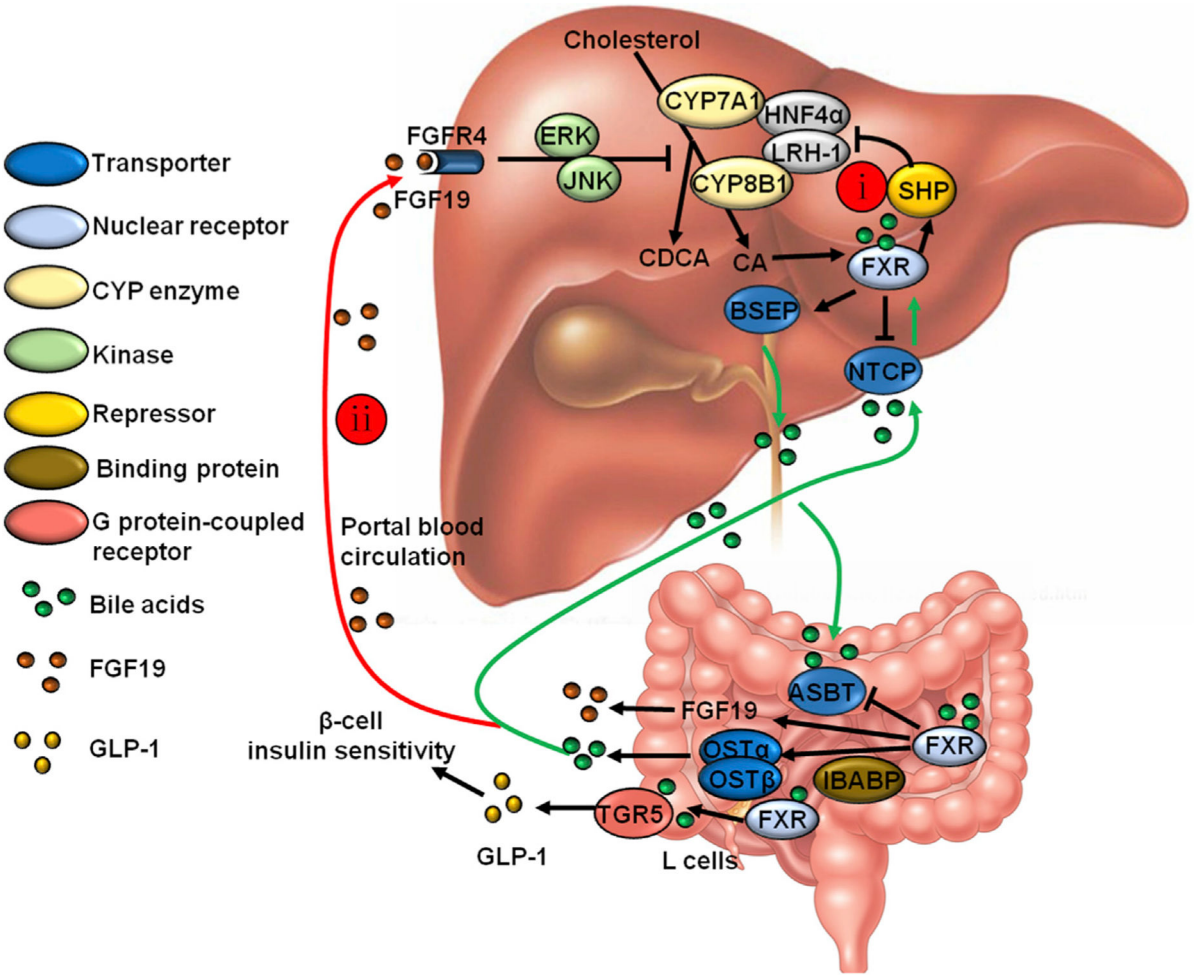

Fig. 3. Bile acid feedback regulation of bile acid synthesis via enterohepatic circulation of bile acids.

(i) Bile acids activate the nuclear receptor FXR in hepatocytes to inhibit bile acid synthesis and promote excretion of bile acids into bile. FXR induces the negative co-repressor small heterodimer partner (SHP) to inhibit hepatocyte nuclear factor 4 alpha (HNF4α)/LXR-mediated activation of CYP7A1. FXR also activates bile salt export pump (BSEP) to efflux bile acids into gallbladder bile, and FXR inhibits sodium taurocholate co-transport peptide (NTCP), preventing the accumulation of bile acids in the liver. (ii) In the intestine, bile acids activate FXR and induce the release of fibroblast growth factor 19 (FGF19, Fgf15 in mice). FGF19 released into portal circulation inhibits bile acid synthesis via extracellular signal-regulated kinase (ERK)/c-Jun N-terminal kinase (JNK)-mediated blockade of CYP7A1. Also, in the intestine, bile acids activate the G protein-coupled receptor TGR5, stimulating release of glucagon-like peptide 1 (GLP-1) and improving insulin tolerance. Abbreviations: FXR, farnesoid X receptor; CYP7A1, cholesterol 7 alpha-hydroxylase; LXR, liver X receptor; TGR5, Takeda G protein-coupled receptor 5; LRH-1, liver-related homologue-1; CDCA, chenodeoxycholic acid; CA, cholic acid; ASBT, apical sodium-dependent bile acid transporter; OST, organic solute transporter; IBABP, ileum bile acid binding protein.

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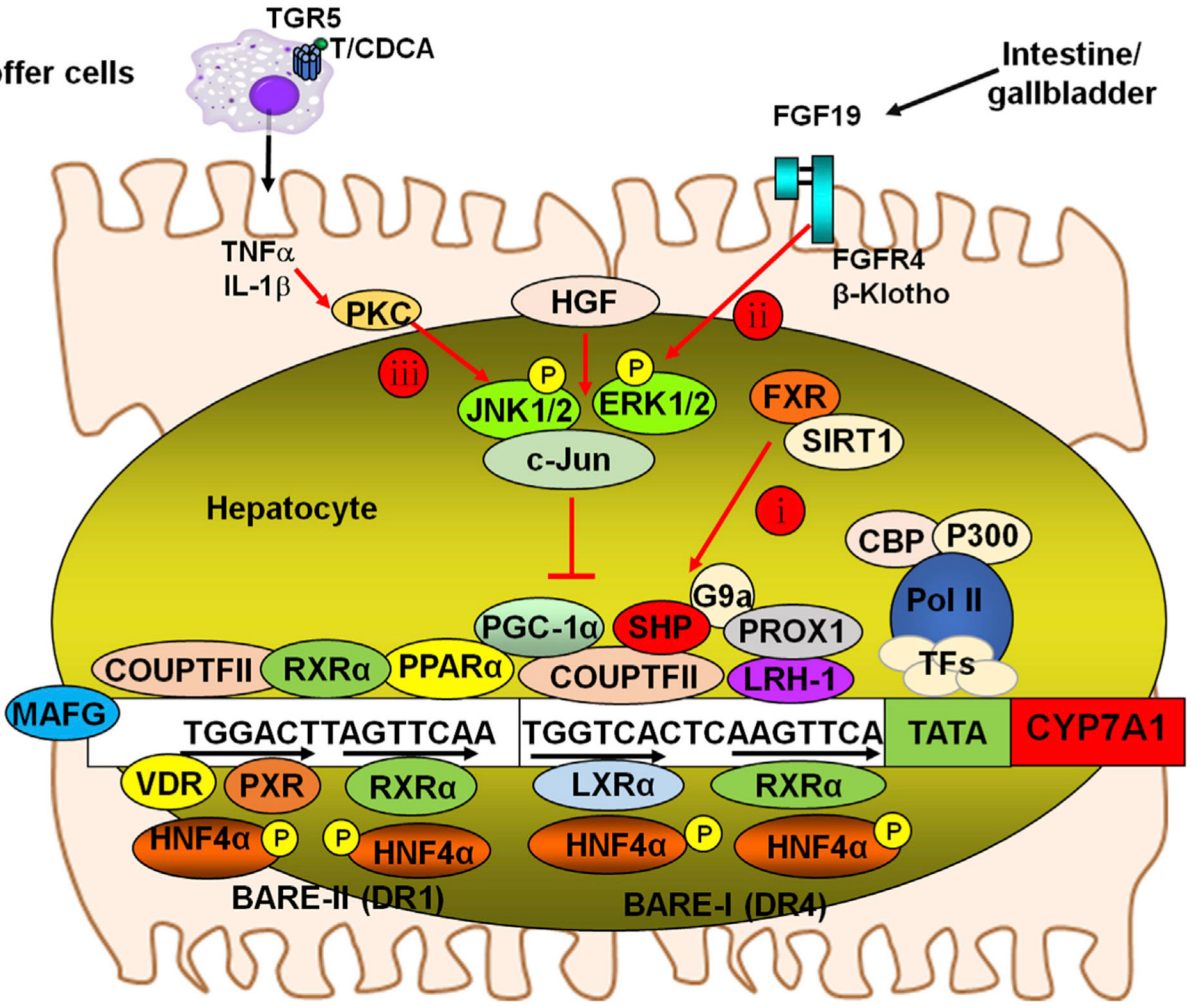

Fig. 4. Mechanism of regulation of the CYP7A1 gene.

The CYP7A1 gene proximal promoter contains BARE, which binds the nuclear receptors shown. Three mechanisms for bile acid regulation of *CYP7A1* gene transcription are illustrated here. The *CYP7A1* promoter contains two bile acid responsive elements, BARE-I and BARE-II, which contains a direct repeat with 4 bases spacing (DR4) and 1 base spacing (DR1) motif, respectively. Nucleotide sequences shown are rat *Cyp7a1* gene. Mechanisms 1 and 2 are FXR-dependent and mechanism 3 depicts an FXR-independent cell signaling pathway. **Mechanism i:** Bile acid activation of FXR induces the repressor SHP to inhibit HNF4α and LRH-1-mediated transcriptional activation of CYP7A1. The SHP-mediated mechanisms may also inhibit PXR/RXRα (BARE-II), COUP-TFII/RXRα (BARE-I and BARE-II), LXR/RXRα (BARE-I) and PPARa/RXRα (BARE-II) binding to the *CYP7A1* promoter to suppress *CYP7A1* gene transcription. FXR also induces MAFG to inhibit CYP7A1. PXR, VDR and PPARa inhibit *CYP7A1* gene transcription via competition for HNF4α binding to the BARE-II on the *CYP7A1* gene promoter. **Mechanism ii:** Bile acid activation of intestinal FXR induces FGF19, which is transported from the intestine to the liver and activates the FGFR4/β-Klotho complex to phosphorylate and activate JNK1/2 and ERK1/2 signaling pathways. Phosphorylation of HNF4α inhibits its dimerization and

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binding to the *CYP7A1* gene, resulting in reduced *CYP7A1* gene transcription. Phosphorylation of PGC-1α, a co-activator of PPARα, and other nuclear receptors also inhibit trans-activation of the *CYP7A1* gene. **Mechanism iii:** Taurodeoxycholic acid (TDCA)/CDCA activates macrophages and induces the release of inflammatory cytokines including TNFα and IL-1β, which cross the sinusoidal membrane to activate protein kinase C (PKC) and JNK signaling pathways to inhibit *CYP7A1* gene transcription. **Epigenetic regulation:** Histone acetylases (HATs), such as CBP/P300 and histone deacetylases (HDACs) such as sirtuin 1 (SIRT1), regulate FXR, SHP and CYP7A1/CYP8B1 by epigenetic mechanisms. The methytransferase G9a is recruited by SHP and Prox-1 to inhibit CYP7A1 by histone methylation. Abbreviations: CYP7A1, cholesterol 7 alpha-hydroxylase; BARE, bile acid response elements; HNF4α, hepatocyte nuclear factor 4 alpha; FXR, farnesoid X receptor; FGFR4, fibroblast growth factor receptor 4; CDCA, chenodeoxycholic acid; JNK, c-Jun N-terminal kinase; SHP, small heterodimer partner; LRH-1, liver-related homologue-1; PXR, pregnane X receptor; VDR, vitamin D receptor; PPAR, peroxisome proliferator-activated receptor.

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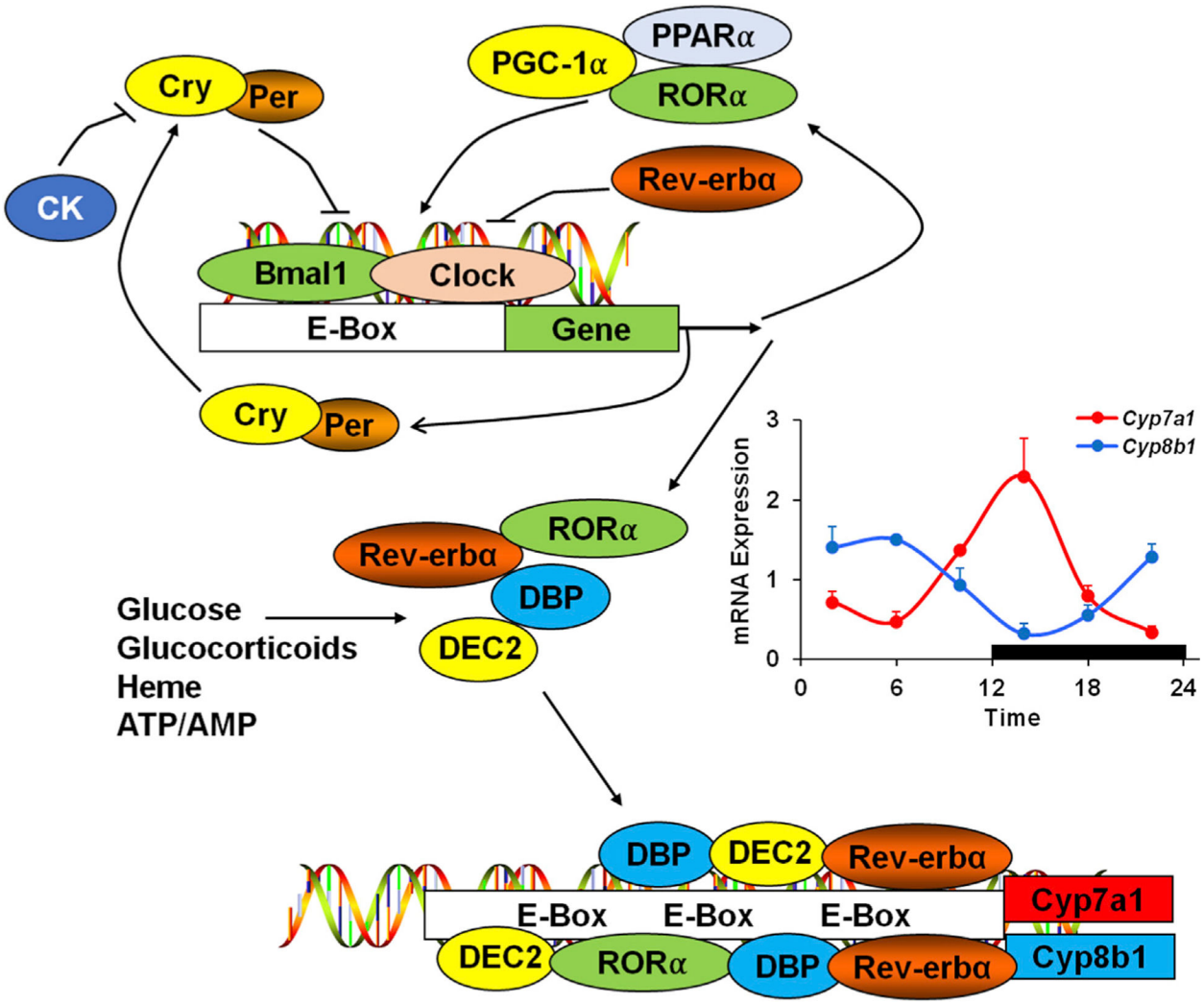

Fig. 5. Circadian regulation of CYP7A1.

Top: The molecular circadian clock exists in nearly all cells and consists of a cycling transcriptional-translational feedback loop. The core clock proteins Clock and Bmal1 activate the transcription of the negative clock regulators Per and Cry. Per and Cry heterodimers inhibit the Clock/Bmal1 forward limb of the clock, thus regulating Per/Cry synthesis. Per/Cry accumulation leads to phosphorylation by casein kinase, which tags the proteins for degradation and releases the inhibition of Clock/Bmal1. Middle: Clock/Bmal1 also regulates the transcription of clock-controlled genes involved in glucose and energy metabolism. Several of these genes play roles in regulating CYP7A1 and CYP8B1, which exhibit inverse rhythms of gene expression. Bottom: Several clock/controlled transcription factors regulate *CYP7A1* and *CYP8B1* gene transcription, including D-site binding protein (DBP), DEC2, and Reverb-α and RORα, which also negatively and positively regulate the core clock mechanism above, respectively. Clock proteins bind to the E-box motifs (CANNTG) on the gene promoters. Abbreviations: CYP7A1, cholesterol 7 alpha-

hydroxylase; CYP8B1, sterol 12 alpha-hydroxylase; Bmal1, brain and muscle ARNT-like 1; Per, Period; Cry, Cryptochrome; RORα, retinoic acid-related orphan receptor alpha.

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