thematic review

Regulation of cholesterol-7α-hydroxylase: BAREly missing a SHP

Roger A. Davis,[1] Jon H. Miyake, To Yuen Hui, and Nathanael J. Spann

Cell and Molecular Biology Laboratory, Department of Biology, San Diego State University, San Diego, CA 92182

Abstract Cholesterol-7α-hydroxylase (CYP7A1) regulates the pathway through which cholesterol is converted into bile acids. The unique detergent properties of bile acids are essential for the digestion and intestinal absorption of hydrophobic nutrients. Bile acids have potent toxic properties (e.g., membrane disruption) and there are a plethora of mechanisms to limit their accumulation in blood and tissues. The discovery of farnesoid X receptor (FXR), the nuclear receptor activated specifically by bile acids, has opened new insights into these mechanisms. Bile acid activation of FXR has been shown to repress the expression of CYP7A1 via increasing the expression of small heterodimer partner (SHP), a non-DNA binding protein. The increased abundance of SHP causes it to associate with liver receptor homolog (LRH)-1, an obligate factor required for transcription of CYP7A1. Recent studies show there is an "FXR/SHP-independent" mechanism that also represses CYP7A1 expression. This "FXR/SHP-independent" pathway involves the interaction of bile acids with liver macrophages (i.e., Kupffer cells), which induces the expression, and secretion of cytokines. These inflammatory cytokines, which include tumor necrosis factor α and interleukin-1β, act upon liver parenchymal cells causing a rapid repression of the CYP7A1 gene.—Davis, R. A., J. H. Miyake, T. Y. Hui, and N. J. Spann. **Regulation of cholesterol-7α-hydroxylase: BAREly missing a SHP.** ***J. Lipid Res.*** **2002.** 43: **533–543.**

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The conversion of cholesterol to bile acids produces sufficient amounts of detergent necessary for the digestion and absorption of lipid nutrients (e.g., triglycerides and fat-soluble vitamins) and provides a metabolic pathway through which excessive cholesterol can be removed from the body. These two seemingly unconnected processes link the acquisition of exogenous nutrients to endogenous metabolism.

There are two distinct bile acid biosynthetic pathways

Cholesterol-7α-hydroxylase (CYP7A1; EC 1.14.13.17) is a liver specific enzyme and is the only mammalian enzyme capable of initiating the multi-organelle pathway through which cholesterol is converted into bile acids (**Fig. 1**). This bile acid biosynthetic pathway is commonly referred to as the neutral or classic pathway (1, 2). Within the endoplasmic reticulum CYP7A1 places a hydroxyl group onto the 7α position of the cholesterol (Fig. 1). The resulting 7α-hydroxycholesterol can be hydroxylated in the 12α position via the microsomal enzyme CYP8B1 (3). The aliphatic side-chain is hydroxylated in the 27 position via the mitochondrial cytochrome P450 enzyme CYP27 (4). The side chain is also hydroxylated in the 24 position by the peroxisomal multifunctional protein-2 (5). Following oxidation-dependent cleavage of the side chain, the C24 position is converted to a carboxylic acid group. Almost all bile acids are secreted by the liver as amides conjugated with either taurine or glycine.

Bile acids can also be synthesized via the 7α-hydroxylation of several different oxysterols; this pathway is referred to as the "acidic" or "alternative" bile acid synthetic pathway (6). There are at least two different enzymes that 7α-hydroxylate oxysterols: CYP7B1, which 7α-hydroxylates several different oxysterols (e.g., 25-hydroxycholesterol, 27-hydroxycholesterol) (7, 8) and CYP39A1, which appears to react only with 24-hydroxycholesterol (9). The production of 24-hydroxycholesterol by CYP46 accounts for the major metabolic pathway for cholesterol in the brain (10).

In mice the alternative/acidic bile acid biosynthetic pathway is estimated to account for about 45% of the total bile acid production (11), while in humans the alternative/acidic bile acid biosynthetic pathway is estimated to account for only ~10% of total production (12). In humans, the two major bile acids produced via these pathways are cholic acid and chenodeoxycholic acid. Mice also produce significant quantities of 6β-hydroxylated bile acids (mainly β-muricholic acid and murideoxycholic acid). Interestingly, the enzyme that catalyzes 6β-hydroxylation

Abbreviations: BARE, bile acid response elements; CPF, CYP7A promoter binding factor; CYP7A1, cholesterol-7α-hydroxylase; FXR, farnesoid X receptor; HNF, hepatocyte nuclear factor; IL, interleukin; JNK, c-Jun N-terminal kinase; LXR, liver X receptor; LRH, liver receptor homolog; PKC, phorbol ester protein kinase C; SHP, small heterodimer partner; TNF, tumor necrosis factor; UTR, untranslated region.

[1] To whom correspondence should be addressed.
e-mail: rdavis@sciences.sdsu.edu

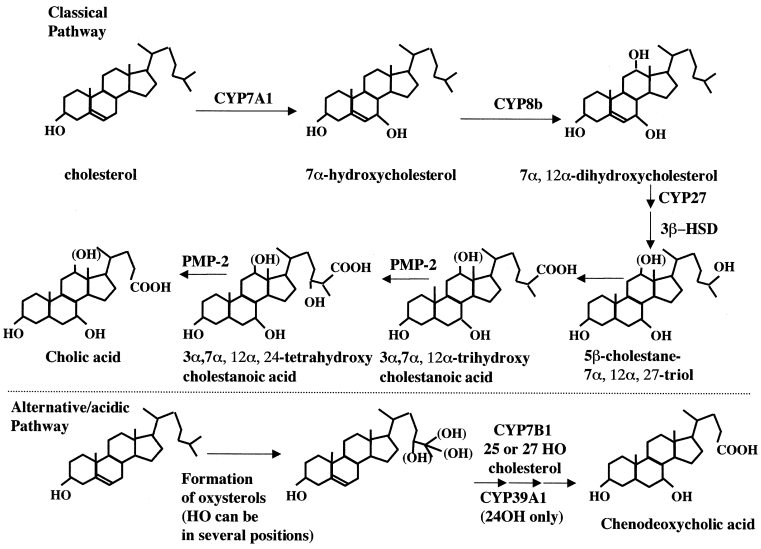

Fig. 1. Bile acid biosynthetic pathways. Bile acids are either synthesized from cholesterol [the classical bile acid synthetic pathway regulated by cholesterol-7α-hydroxylase (CYP7A1)] or by the alternative/acidic pathway regulated by either CYP7B1, which 7α-hydroxylates several different oxysterols, and CYP39A1, which appears to react only with 24-hydroxycholesterol (formed mainly in the brain). The 12α-hydroxyl group is placed on 7α-hydroxycholesterol via microsomal CYP8B1.

of lithocholic acid (i.e., CYP3A1) (13) also carries out 6β-hydroxylation of steroid hormones (14).

CYP7A1 can also 7α-hydroxylate several different oxysterols (e.g., 20(*S*)-hydroxycholesterol, 25-hydroxycholesterol, and 27-hydroxycholesterol) (15, 16). The metabolism of oxysterols by CYP7A1 has been shown to markedly influence several aspects of lipid metabolism via increasing the expression of genes whose transcription is regulated by SREBP (17–19). Oxysterols are potent inhibitors of the proteolytic activation of SREBPs (20). Placing a hydroxyl group in the 7-position of oxysterols blocks their ability to act as repressors of SREBP processing (17, 21, 22). Cell culture studies showed that transgenic expression of CYP7A1 led to an induction of the LDL receptor expression and increased metabolism of [^{3}H]25-hydroxycholesterol (17). These findings led to the proposal that 7α-hydroxylation of endogenous oxysterols inactivated their ability to block SREBP processing, allowing the liver to maintain expression of LDL receptors (17). While this hypothesis was initially challenged by results showing that CYP7A1 displayed no enzymatic activity toward oxysterols (23), subsequent studies by the same laboratory using high resolution mass spectroscopy showed that CYP7A1 could 7α-hydroxylate 20(S)-hydroxycholesterol, 25-hydroxycholesterol, and 27-hydroxycholesterol (15,16). These findings resolve the mechanism through which variations in the expression of CYP7A1 correlate with changes in the hepatic expression of SREBP-regulated genes (17–19, 24).

Bile acids are effective detergents

Molecular modeling of cholic acid emphasizes its ability to a form rigid amphipathic structure. The planar hydrophobic surface is opposed by a hydrophilic surface having the freedom to hydrogen bond with the water interface using the 3α-, 12α-, and 7α-hydroxyl groups that are directed away from the steroid nucleus (**Fig. 2**). This rigid amphipathic planar structure facilitates the ability of bile acids to act as effective detergents necessary for the digestion and intestinal absorption of hydrophobic nutrients (e.g., lipids and fat soluble vitamins). Perhaps the most compelling evidence demonstrating the functional importance of the "amphipathic sidedness" of the steroidal planar structure of bile acids is that biotransformation of chenodeoxycholic acid into ursodeoxycholic acid via changing the 7α-hydroxy group to a 7β-hydroxy group markedly decreases its amphipathic/detergent properties (25, 26).

Most bile acids secreted by the liver are in the form of C24 amides that contain either taurine or glycine (27). The formation of these amides lowers the pK_a to <3; the lower pKa may facilitate their solubility in the lumen of the intestine, proximal to the stomach. Conjugated bile acids are transported from the hepatocyte cytosol across the canalicular membrane into bile via the ATP-binding

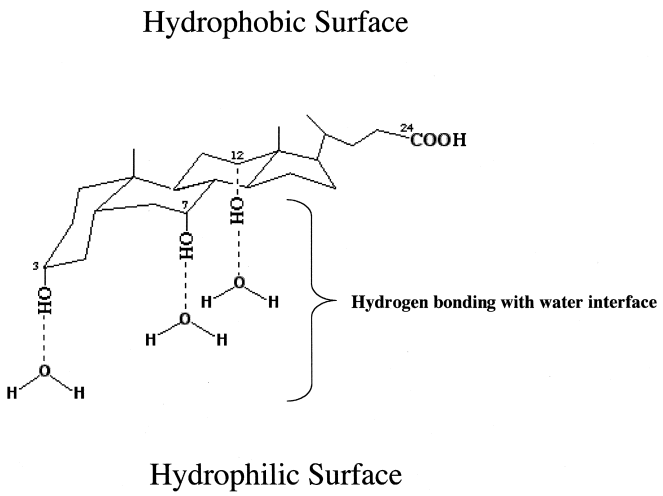

Fig. 2. The unique structure of cholic acid, provides an excellent amphipathic molecule. The ability of primary bile acids to form a rigid planar structure having a hydrophobic surface separated from the hydrophilic surface allows them to function as excellent detergents. This property is important for the digestion and intestinal absorption of lipid nutrients. It also may be important in disrupting membranes and activating an inflammatory response by macrophages.

cassette (ABCB11) protein originally designated as sister of P-glycoprotein (28). The excretion into bile of amphipathic bile salts drives the excretion of phosphatidyl choline and free cholesterol as mixed micelles, which are stored in the gallbladder (29). The relative composition of these mixed micelles has important implications in the formation of cholesterol gallstones. If the concentration of biliary cholesterol relative to phosphatidyl choline and bile salts exceeds its solubility, cholesterol will precipitate as insoluble crystals (i.e., cholesterol gallstones) (30). Soon after eating, the gallbladder contracts, releasing bile into the proximal intestinal lumen via the common duct. The addition of bile salts to pancreatic digestive enzymes (e.g., lipases and proteases) facilitates the hydrolysis of glycerolipids into fatty acids, cholesterol esters into free cholesterol, and fatty acids and hydrophobic segments of proteins into amino acids.

Conjugated bile salts are efficiently hydrolyzed to bile acids by anaerobic bacteria residing within the proximal intestine (31). In addition, the 7α-hydroxyl group can be removed via dehydrogenase produced by anaerobic bacteria (32). As a result of dehydrogenase-dependent loss of the α-hydroxyl group, cholic acid is converted into the more hydrophobic bile acid deoxycholic acid, whereas chenodeoxycholic acid is converted into lithocholic acid, a bile acid that in sufficient quantities can cause hepatic inflammation (33).

The essential role of bile acids in lipid absorption is dramatically exemplified by mice that have a genetic deletion of CYP7A1 (6, 34). These mice display severe neurological developmental abnormalities that can be prevented by supplementation with fat-soluble vitamins and cholic acid (34).

Expression of CYP7A1 in pericentral parenchymal cells in the livers of fed animals

In rats, the expression of CYP7A1 varies as a gradient having highest concentrations in a subset of adult liver parenchymal cells that surround the central vein of the hepatic acinus, and lowest concentrations in the cell localized to the periportal regions (35–37). A recent report suggests that pancreatic cells isolated from copper-deficient rats are able to express CYP7A1 and some other "liver-specific" gene products (38). When hepatic CYP7A1 expression is induced in response to the administration of the bile acid sequestrant cholestyramine (36) or during development (37), the number of hepatocytes expressing "high" levels of CYP7A1 increases, causing the distribution to include cells closer to the periportal region. This cell type specific expression of CYP7A1 extends to stable lines of cultured hepatocytes. While most stable lines of cultured hepatocytes express several liver specific gene products [e.g., the apolipoprotein B-100 lipoprotein secretion pathway (39), to our knowledge the only stable cell lines that express CYP7A1 are HepG2 cells (40) and L35 cells (41). The level of CYP7A1 expression by L35 cells is 10 times that of HepG2 cells and similar to the level expressed by rat liver (41). The finding that transgenic expression of CYP7A1 in either non-hepatic CHO cells (17, 19) or in McArdle rat hepatoma cells, which normally do not express CYP7A1 (18) has no apparent adverse effect suggests that CYP7A1 expression is not deleterious to normal cellular functions. Thus, it is more likely that the basis for the regulated and zonal expression of CYP7A1 relates to the environmental factors controlling its gene expression. As discussed below, CYP7A1 mRNA expression is controlled by both transcription and post-transcriptional (i.e., mRNA stability) mechanisms.

CYP7A1 expression displays a circadian cycle

It is well-established that in rats maintained on a 12 h light cycle, bile acid synthesis and the expression of CYP7A1 exhibit a circadian cycle in which expression is greatest just prior the time when food is most actively consumed (when lights are turned off) (42, 43). Glucocorticoids appear to increase the amplitude of the changes in the expression of CYP7A1 (44). Throughout the circadian cycle, transcription of the CYP7A1 gene correlates with the hepatic expression of the basic leucine zipper transcription factor albumin promoter D-site binding protein (45–47). The circadian increase in expression of CYP7A1 is associated with greater levels of expression by cells extending from the pericentral vein toward the periportal region (48). Two studies have noted that the changes in CYP7A1 mRNA stability also contribute to the circadian cycle of expression (48, 49).

The circadian cycle of CYP7A1 expression is coincident with the circadian cycle for the expression of HMG-CoA reductase (50), the LDL receptor (51), CYP27 (52), and the relative content of hepatic endoplasmic reticulum (53). Liver-specific transgenic expression of CYP7A1 increases cellular content of mature SREBP (18) as well as the expression of the LDL receptor (17, 18) and several lipid biosynthetic enzymes (17, 19). Thus, by metabolizing and inactivating oxysterol repressors (17–19, 24), CYP7A1 coordinately links the expression of genes regulated by SREBP to circadian cycle. It is interesting to note that levels of NADPH, a co-factor for the CYP7A1 reaction, correlate with the ability of neuronal transcription factors controlling circadian cycle to bind to specific promoter sequences on the lactate dehydrogenase promoter (54). Thus, nutritional status may entrain the circadian cycle via redox potential.

End-product (bile acid) negative feedback regulation of CYP7A1

One of the first studies examining the regulation of bile acid synthesis showed that diverting bile from the enterohepatic circulation via cannulation of the bile duct caused a marked increase in the excretion of bile acids (55). More detailed studies indicated that as the duration of the removal of bile from the rat increased, the amount of bile acid that was excreted increased up to a maximal level (56). The findings showing higher rates of bile acid synthesis and/or expression of CYP7A1 in animals fed bile acid sequestrants (e.g., cholestyramine), which bind bile acids and prevent their reabsorption via the intestine, pro-

vides further evidence for bile acid negative feedback regulation (57–59).

Bile acid negative feedback regulation of bile acid synthesis and CYP7A1 have also been demonstrated by administering bile acids to animals via either feeding or infusing them into the intestine and/or the circulation. One of the first published studies demonstrating bile acid negative feedback regulation of bile acid synthesis involved injecting a bolus of bile acids into rats and observing a rapidly reduced rate of biliary excretion (60). Subsequent studies showed that infusing taurocholate into the intestine of rats reduced the rate of bile acid synthesis (61) by blocking the 7α-hydroxylation of cholesterol (62). The structure of the infused bile acid is an important determinant of its effect on bile acid synthesis and the expression of CYP7A1 (26, 63). For example, infusing taurocholate markedly decreases bile acid synthesis, whereas tauroursodeoxycholate causes a slight increase (26). Further analysis shows that the ability to decrease bile acid synthesis correlates with the relative hydrophobicity, as determined by elution of the bile acid on reverse-phase chromatography (63). Administration of taurocholate in the rat diet decreased bile acid synthesis and the expression of CYP7A1 mRNA and enzyme activity (58, 64). The ability of individual bile acids, fed as a dietary component, to decrease CYP7A1 also correlates with the relative amount of hydrophobicity (65).

There is controversy in being able to demonstrate that infusing bile acids into rats decreases CYP7A1. In three independent studies performed by different groups, infusing taurocholate into the intestine of rats did not reduce bile acid synthesis and/or the activity of CYP7A1 (66–68). In one study, infusing taurocholate into the intestine inhibited CYP7A1 expression, whereas infusing the same amount of taurocholate into the portal vein has no effect (68). The mechanistic basis for these discrepancies has not been resolved.

There exist several highly regulated processes that can efficiently bind, transport and metabolize bile acids. These processes prevent bile acids from accumulating in amounts that are sufficient to disrupt cells and/or membranes. Physiologic responses associated with the administration of bile acids are likely to reflect the induction of mechanisms to prevent and/or reduce their detergent (membrane disruptive) nature. If the amount of bile acids that are administered exceed the capacity of any one of these processes, the phenotype produced may include side effects caused by their detergent (toxic) effects.

Induction of CYP7A1 and bile acid synthesis by dietary cholesterol

The cholesterol molecule co-evolved with atmospheric oxygen and respiration to allow the formation of phospholipid/cholesterol membranes capable of carrying out functions essential for eukaryotes (69, 70). Clearly, the relative content of free cholesterol/phospholipid is one of the major determinants of membrane viscosity, an important determinant of membrane function (71, 72). The importance of maintaining the relative level of cholesterol and phospholipids in specific membranes and microdomains (e.g., lipid rafts or caveolae) at precise levels is perhaps best emphasized by the plethora of gene products and mechanisms that are involved (73). One of the first lines of defense preventing alterations in membrane cholesterol/phospholipid content is at the level of the intestine where the amount of dietary cholesterol that enters the body is regulated. Recent studies showed that a synthetic activator of the nuclear receptor liver X receptor (LXR)α decreased the intestinal absorption of cholesterol, presumably through an increase in the expression of ABC transporters responsible for cholesterol export (74). Subsequent studies have shown that induced expression of ABCG5 and ABCG8, the genes whose functional inactivation is responsible for β-sitosterolemia (75–77), is associated with reduced cholesterol absorption. These combined data suggest that the intestine contributes to the maintenance of cholesterol homeostasis by inducing the expression of ABCG5 and ABCG8 (as well as other gene products) that reduce cholesterol absorption by transporting the sterol out of the enterocyte into the intestinal lumen.

The liver also acts to promote cholesterol homeostasis via the excretion of cholesterol and bile acids. It has been proposed that the ability of certain species, strains, and individuals to increase the production of bile acids in response to augmented ingestion of cholesterol is a major predictor of their susceptibility to diet-induced hypercholesterolemia (78). Rats and mice, which are generally noted as being resistant to diet-induced hypercholesterolemia, display increased expression of CYP7A1 and the production of bile acids in response to cholesterol-rich diets (58, 79–86). The induction of CYP7A1 and bile acid synthesis by dietary cholesterol has also been observed in dogs (87) and in a hypercholesterolemia-resistant strain of rabbits (88). The ability of individual strains of rabbits to increase the production of bile acids in response to a cholesterol-rich diet correlated with their resistance to diet-induced hypercholesterolemia (89, 90). Species that are susceptible to diet-induced hypercholesterolemia [hamsters (91), guinea pigs (90) and African green monkeys (92)] apparently lack the ability to induce the expression of CYP7A1 as a means to compensate for increased ingestion of cholesterol.

Based on the observations that humans display variable responses to dietary cholesterol in regard to CYP7A1 and bile acid synthesis (93–96), it is reasonable to propose that genetic background determines the response. Early cholesterol balance studies showed that feeding cholesterol-enriched diets to hyperlipidemic patients decreased cholesterol absorption and biosynthesis, but had no effect on bile acid production (93). Feeding gallstone patients cholesterol-enriched diets decreased intestinal cholesterol absorption and cholesterol biosynthesis but increased the excretion of biliary cholesterol while decreasing bile acid production (94). In certain groups of human subjects who are resistant to dietary cholesterol-induced hypercholesterolemia, bile acid production increases in response to dietary cholesterol (95, 96). Furthermore, feeding gall-

stone patients cholesterol-enriched diets increased the enzymatic activity of CYP7A1 (97). Based on these findings, it is reasonable to conclude that, similar to rodents, humans have the capacity to display induced CYP7A1 as a means to compensate for increased dietary cholesterol.

One important consideration in evaluating individual responses to dietary cholesterol is the relative amount that is administered. In general, cholesterol feeding studies in rodents consist of feeding a diet containing ~2% cholesterol (58, 86), which represents a ~100-fold increase compared with normal rodent diets. In contrast, humans are usually given much smaller increments in dietary cholesterol (~3-fold increases over normal intake) (98). It is likely that species and individual differences in response to dietary cholesterol may be caused by differences in the amount of cholesterol consumed relative to the control diet.

Post-transcriptional regulation of CYP7A1 expression (i.e., mRNA stability)

The finding that the diurnal variation in CYP7A1 mRNA, protein, and enzyme activity occurs concomitantly suggested that the rate of turnover of the protein and mRNA was rapid and regulation was at the mRNA level (transcription and/or stability) (49). The identification of AUUUA elements in the 3′ untranslated region (UTR) of the CYP7A1 mRNA led to the proposal that regulated mRNA stability may contribute to the regulation of CYP7A1 mRNA levels (49, 99). Kinetic analysis showed that the rate of CYP7A1 mRNA turnover was unusually rapid (<5 min) (100). Furthermore, adding the 3′UTR of the rat CYP7A1 mRNA to the coding regions of two reporters (luciferase and green fluorescent protein) caused a rapid rate of degradation of the chimeric mRNA (100). In other studies, the 3′UTR of the mouse CYP7A1 caused a chimeric mRNA to display increased rate of degradation in response to bile acids (101). The regulated turnover of CYP7A1 mRNA is likely to act in concert with changes in transcription, thus providing rapid changes in physiologic expression.

Identification of the trans-acting factors that regulate the transcription of CYP7A1

Transcription of CYP7A1 is regulated by a number of effectors, insulin, steroid/thyroid hormones, endotoxin, cytokines, cholesterol, and bile acids. Due to space limitations, we will confine our review to the remarkable progress that has been made in understanding transcriptional control of CYP7A1 by cholesterol and bile acids.

Cholesterol/oxysterols

Early cell culture studies suggested that hydroxylated derivatives of cholesterol, rather than cholesterol itself, were responsible for regulating "negative-feedback" of cholesterol synthesis as well as cellular cholesterol homeostasis (102–104). While this hypothesis was initially viewed with skepticism, over 20 years of subsequent studies have provided supportive biochemical and genetic data (17, 22, 105–106). It is now generally accepted that oxysterols play important roles in regulating *1*) the activation of ACAT; *2*) transcriptional activation of genes via LXRα and LXRβ; and *3*) the proteolytic processing and activation of SREBPs, a family of transcription factors common to the control of many carbohydrate, energy, and lipid metabolic pathways (107–110). Oxysterols produced and metabolized by many of the individual processes involved in the "classical" and "alternative/acidic" bile acid synthetic pathways (Fig. 1) may also link the expression of CYP7A1 to cellular cholesterol homeostasis via all three mechanisms.

Role of LXRα and LXRβ in the regulation of CYP7A1 and cholesterol homeostasis

Transfection assays using rat/mouse CYP7A1 promoter/reporter constructs indicate the presence of a LXR binding site that functionally activates transcription in response to dietary cholesterol and/or oxysterol agonists (111). In contrast, functionally active LXR sites are absent in the corresponding sequences in the human CYP7A1 promoter (112–114). This region of the human CYP7A1 promoter contains an hepatocyte nuclear factor-1 (HNF1) binding site (113). Differences in LXRα activation sites in the CYP7A1 promoter may explain the species-specific differences in response to dietary cholesterol.

Perhaps the most compelling evidence linking LXR-dependent transcriptional regulation to maintenance of cholesterol homeostasis are studies showing that genetic deletion of functional LXR receptors is associated with a marked toxicity to diets containing cholesterol (106). These studies were interpreted to indicate the existence of an LXR-dependent feed-forward regulatory pathway for sterol metabolism (106). This conclusion was further supported by subsequent findings showing oxysterols/LXR can induce the expression of genes that export cholesterol out of macrophages and other cell types via ABCA1 (19, 115–117) and ABCG1 (118), and liver and intestinal cells via ABCG5 and ABCG8 (75).

The multiple processes that act in concert to maintain cholesterol homeostasis via oxysterol activation of LXR-dependent transcription may explain how species that lack the ability to induce CYP7A1 expression can resist the toxicity associated with dietary cholesterol displayed by $LXR^{-/-}$ mice (106). For example, even in the absence of an induction of CYP7A1, an induction in one of more of the putative ABC transporters that has been proposed to decrease cholesterol absorption may provide a means to maintain cholesterol homeostasis in response to dietary cholesterol.

FXR-dependent induction of short heterodimer partner represses CYP7A1

Bile acids are the major natural ligands responsible for activating farnesoid X receptor (FXR)-dependent transcription of a number of genes that are involved in the transport and metabolism of bile acids and lipoproteins. The discovery that the human transcription factor CYP7A promoter binding factor (CPF) was required for the ex-

pression of CYP7A1 provided new insights into the mechanisms controlling the transcription of CYP7A1 (119). CPF is a member of the Fushi-tarazu factor-1 family, which includes SF-1 (120) and the murine homolog of CPF, LRH-1 (121).

The role of CPF/LRH-1 in bile acid negative feedback regulation of CYP7A1 was revealed by studies showing that the rat CYP7A1 promoter contained at least two bile acid response elements (BARE) (122, 123). The upstream BARE (BAREII) is responsive to FXR (124). Surprisingly, while the transcription factors HNF4, LRH-1 (125), COUP-TFII, and RXR (123, 125), and basic transcription element binding protein (126) (a GC box-binding protein of Sp1 family transcription factors) all bind to the upstream BAREII element, FXR does not (124). These data raised the possibility that FXR repressed the transcription of CYP7A1 via an indirect mechanism (124). The subsequent discovery that FXR induces the transcription of the nuclear receptor factor short heterodimer partner (SHP) provided a mechanism linking bile acids to repression of CYP7A1 (127, 128). The transcription inactivator SHP does not bind DNA, but rather forms heterodimers with several nuclear receptors, thereby blocking their ability to activate transcription (129). Transfection experiments using a CYP7A1 promoter/luciferase reporter showed that SHP blocked the ability of CPF/LRH-1 to activate transcription (128). From these and additional experiments it was proposed that bile acids activate FXR-dependent transcription of SHP, which subsequently blocks CYP7A1 transcription by interfering with the ability of LRH-1 to activate transcription (127, 128) (**Fig. 3**). The finding $FXR^{-/-}$ mice fed bile acids do not display a repression of CYP7A1 nor an induction of SHP expression by the liver supports the role of FXR and SHP in bile acid negative feedback regulation of CYP7A1 (130) (Fig. 3).

Role of Kupffer cells and cytokines

Kupffer cells are resident macrophages that reside within the liver at the interface between the circulation and parenchymal cells, allowing them to activate innate immunity in response to the entrance of bacteria and bacterial products from the intestinal tract (e.g., bacterial lipopolysaccharides) (131, 132). Kupffer cell-derived cytokines play fundamental roles in controlling hepatic parenchymal cell function: expression of cytochrome P450s (133), lipid metabolism (134), inflammation (131), viral propagation (135), apoptosis, and regeneration (136, 137). Recent studies suggest that Kupffer cells act as sentinels to sense the relative concentration of bile acids entering the liver via the enterohepatic circulation (138)

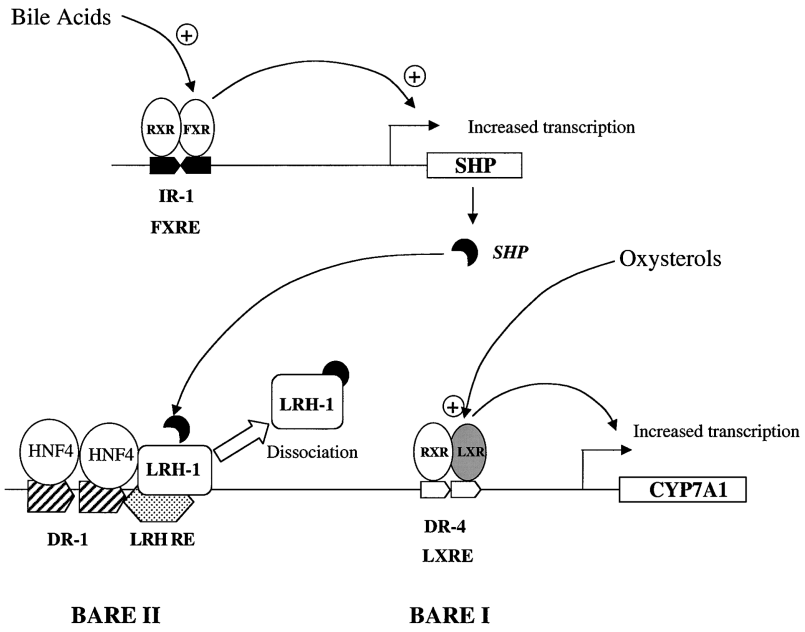

Fig. 3. The CYP7A1 promoter contains two highly conserved bile acid response elements (BARE). The rodent (rat and mouse) CYP7A1 gene contains a BAREI, a DR4 element (not found in the human promoter), which has been shown to be the binding site for the liver X receptor α/retinoid X receptor LXRα/RXR heterodimer. Oxysterols increase CYP7A1 transcription through the activation of LXRα. The inhibitory action of bile acids through farnesoid X receptor (FXR) on CYP7A1 transcription is mediated through upstream BARE. However, CYP7A1 promoter does not contain any FXR binding sites. BARE II contains a conserved DR1 element for hepatocyte nuclear factor 4 binding and a liver receptor homolog response element for LRH-1 binding. Binding of these two factors is essential for liver-specific basal expression of CYP7A1. Bile acids mediate their repression on CYP7A1 through an indirect mechanism. FXR binds to the IR1 element in the small heterodimer partner (SHP) promoter and in response to bile acids SHP transcription is increased. SHP, in turns, interacts with the competence factor LRH-1. This interaction represses the transcription activation by LRH-1. As a result, CYP7A1 expression is decreased.

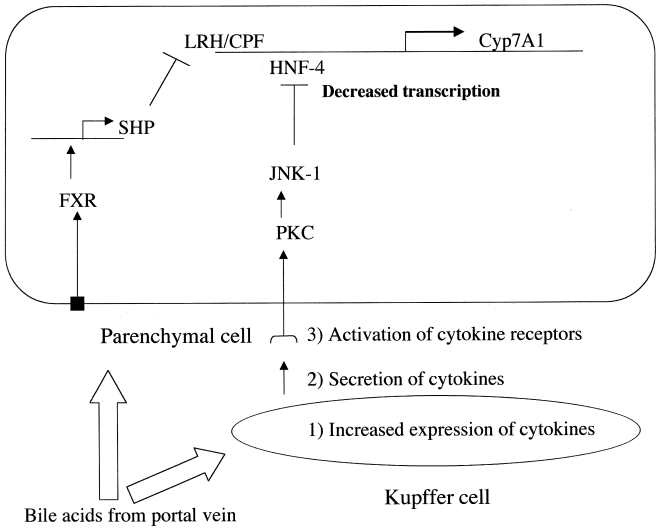

Fig. 4. Bile acids regulate CYP7A1 gene expression via FXR/SHP-dependent and -independent mechanisms. Bile acids returning to the liver via the portal blood can act directly upon the liver via FXR or can act on liver macrophages (Kupffer cells) to initiate "negative feedback" regulation of CYP7A1. The bile acid receptor FXR is activated upon ligand (bile acid) binding. FXR then transactivates the SHP expression. SHP then represses CYP7A1 by interacting with liver-related homolog/CYP7A promoter binding factor and preventing its binding to the CYP7A1 promoter. Bile acids can also interact with liver macrophages (Kupffer cells) and activate cytokine production. The cytokines can then activate protein kinase C in the hepatocyte and initiate a cascade that activates c-Jun N-terminal kinase, which decreases the transcription of CYP7A1.

(**Fig. 4**). As a result of interaction with macrophages, bile acids induce the expression of inflammatory cytokines [(e.g., tumor necrosis factor (TNF)α and interleukin (IL-1β)] that are subsequently secreted into the sinusoids. These cytokines are recognized by high affinity receptors on hepatic parenchymal cells, which respond by blocking the transcription of CYP7A1 (138) (Fig. 4). The cytokine-mediated regulation of CYP7A1 expression is supported by the following data obtained from in vivo studies: *1)* In response to feeding a bile acid-containing diet, inbred C57BL/6 mice display a rapid induction of TNFα and IL-1β mRNA expression by Kupffer cells and a concomitant repression of CYP7A1. In contrast, inbred C3H/HeJ mice display no induction of TNFα and IL-1β mRNA expression by Kupffer cells, and no repression of CYP7A1 (138). *2)* Treating C57BL/6 mice with Rosiglitazone, a known inhibitor of cytokine activation (139), blocks the ability of dietary bile acids to both induce the hepatic expression of TNFα and IL-1β mRNA, and repress CYP7A1 (138). *3)* Feeding C57BL/6 mice with tauroursodeoxycholic acid, a bile acid shown not to inhibit CYP7A1 (26), results in no induction of TNFα and IL-1β mRNA expression by Kupffer cells and no repression of CYP7A1 (138).

Additional studies indicate that bile acid activation of TNFα and IL-1β expression by Kupffer cells and the subsequent repression of CYP7A1 by hepatic parenchymal cells occur via a FXR/SHP-independent mechanism (S-L. Wang, R. Davis, unpublished observations).

Cell culture studies

L35 rat hepatoma cells exhibit many of the functional and morphological characteristics unique to adult hepatic parenchymal cells (41, 140, 141). L35 cells express CYP7A1 at levels similar to those of in vivo rat liver (41, 140, 141). Since bile acids induced FXR-dependent transcription (S-L. Wang, R. Davis, unpublished observations), but had no effect on the expression of CYP7A1, it was concluded that these cells lacked a factor present in liver necessary to mediate bile acid repression (140). Subsequent studies showed that L35 cells displayed bile acid repression of CYP7A1 when they were co-cultured with THP-1 human macrophages (138). Further studies revealed that the missing factors necessary for L35 cells to show bile acid repression of CYP7A1 were TNFα and IL-1β, whose expression by THP-1 macrophages were induced by bile acids (138). These studies indicated that bile acids activate the expression of TNFα and IL-1β by Kupffer cells.

Cytokine mediation of bile acid negative feedback regulation of CYP7A1 can account for involvement of the same group of kinases that are activated by stress and inflammation. The BAREI in the hamster CYP7A1 promoter contains a phorbol ester protein kinase C (PKC) response sequence (142). The same authors showed that the BAREII in the rat CYP7A1 promoter contains an HNF4 binding site, which is necessary for transcription (143). Bile acids and TNFα activate various members of the mitogen-activated protein kinase signaling cascade, causing a reduction in the binding of HNF4 to the BAREII and decreased expression of CYP7A1 (143). Studies using the PKC activator PMA have shown that it can reduce CYP7A1 mRNA levels (144). In other studies it was observed that bile acids activated PKC and this was associated with a repression of CYP7A1 (145). Notably, the more hydrophobic bile acids activated PKC consistent with their rank order of repression of CYP7A1. The additional finding showing that phorbol esters, which are potent activators of PKC, also repressed CYP7A1 expression led to the proposal that PKC mediated bile acid "negative feedback" (145).

Additional studies have shown that bile acids, as well as the cytokine TNFα, activate the c-Jun N-terminal kinase (JNK) pathway in primary cultures of rat hepatocytes, resulting in an induction of SHP mRNA and a repression of CYP7A1 (146). Over-expression of a dominant-negative form of JNK1 or a transactivating domain mutant of c-Jun significantly blocked the ability of bile acids to down-regulate CYP7A1 mRNA (146). These combined findings were interpreted to suggest that bile acids and/or cytokines repress CYP7A1 via JNK1 activation of SHP (146). The importance of HNF4 for expression of rodent CYP7A1 expression is underscored by the findings that HNF4 liver-specific knockout is associated a marked decrease in CYP7A1 expression (147).

Summary and conclusions

The discovery of the role of LXR and FXR in regulating many different aspects of bile acid metabolism and transport has opened new insights into how a complex pathway can be coordinately regulated. It is clear that both LXR and FXR have many gene targets whose products regulate

the transport of both cholesterol and bile acids. Since both cholesterol and bile acids influence CYP7A1 expression, it is difficult to distinguish direct effects on CYP7A1 transcription from indirect effects. While gene knockout experiments can provide new insights into the obligatory role of specific genes in specific processes, it may be difficult to distinguish direct effects from secondary effects.

Based on studies showing that SHP and FXR are not involved in the cytokine-mediated repression of CYP7A1, we propose that the SHP/FXR regulatory pathway is distinct from the cytokine/FXR-independent regulatory pathway, which is activated by the detergent properties of bile acids (138). The recent discovery of a non-steroidal FXR agonist (GW4064) which lacks detergent properties (127, 148, 149) provides an experimental tool to distinguish the SHP/FXR regulatory pathway from the detergent-induced regulation pathway.

Recent studies have shown that transgenic expression of rat CYP7A1 in the livers of C57BL/6 mice resulted in a 5-fold increase in CYP7A1 enzyme activity, and a doubling of the bile acid pool size, but no effects on size, growth, fecundity, or longevity (24). Rather than being detrimental, "over-expression" of CYP7A1 in the livers of transgenic mice blocked the formation of a bile acid diet-induced atherosclerosis and gallstone formation (150). These findings suggest that CYP7A1 may be an excellent therapeutic target. The new insights into the mechanisms regulating the expression of CYP7A1 reviewed herein should provide clues toward developing effective therapeutics.

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