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REGULATION OF BILE ACID SYNTHESIS

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1. ABSTRACT

Bile acids are important physiological agents required for disposal of cholesterol and absorption of vitamins and fats. Bile acids are synthesized from cholesterol in the liver. Enterohepatic circulation of bile acids is very efficient and plays an important physiological role in lipid absorption and secretion, and regulation of bile acid biosynthesis and cholesterol homeostasis. Conversion of cholesterol to bile acids requires 15 different enzymatic steps. Four cytochrome P450 enzymes play important roles in bile acid biosynthesis. The classic bile acid biosynthesis pathway starts with modification of the sterol ring and followed by side chain cleavage reactions to synthesize cholic acid (CA) and chenodeoxycholic acid (CDCA), the primary bile acids in most species. The first and rate-limiting enzyme in this pathway is cholesterol 7alpha -hydroxylase, a microsomal cytochrome P450, CYP7A. Another microsomal cytochrome P450 sterol 12alpha-hydroxylase (CYP12) is required for the synthesis of cholic acid. Mitochondrial cytochrome P450 sterol 27-hydroxylase (CYP27) catalyzes sterol side chain oxidation to convert C27 sterol to C24 bile acids. An alternative bile acid biosynthesis pathway (acidic) has been known for sometime but only recently has attracted much attention. In this pathway, side chain oxidation precedes modification of the sterol ring. Mitochondrial sterol 27-hydroxylase (CYP27) catalyzes the first reaction and followed by 7alpha-hydroxylation catalyzed by a microsomal oxysterol 7alpha-hydroxylase (CYP7B). Recent advances in purification and cloning of these major enzymes in the pathways have led to better understanding the molecular basis of regulation of bile acid synthesis and physiological role of the alternative pathways.

2. INTRODUCTION

Conversion of cholesterol to bile acids in the liver is the most significant pathway for the elimination of cholesterol from the body. Bile acids are important physiological detergents which induce bile flow and biliary lipid secretion, facilitate the absorption of dietary fat, cholesterol, and lipid-soluble vitamins in the small intestine, and secretion of cholesterol and other compounds into feces. Bile acids are quantitatively reabsorbed in the ileum by active transport systems, and are transported back to the liver via the portal venous circulation (1). In the liver, bile acids are taken up by Na+-taurocholate cotransporter and Na+-independent organic anion transporting polypeptide located in the sinusoidal membrane (basolateral), shuttled vectorially by bile acid binding proteins across the hepatocyte to the canalicular surface, and secreted into the bile canalicular (apical) by canalicular multiorganic anion

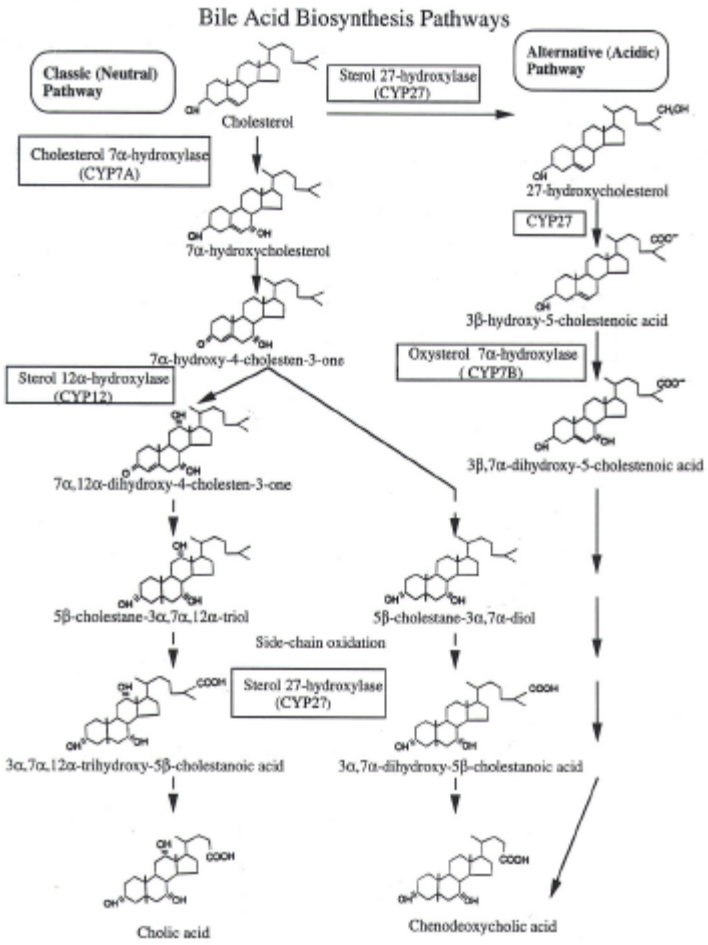

Figure 1: Major bile acid biosynthesis pathways. Only major enzymes and their substrate and metabolites are shown.

transporter and ATP-dependent bile salt transporter. This enterohepatic circulation of bile acids is remarkably efficient, with only 5% bile acids lost in feces which is compensated for by biosynthesis from cholesterol. This feedback mechanism not only regulates bile acid synthesis and bile flow, but also is important in regulating cholesterol synthesis by inhibiting the rate-limiting enzyme, 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase and hepatic uptake of low density lipoprotein (LDL) cholesterol by LDL receptor. Excess cholesterol in extrahepatic tissues is excreted into serum and transported by reverse cholesterol transport involving HDL to the liver. Scavenger receptor SR-BI in hepatocytes uptakes HDL-cholesterol and convert it to bile acids (2, 3). Conversion of cholesterol to bile acids accounts for the catabolism of about 50% of cholesterol in the body and bile acids are also required for the disposal of 40% of cholesterol in feces. Bile acid biosynthesis pathway thus plays an important role in maintaining cholesterol homeostasis in mammals. Bile acids are toxic biological detergents, the synthesis of which has to be tightly regulated to prevent damage of biological membranes and maintaining membrane structure and function. In past few years, knowledge on the regulation of the first and rate-limiting enzyme of the classic bile acid biosynthesis pathway, cholesterol 7alpha-hydroxylase, has been greatly advanced by molecular cloning of the gene. Much advances also has been made on the role of the alternative pathway starting with 27-hydroxylation of cholesterol. This review will focus on the molecular mechanisms of regulation of the rate-limiting enzyme, cholesterol 7alpha-hydroxylase, and other major cytochrome P450 enzymes in the pathways, sterol 27-hydroxylase, sterol 12alpha-hydroxylase and oxysterol 7alpha-hydroxylase.

3. BILE ACID BIOSYNTHESIS PATHWAYS

Cholesterol degradation to primary bile acids can be initiated by either a microsomal cholesterol 7alpha-hydroxylase (the classic or neutral pathway) or by mitochondrial sterol 27-hydroxylase (acidic pathway) (figure 1). The classic pathway with modifications of cholesterol nucleus preceding oxidation and cleavage of the side chain is believed to be the major pathway in the rat. In humans,

alternative pathways were proposed to explain the presence of many bile acid metabolites in human plasma which could not be formed by the classic pathway (4, 5). Axelson and Sjovall (5) studied plasma concentration of c27-acids in patients with liver disease and proposed two pathways of bile acid synthesis in man. They referred the classic pathway as a neutral pathway which is most important when cholesterol 7alpha-hydroxylase activity is high. When cholesterol 7alpha-hydroxylase activity is low as in liver disease, the alternative pathway, referred as "acidic" or "26-oxygenated" pathway predominant to produce chenodeoxycholic acid. The neutral pathway is connected to the acidic pathway through some intermediates. The end products of cholesterol degradation via the neutral pathway are cholic and chenodeoxycholic acids, which are synthesized in roughly similar amounts in humans. By contrast, the end product of the acidic pathway is predominantly chenodeoxycholic acid (6). Several recent studies have suggested that the alternative pathway may account for nearly 50% of total bile acid synthesis (7, 8). Lund *et al.* (9) studied the flux of 27-hydroxylated products of cholesterol in macrophages and estimated that 10% of cholesterol was excreted as 27-oxygenated products which were converted to bile acids in the liver and accounted for about 4% of total bile acids synthesis. It appears that the neutral pathway may be the main pathway under most physiologic conditions and the dominant pathway when bile acid feedback is interrupted, i.e. treatment with bile acid sequestrant or chronic biliary diversion, while acidic pathway might become the major pathway to compensate for the low rate of bile acid synthesis in certain liver diseases (5), and in transgenic mice lacking cholesterol 7alpha-hydroxylase (10). The acidic pathway may be less important in rats and rabbits than in humans, since 27-hydroxycholesterol level in circulation serum is much lower in the former species which has cholic acid as the predominant bile acids in the bile. Javitt (11) has hypothesized that the 27-hydroxylase pathway might even serve as a regulatory function since the major end product chenodeoxycholic acid is a potent inhibitor of cholesterol 7alpha-hydroxylase, that 27-hydroxycholesterol has potent biological effect on cholesterol metabolism and atherosclerosis, and that defect of sterol 27-hydroxylase causes genetic disease cerebrotendinous xanthomatosis (CTX) in humans (11).

3.1. Classic (neutral) Pathway

The biosynthesis of bile acids from cholesterol via the neutral pathway requires at least fifteen different steps catalyzed by a variety of enzymes located in cytosol, microsomes, mitochondria and peroxisomes. However, in different species, bile acid biosynthesis pathways and types of bile acids synthesized vary somewhat. The first step in cholesterol degradation to bile acids is hydroxylation of cholesterol at the C-7 position to form 7alpha-hydroxycholesterol (Figure 1). This reaction is catalyzed by microsomal cholesterol 7alpha-hydroxylase (CYP7A) which is thought to be the only rate-determining enzyme in the bile acid biosynthetic pathway. This enzyme is localized in the smooth endoplasmic reticulum and is believed to be a cytochrome P-450 enzyme based on its sensitivity to carbon monoxide inhibition, and the requirement for NADPH, oxygen, lecithin and NADPH-cytochrome P-450 reductase (1). This enzyme is believed to be feedback repressed by the flux of hydrophobic bile acids returning to the liver via portal circulation. The 7alpha-hydroxycholesterol formed is converted to 7alpha-hydroxy-4-cholesten-3-one by a microsomal 3beta-hydroxy-delta^5-C27-steroid oxidoreductase\ isomerase (12). 7alpha-Hydroxy-4-cholesten-3-one is a branch point intermediate in the bile acid biosynthesis pathway which can be converted into the precursors of either cholic or chenodeoxycholic acids (Figure 1). This intermediate may be hydroxylated at the C-12 position by a microsomal cytochrome P-450 monooxygenase (sterol 12alpha-hydroxylase, CYP12) and subsequently reduced by two cytosolic enzymes delta^4-3-ketosteroid- 5beta-reductase and 3alpha-hydroxysteroid dehydrogenase to 5beta-cholestane-3alpha, 7alpha, 12alpha-triol (figure 1). Alternatively, 7alpha-hydroxy-4-cholesten-3-one may be reduced to 5beta-cholestane-3alpha, 7alpha-diol which is ultimately converted to chenodeoxycholic acid (Figure 1). The next step in this pathway is the reduction of the 3-oxo group of 7alpha-hydroxy-5beta-cholestan-3-one and 7alpha,12alpha-dihydroxy-5beta -cholestan -3-one to the corresponding 3alpha-hydroxy group. This biotransformation is catalyzed by a soluble NADPH-dependent 3alpha-hydroxysteroid oxidoreductase. This sequence of reactions completes the alteration of the sterol nucleus of cholesterol.

The initial step in the side-chain oxidation involves hydroxylation at C27 position which is catalyzed by mitochondrial cytochrome P-450 monooxygenase, sterol 27-hydroxylase (CYP27) (figure 1). Sterol 27-hydroxylase has the unique ability to both hydroxylate the C27 methyl group to the CH2OH group and the CH2OH to COOH (13). The next step in side-chain cleavage involves the ligation of 3alpha,7alpha,12alpha-trihydroxy-5beta-cholestanoic acid or 3alpha,7alpha- dihydroxy-5beta-cholestanoic acid to coenzyme A by an ATP-dependent microsomal coenzyme A ligase (12). The final steps in side-chain oxidation occur mainly in the liver peroxisomes and are believed to be similar to the beta-oxidation of fatty acids including introduction of a double bond at C24-C25 by an oxidase, hydration and dehydrogenation to form oxo-derivatives by a bifunctional hydratase/dehydrogenase, followed by thiolysis yielding propionyl-CoA and cholyl-CoA or chenodeoxycholyl-CoA. Prior to secretion from the hepatocyte, free bile acids are conjugated at C-24 to either glycine or taurine. This reaction involves the initial activation of cholic acid and chenodeoxycholic acid to CoA by a microsomal CoA synthetase. The final conjugation to either glycine or taurine is catalyzed by bile acid CoA: amino acid N-acyltransferase.

3.2. Alternative (acidic) pathway

Bile acid biosynthesis via acidic pathway is initiated by mitochondrial sterol 27-hydroxylase (CYP27) toconvert cholesterol to 27-hydroxycholesterol and 3beta-

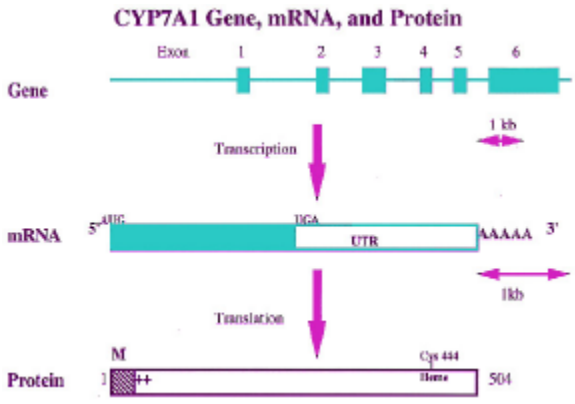

Figure 2: Structures of cholesterol 7alpha-hydroxylase gene, mRNA and protein

hydroxy-5-cholestenoic acid (figure 1). Both 27-hydroxycholesterol and 3beta-hydroxy-5-cholestenoic acid are 7alpha-hydroxylated by enzyme activity in human liver microsomes, whereas 3beta-hydroxy-5-cholestenoic acid is 7alpha-hydroxylated in human liver mitochondria to 3beta, 7alpha-dihydroxy-5-cholestenoic acid (14, 15). The sterol 7alpha-hydroxylase activity, different from cholesterol 7alpha-hydroxylase, converts 27-hydroxycholesterol to 7alpha,27-dihydroxycholesterol in hamster liver microsomes (16). Recently, a cytochrome P450 enzyme named ?oxysterol 7alpha-hydroxylase or CYP7B (Figure 1), originally cloned from mouse hippocampus, has been shown to be able to catalyze 7alpha-hydroxylation of 25- and 27-hydroxycholesterol when transfected into kidney 293 cells (17, 18). Microsomal 3beta-hydroxysteroid oxidoreductase/ isomerase then converts 3beta,7alpha-dihydroxy-5- cholestenoic acid to 7alpha-hydroxy-3-oxo-4-cholestenoic acid, a precursor of chenodeoxycholic acid. However, little is known about remaining enzymes involved in the conversion of this metabolite to chenodeoxycholic acid. Recent observation that both cholic acid and chenodeoxycholic acid can be formed when cholesterol 7alpha-hydroxylase is inhibited in rat hepatocytes provided a suggestive evidence that a pathway may also hydroxylate intermediates at 12alpha position to produce cholic acid via the acidic pathway (8).

4. MAJOR REGULATORY ENZYMES IN BILE ACID SYNTHESIS

Most enzymes in classic bile acid biosynthesis pathway have been purified. The first and rate-limiting enzyme in the pathway, cholesterol 7alpha-hydroxylase, has been the subject of intense study in last thirty years. Since the comprehensive review of cholesterol 7alpha-hydroxylase by Myant and Mitropoulos (1) in 1997, much progress has been made by purification and cloning of this important enzyme. A detailed review of literatures published since 1990 on CYP7A is described here. Sterol 27-hydroxylase has been the subject of increasing study in this decade because the link of the disease CTX to mutation of the *CYP27* gene. Sterol 12alpha-hydroxylase may be important in regulating the ratio of cholic acid to chenodeoxycholic acid. More recently, another microsomal 7alpha-hydroxylase, oxysterol 7alpha-hydroxylase has been identified to play a role in bile acid synthesis. However, not much is known about molecular mechanisms of regulation of CYP27, CYP12 and CYP7B.

4.1. Cholesterol 7alpha-hydroxylase

Early studies using bile fistula rat model established that cholesterol 7alpha-hydroxylase is the only rate-limiting enzyme in the entire pathway (review in Refs.1, 19-22). The liver microsomal enzyme activity requires NADPH and molecular oxygen and is inhibited by carbonmonoxide. These properties suggest that cholesterol 7alpha-hydroxylase activity is catalyzed by a microsomal cytochrome P450 monooxygenase. This was confirmed by purification of a cytochrome P450 from rat liver microsomes and reconstitution of cholesterol 7alpha-hydroxylase activity using this purified cytochrome P450 and NADPH-cytochrome P450 reductase (23, 24). The cloning and sequencing of the cDNAs encoding cholesterol 7alpha-hydroxylase provides further evidence that cholesterol 7alpha-hydroxylase is a unique cytochrome P450 which was designated CYP7A (25-27). Mono-specific antibodies against purified CYP7A were obtained and used to demonstrate that CYP7A enzyme protein levels change in parallel with the activity in rat liver microsomes upon the treatment with cholestyramine (24). Both CYP7A activity and protein levels were rapidly reduced upon the removal of cholestyramine from the diet or treatment with diet containing bile acids. CYP7A activity and protein levels in rat liver microsomes exhibit a significant diurnal rhythm. The enzyme has a very short half-life which was estimated to be about 2 to 3 hours.

Specific antibodies against purified CYP7A were used as probes to screen liver cDNA expression libraries to clone cDNAs encoding rat liver CYP7A by Noshiro *et al.* (27) and Li *et al.* (25). Degenerated oligonucleotide probes based on the internal amino acid sequences were used to clone rat liver CYP7A cDNAs by Jelinek *et al.* (26). The cDNA clones and nucleotide sequences obtained independently by these three laboratories were essentially the same and thus confirmed the authenticity of these cDNA clones. Multiple mRNAs ranging from 4.0 to 1.8 kb were detected in rat liver, most likely are derived from the use of different polyadenylation signals (25). The mRNAs translate a polypeptide of 503 (rat) or 504 (human) amino acid residues the sequences of which are typical of microsomal cytochrome P450 isozymes and are highly conserved among different species (28, 29). Figure 2 shows the structures of the rat *CYP7A* gene, mRNA and protein.

The *CYP7A*genes were cloned by screening genomic libraries using rat CYP7A cDNA as hybridization probes. The *CYP7A* gene spans about 11 kb of the genome

and consists of six exons and five introns, and was mapped to q11-q12 of the human chromosome 8 (30) and mouse chromosome 4 (31). The *CYP7A* gene belongs to the P450 super gene family (32). The 5'-flanking sequences of the rat (33, 34), human (30, 35-37), hamster (29), rabbit (38), and mouse (39) have been determined. Sequences from transcription start site to about 250 bp upstream are highly homologous among different species. The *CYP7A* promoter contains a TATA box sequence located about 30 bp upstream of the transcription start site (+1) which is typical for eukaryotic genes transcribed by a DNA-dependent RNA polymerase II.

Recently, mice deficient of *CYP7A* have been obtained by gene knockout (10). These mice displayed complex phenotypes including oily coats, hyperkeratosis, vision defects, and behavioral irregularities, consistent with malabsorption of vitamins E and D3. Homozygous animals (Cyp7-/-) died within 18 days; 40% of them died between days 1 and 4, and 45% died within days 11 and 18. Vitamin supplement to nursing mothers could prevent deaths in the early period and bile acid supplement prevented deaths in the later period. Cyp7-/- mice have low levels of vitamin E and D3 and elevated stool fat content (40). Several 7alpha-hydroxylated bile acids were detected in the bile and stool of adult Cyp7-/- mice. Apparently, a hepatic oxysterol 7alpha-hydroxylase activity expressed after weaning may account for the synthesis of 7alpha-hydroxylated bile acids.

4.1.1. Bile acid feedback

More hydrophobic bile acids such as deoxycholic acid and chenodeoxycholic acid are potent repressors of the CYP7A activity, whereas hydrophilic bile acids such as ursodeoxycholic acid (UDCA) do not have much effect (41-43). The relationship between the hydrophobicity and 7alpha-hydroxylation of cholesterol was recently confirmed in humans (44). It has been suggested that secondary bile acids formed in the colon might be the physiological regulators (43, 45,46). Recent *in vivo* studies suggest that deoxycholate is a more potent regulator than cholate, and deoxycholate and cholate might modulate bile acid synthesis by different mechanisms (45,47). Twisk *et al*. (48) studied effects of bile acid metabolites on cholesterol 7alpha-hydroxylase mRNA levels in rat primary hepatocytes and found a weak correlation between hydrophobicity of bile acids and potency of inhibition of mRNA expression. They postulated that the hydroxyl groups in the steroid rings may form a hydrophilic microenvironment in the otherwise hydrophobic molecules. The steriostructure of bile acids may be important for binding to a transcription factor which interacts with regulatory sequence in the *CYP7A* gene.

Intraduodenal infusion of hydrophobic bile acids to bile fistula rat down-regulates cholesterol 7alpha-hydroxylase activity, mRNA level and protein mass in parallel (49). Nuclear run-on transcription assay of the rate of *CYP7A* gene transcription reveals that cholesterol 7alpha-hydroxylase is regulated primarily at the level of gene transcription by bile acids (50). Taurocholate (TCA) represses CYP7A mRNA transcription in rat primary hepatocyte cultures (51,52), however, it fails to suppress mRNA level in HepG2 cells (53,54).

Several experimental observations have raised some doubt about the bile acid feed back mechanism. Obstruction of the common bile duct, which excluded bile salts from the intestine, paradoxically up-regulated CYP7A and HMG-CoA reductase in rats, despite a marked increase in hepatic concentrations of bile salts and cholesterol (55). Feeding of rat with 1% deoxycholate reduced CYP7A activity, but drastically stimulated both HMG-CoA reductase and CYP7A mRNA expression (56). This high dose of deoxycholate induces liver cholestasis and necrosis. It is likely that deoxycholate induced liver regeneration which cause the indution of CYP7A mRNA expression, but enzyme activity was repressed by deoxycholate. A marked increase in the specific activities of CYP7A after creation a thoracic duct lymph fistula in rats was observed and was attributed to the loss of a lymp-transmitted intestinal inhibitory factor normally released in response to bile salts in the intestinal lumen (57). In bile fistula rats, intravenous infusion of taurocholate failed to down-regulate CYP7A (58). It was concluded that passage of taurocholate through the intestine strongly potentiated negative feedback regulation of the *CYP7A*. A putative intestinal factor, released or absorbed in the presence of bile acids in the intestinal lumen, may play a role in the regulation of bile acid synthesis in the rat. In HepG2 cells, tauro-CDCA (TCDCA) and tauro-DCA (TCDCA) strongly repressed CYP7A mRNA expression but tauro-CA (TCA) and tauro-UDCA (TUDCA) had no effect (53) indicating that extrahepatic factors were not required for bile acid repression. More recent experiment in hamster demonstrated that overexpression of CYP7A by adenovirus-mediated gene transfer, or obstruction of common bile duct, or intravenous infusion of TCA prevented the derepression of *CYP7A* expression by bile diversion (59). These investigators suggested that primary bile acid synthesized in the liver repressed cholesterol 7alpha-hydroxylase expression at the level of hepatocytes and intestinal factor might not be needed for bile acid feedback. Trawick et al. (60) found that bile acids did not repress *CYP7A* expression in a rat hepatoma L35 cells which were insensitive to the toxicity of 25-hydroxycholesterol. These contradictory results can only be explained by differences in animal species, models and cell lines used in studying regulation of bile acid synthesis and *CYP7A* expression.

4.1.2. Role of cholesterol

Under most physiologic and pathophysiologic circumstances, HMG-CoA reductase and cholesterol 7alpha-hydroxylase specific activities change in tandem (review in Refs 19, 61). The exception is that cholesterol is a potent repressor of HMG-CoA reductase, but appears to up-regulate cholesterol 7alpha-hydroxylase activity in the rat. However, it was suggested that

cholesterol feeding might affect cholesterol 7alpha-hydroxylase indirectly, i.e. by altering bile acid absorption in the intestines, similar to the effect of cholestyramine (62). In contrast, high cholesterol diet suppressed cholesterol 7alpha-hydroxylase activity and mRNA level in African green monkeys (63) but had no effect in the hamsters (64). In Watanabe rabbits, a model of familial hypercholesterolemia with defective LDL receptor, cholesterol 7alpha-hydroxylase activity was found to be much lower than that in New Zealand white rabbits and high cholesterol diet did not further reduce enzyme activity (65). In hypercholesterolemia-resistant rabbits (CRT/mlo), cholesterol 7alpha-hydroxylase activity and mRMA levels were substantially higher than in normal New Zealand rabbits which are susceptible to hypercholesterolemia (38). In humans, increased bile acid synthesis apparently is the major compensatory response to increased dietary cholesterol and reduced bile acid absorption (66). In gallstone patients, cholesterol and bile acid homeostasis is altered and dietary cholesterol increases biliary cholesterol secretion and decreases bile acid synthesis and pool (67). It seems that the response to dietary cholesterol input varies greatly with different species and individuals (68). It appears that the rat has the ability to maintain cholesterol homeostasis efficiently by high rate of bile acid synthesis, less efficient feedback inhibition and lower rate of cholesterol synthesis, thus less susceptible to diet-induced atherosclerosis.

Several lines of evidence suggest that availability of cholesterol, especially the newly synthesized cholesterol, is the preferred substrate for cholesterol 7alpha-hydroxylase (1, 69). Other experiments indicated that specific lipoproteins might be preferentially utilized to provide cholesterol for cholesterol 7alpha-hydroxylase. In HepG2 cells, beta-VLDL stimulated cholesterol 7alpha-hydroxylase mRNA level and 25-hydroxycholesterol decreased mRNA level slightly (54). LDL cholesterol stimulated the promoter activity in differentiated mouse H2.35 cells transfected with CYP7A promoter/reporter genes (70). Cholesterol synthesis has been found to regulate cholesterol 7alpha-hydroxylase activity and mRNA expression. Inhibition of HMG-CoA reductase by lovastatin led to the down-regulation of CYP7A gene transcription, and mevalonate, a precursor of cholesterol, could prevent down-regulation in bile fistula rats (71). In intact rats, mevalonate stimulated CYP7A activity and mRNAs (71-73). In contrast, little or no effect of HMG-CoA reductase inhibitors on cholesterol 7alpha-hydroxylase expression in intact rats was observed (74). Squalestatin or zaragozic acid A, an inhibitor of squalene synthase, and AY9994, an inhibitor of 7-dehydrocholesterol ?7-reductase, strongly inhibited cholesterol 7alpha-hydroxylase activity and mRNA levels in rat liver or primary rat hepatocyte, and this effect could be prevented by cholesterol but not mevalonate (75-77). These results suggest that intermediates in cholesterol synthesis do not regulate CYP7A expression. Differences in animal species and experimental models may account for differences in response to cholesterol and its precursors.

Taurocholate repressed cholesterol 7alpha-hydroxylase transcription even in the presence of excess mevalonate (49, 51). Taurocholate may repress cholesterol 7alpha-hydroxylase directly rather than indirectly by suppressing the HMG-CoA reductase. In inbred strains of mice, taurocholate suppressed both the activity and mRNA of CYP7A and adding of taurocholate to the cholesterol-rich diet suppressed both the activity and mRNA of CYP7A (78). Therefore, the repressive effect of taurocholate overcomes the inductive effect of cholesterol on 7alpha-hydroxylase activity and mRNA. In contrast, Spady and Cuthbert (79) have reported that sodium cholate repressed cholesterol 7alpha-hydroxylase activity and mRNA levels in the rat and cholate did not suppress CYP7A activity when fed to rats on a high cholesterol diet. They concluded that the effect of cholesterol dominated the effect of cholate on 7alpha-hydroxylase in rat liver. The most plausible explanation is that dietary or de novo synthesized cholesterol may be responsible for a basal level of gene expression while the hydrophobic bile acids play a major role in modulating the level of CYP7A gene expression in vivo.

4.1.3. Hormonal regulation

Thyroid hormone rapidly stimulated cholesterol 7alpha-hydroxylase mRNA expression in hypophysectomized rats (80, 81). In primary rat hepatocytes, cholesterol 7alpha-hydroxylase mRNA level and activity are declined within 72 hrs of culture and both T4 and dexamethasone are required to stimulate and maintain cholesterol 7alpha-hydroxylase mRNA level (82). Adrenalectomy plus thyroidectomy or hypophysectomy led to decreases in cholesterol 7alpha-hydroxylase activity and mRNA levels, and transcriptional activity (83). Neither adrenalectomy nor thyroidectomy alone affected transcriptional activity. Dexamethasone was shown to be required to maintain activity in primary rat hepatocyte (84) and to stimulate expression of mRNA in a 25-hydroxycholesterol resistent rat cell line (85). Recent transient transfection assay in HepG2 cells have mapped two regions responsive to dexamethasone stimulation, but failed to demonstrate the stimulatory effect of thyroid hormones on *CYP7A* promoter activity (86, 87). It is likely that primary hepatocyte is rapidly dedifferentiated and thyroxine and dexamethasone are required to maintain the expression of liver-specific genes in serum free medium (88). In highly differentiated HepG2 cells, thyroxine may not be needed for the expression of the *CYP7A* gene. Insulin strongly suppressed cholesterol 7alpha-hydroxylase activity in the rat (89). It has been reported that bile acid pool size, and rate of synthesis and excretion were increased in humans with diabetes mellitus (90) and in rats treated with streptozotocin (91). Thus, insulin may play an important role in the regulation of *CYP7A* gene expression and cholesterol homeostasis. Retinoic acid strongly stimulated *CYP7A* promoter activity and retinoid response elements for binding of retinoid receptors, RAR/RXR, have been identified in the rat *CYP7A* promoter (86, 92). Retinoic acids are known to regulate cell differentiation and growth. In addition, cAMP or the catalytic subunit of protein kinase A stimulated the rat *CYP7A* promoter in transient transfection assay (86). Both positive and negative cAMP regulatory regions were mapped

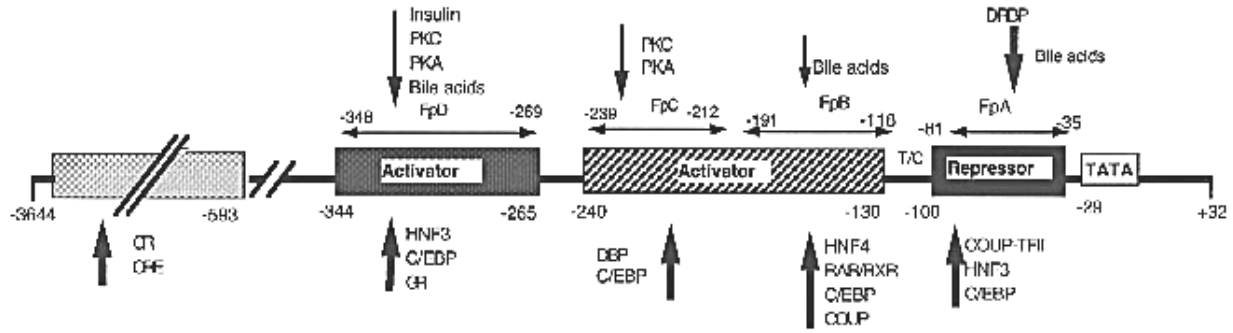

Figure 3: Mapping of regulatory regions and transcription factors binding sites in the rat CYP7A promoter.

in the rat promoter. Glucagon or cAMP increased bile acid synthesis (1), but strong down-regulation of CYP7A activity and mRNA was observed in rat primary hepatocytes (51, 82). Growth hormones stimulated CYP7A activity in the rat (93). Thus, the *CYP7A* gene is regulated by multiple hormones and mechanisms of regulation by these physiological stimuli are extremely complicated.

4.1.4. Diurnal rhythm

Expression of cholesterol 7alpha-hydroxylase activity in the liver is the highest at the middle of dark and the lowest at the middle of light cycle. This diurnal variation of activity follows the circadian pattern of serum concentration of corticosterones with a lag of about 4 hours suggesting corticosterones may play a role in diurnal rhythm of cholesterol 7alpha-hydroxylase activity (1). Hypophysectomy abolished diurnal rhythm of this enzyme activity. The circadian rhythm is free running and is independent of external stimuli such as light and feeding. The variation of activity paralleled the level of protein, mRNA and transcription of the *CYP7A* (25, 94). Lavery *et al*. (95) reported that circadian transcription of the *CYP7A* gene requires DBP, a PAR family of clock genes. Transcription of DBP exhibits a stringent circadian rhythm in the liver. Another PAR gene human leukemia factor 43 (HLF43) also stimulates the *CYP7A* transcription (96). DBP is a member of basic leucine zipper transcription factors enriched in the liver. Several DBP binding sites have been mapped in the rat *CYP7A* gene, which also bind C/EBPbeta(LAP) (97). DBP gene is regulated by suprachiasmatic nuclei in the hypothalamus and destruction of which abolished circadian rhythm of DBP and HMG-CoA reductase, presumably also the *CYP7A* (98). DBP and C/EBPbeta may compete for overlapping binding sites to determine the relative rate of basal vs. diurnally regulated *CYP7A* gene expression (97).

4.1.5. Other factors

Endotoxin, TNF and IL-1 strongly decreased CYP7A mRNA level and activity (99). The *CYP7A* is a negative acute phase response gene, in contrast to LDL receptor and HMG-CoA reductase gene (100). CYP7A activity was decreased on days 1 and 2 after partial hepatectomy and returned to the preoperative level on day 3 (101). In acute phase, cholesterol level is reduced and cholesterol synthesis is up-regulated but bile acid synthesis is reduced to maintain cholesterol level. During liver regeneration, bile acid synthesis may be up-regulated to compensate the decreased bile acid synthesis during hepatectomy. Dietary fats could modulate the *CYP7A* expression (102). Fibrates reduced CYP7A activity in gallstone patients (103) and in hyperlipoproteinemia patients (104). Sulfhydryl reducing agents (DTT, glutathione) are required for dexamethasone induced CYP7A expression in L35 cells (105). DTT could reverse repression of CYP7A expression by insulin but not by phorbol ester. The redox state of hepatocytes may regulate CYP7A expression. Phenobarbital induced CYP7A activity and resulted in stimulation of HMG-CoA reductase activity (106). This is consistent with the stimulation of HMG-CoA reductase expression in COS cells overexpressing CYP7A (107).

4.1.6. Regulation of the *CYP7A* gene expression

Potential regulatory sequences in *CYP7A* upstream region has been identified by DNase I footprinting assay, transient transfection assay of *CYP7A* promoter/reporter genes, and electrophoretic mobility shift assay. Figure 3 illustrates the mapping of regulatory regions in the rat *CYP7A* proximal promoter. A footprint (FpA) from nt -81 to -35 contains a repeated sequence and consensus binding sites for liver-enriched hepatocyte nuclear factors HNF3, C/EBP and COUP-TFII (108, 109). Deletion of the sequence from nt -74 to -54 greatly stimulated promoter activity and indicated that this sequence is a strong negative regulatory element (109). These transcription factors are enriched in hepatocytes and are known to regulate the expression of liver-specific genes, such as albumin, alpha-fetoprotein, alpha-1 antitrypsin during development, differentiation and diseases (110, 111). An activator region (nt -240 and -118) contains two footprints. FpB (nt -186 and -118) is the most conserved region and contains three hexameric hormone response elements (HRE) -like half sites, AGTTCA, which form overlapping direct repeats spaced by one base (DR1) and

five bases (DR5). These HRE motifs are potential binding sites for nuclear receptors HNF4 and RXR/RAR, respectively. HNF3 binding site is also located in this sequence. The major DBP binding site is located in FpC (nt -240 and -216) (95, 97). Another activator region (nt -344 to -265) contains FpD (nt -338 and -269) which has insulin response sequence IRS/TGT4/HNF3, cAMP response element, C/EBP and HNF3 binding sites.

Molowa *et al.* (36) reported that human *CYP7A* gene transcription was stimulated by HNF3 and identified three HNF3 binding sites by DNase I footprints (nt -340/-317, -315/–306, and -276/-260) using rat liver nuclear extracts. Using HepG2 nuclear extracts, nine DNase I footprints (FPs 1 to 9) were mapped in the 5?-flanking regions of the human *CYP7A* gene (112): FP1, FP2, and FP3 exactly match the rat FpA; FP4 (nt -104/-91), FP5 (nt -144/-125) and FP6 (nt -191/-174) match the rat FpB; FP7 (nt -227/-213) matches the rat FpC; FP8 (nt -286/-268) and FP9 (nt -341/-313) match the rat FpD.

Crestani *et al.* (53) studied effects of bile acids and steroid/thyroid hormones on the promotor activity of the *CYP7A* gene fused to the luciferase reporter gene which was transiently transfected into confluent culture of HepG2 cells. These investigators found that taurocholate did not repress promoter activity, whereas taurodeoxycholate and taurochenodeoxycholate suppressed the promoter activity. Bile acid responsive elements were located in the -160 fragment and also in the upstream region. Thyroid and steroid hormones stimulated promoter activity and their responsive elements were located in the upstream region. Endogenous cholesterol 7alpha-hydroxylase mRNA levels were regulated similarly by bile acids and hormones in the confluent cultures of HepG2 cells (53). A putative bile acid responsive element has been mapped to the sequence between nt -79 and -49 of the rat *CYP7A* gene (113, 108). direct repeat sequence (-65TCAAGTTCAAGT-54) was shown to mediate bile acid response when linked to a heterologous promoter and was named bile acid response element (BARE) (108). Electrophoretic mobility shift assay using oligonucleotide based on this sequence specifically shifted one or two bands in rat liver nuclear extracts. This band shift was abolished when nuclear extracts of deoxycholate treated rats were used. These DNA-protein complexes were much stronger when nuclear extracts of human liver or HepG2 cells were used. However, internal deletion of the -74 to -54 sequence from the -416/+32 fragment did not abolish bile acid repression of promoter activity. This suggests that other BARE must be localized in the upstream region, between nt -344 and -222 (in FpD) and -186 and -118 (FpB) of the rat CYP7A gene. A second bile acid responsive element named BARE-II was recently mapped to a sequence between -149 and -118. This BARE-II shares an identical AGTTCAAG core sequence with BARE-I (114). The protein factors bind to this sequence have not been identified.

The human CYP7A gene is resistant to stimulation by hormones and is more responsive to repression by bile acids, insulin and phorbol 12-myristate 13-acetate (PMA) (87). This may explain the much lower level of cholesterol 7alpha-hydroxylase activity and mRNA expression in the human than in the rat liver. Several differences in the liver-specific transcriptional regulation between the rat and human CYP7A genes were noticed. HNF3 is a very strong transcriptional activator of the human gene, but rather a moderate activator of the rat CYP7A gene. COUP-TFII, a nuclear orphan receptor involved in the regulation of the genes in lipid metabolism, is a stronger activator of the rat CYP7A gene but had moderate effect on the human gene. Retinoic acid strongly stimulated rat CYP7A transcriptional activity but had no effect on the human CYP7A gene (92). Dexamethasone stimulate the rat gene but inhibited the human gene (87). Thus species differences in transcriptional regulation of the CYP7A gene exist.

4.1.7. Molecular mechanisms of regulation

Two models (figure 4) have been proposed for bile acid-mediated repression of the CYP7A gene transcription. Chiang and Stroup (108) proposed that bile acid might bind to a bile acid receptor (BAR) which enters into nuclei and interacts with a bile acid responsive protein (BARP). BARP may be a positive transcription factor required for basal level transcription of the CYP7A. Interaction between BAR and BARP prevents the binding of BARP to BARE and represses the CYP7A gene expression. Our recent results suggest that BARP could be liver-enriched transcription factors and orphan nuclear receptor (109, 114). Recent identification of bile acids in human and rat liver nuclei support this mechanism (115). Hydrophobic bile acids or their metabolites may act as ligands for orphan nuclear receptors, similar to many biologically active lipids identified as endogenous ligands for peroxisome proliferator activated receptor (116-119). In another model, hydrophobic bile acids may activate protein kinase Cs which initiate a signal transduction pathway leading to down-regulation of the CYP7A gene transcription (120, 121). However, the down stream signaling molecules have not been identified. These two models may be merged to form a unified model that the bile acid stimulated protein kinase C signaling pathway may lead to phosphorylation of BARP and disrupt its interaction with other DNA-binding proteins and their binding to the BAREs. Further studies are needed to identify transcription factors involved in mediating bile acid effects.

4.2. Sterol 27-hydroxylase

Sterol 27-hydroxylase has a broad substrate specificity and is present in many tissues. Sterol 27-hydroxylase metabolizes 7alpha-Hydroxy-4- cholesten-3-one efficiently (122). In the reconstituted system, the purified, bacterially expressed sterol 27-hydroxylase has a high turnover number of 39 nmol/min/mmol of P450 for 5beta-cholestane-3alpha, 7alpha,12alpha-triol and 1.2 for cholesterol (123) indicating that this bile acid intermediate is the preferred substrates than cholesterol. The catalytic activity of sterol 27-hydroxylase toward cholesterol is also lower than other C27-sterols (122). In addition, sterol 27-hydroxylase has been shown to catalyze both 25-hydroxylation of vitamin D3 and 27-hydroxylation of cholesterol (124, 125). Mitochondrial 27-hydroxylase is able

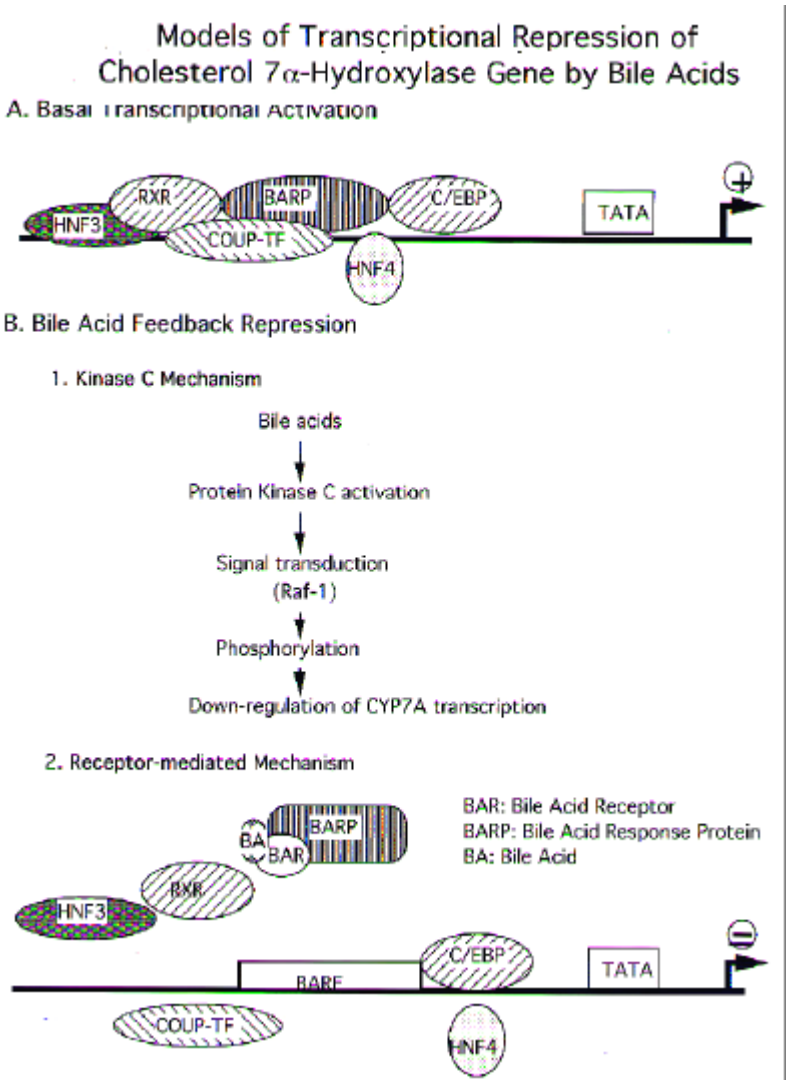

Figure 4: Models of mechanism of transcriptional regulation of the CYP7A gene.

to catalyze 27-, 25-, or 24-hydroxylation of cholesterol in pig liver (126). Human CYP27 is capable to hydroxylate vitamin D analogs at and 1alpha-, 24-, 25-, or 27-position when expressed in COS cells (127). Sterol 27-hydroxylase activity (128), mRNA (129) and protein (130) are present at high level in vascular endothelium and atherosclerotic plaque, and in macrophages (122) and fibroblasts (131). It has been proposed that CYP27 may play important roles in elimination of cholesterol in macrophages and endothelial cells (132), and may act as a defense against atherosclerosis by converting excess cholesterol in macrophages (122) and in the arterial wall (129).

The rabbit, rat and human mitochondrial sterol 27-hydroxylases have been purified, cloned and nucleotide sequences of the cDNAs determined (125, 133-135). Sterol 27-hydroxylase is a product of a P450 gene, *CYP27*, which has been mapped to q33-qter interval of human chromosome 2 (135). The *CYP27* gene contains nine exons and eight introns spanning at least 18.9 kb of DNA (136). The enzyme is located in the inner mitochondrial membrane and requires ferredoxin, ferredoxin reductase and NADPH for activity. The 27-hydroxylase mRNAs (1.9 kb) are present in high levels in liver, duodenum and adrenal gland, and in much lower levels in lung, kidney and spleen (133). The cDNA encodes a protein of 535 amino acid residues consisting the mitochondrial signal peptide of N-terminus 36 amino acid residues. The promoter lacks a TATA-like sequence and is GC-rich, typical of a house keeping gene.

It has been reported that transcription of the rat *CYP27* gene is suppressed by bile acids, although to a much less extent than the *CYP7A* gene (137-140). However, rabbit *CYP27* gene is neither repressed by bile acids nor

stimulated by cholestyramine (141). CYP27 activity was increased with increased bile acid synthesis in cholesterol-fed rabbit, but bile drainage did not affect CYP27 activity in cholesterol-fed rabbits (142). Insulin also suppressed *CYP27* transcription (89). Dexamethasone was required for the expression of sterol 27-hydroxylase activity in rat primary hepatocytes, however, thyroxine did not affect *CYP27* expression (137). It seems clear that the *CYP27* gene is not regulated as much as the *CYP7A* gene.

Mutations of the *CYP27* gene cause cerebrotendinous xanthomatosis (CTX), a rare autosomal recessive defect of cholesterol metabolism manifested by xanthoma, progressive neurologic dysfunction, accumulation of cholesterol in the tissues, premature coronary heart disease and high frequency of cholesterol gallstones (12). Point mutations and frame shift/splice junction mutations in the *CYP27* gene have been identified in CTX patients (134, 136, 143, 144). The defect in CYP27 leads to excessive accumulation of 7alpha-hydroxycholesterol, 7alpha- hydroxy-4-cholesten-3-one, 5β-cholestane-3alpha, 7alpha, 12alpha-triol, cholesterol and cholestanol in CTX. In spite of the link of *CYP27* gene mutations to CTX, the etiology of this disease is still not known. It is possible that conversion of excess cholesterol to 27-hydroxycholesterol is a mechanism which prevents the accumulation of intracellular cholesterol and provides a defense mechanism for atherosclerosis (122). The link of *CYP27* mutations to CTX and oxysterols to the development of atherosclerosis and apoptosis warrant a detailed study of transcriptional mechanism of the *CYP27*.

4.3. Oxysterol 7alpha-hydroxylase

The existence of a different 7alpha-hydroxylase specific for 27- and 25-hydroxycholesterol has been described by several laboratories. Two liver microsomal cytochrome P450 fractions and a mitochondrial 7alpha-hydroxylase fraction capable of 7alpha-hydroxylation of 27-hydroxycholesterol, 3beta-hydroxy-5-cholestenoic acid and 3beta-hydroxy-5-cholenoic acid were isolated from pig liver (145, 146). 7alpha-hydroxylase activity specific for 27-hydroxycholesterol has also been found in microsomes (14, 15) and mitochondria (14) of human liver, human fibroblasts (131), rat ovary (147) and brain (148), and in hamster liver and HepG2 cells (16). Partially purified oxysterol 7alpha-hydroxylase from pig liver microsomes could hydroxylate both 25- and 27-hydroxycholesterol (149). Oxysterol 7alpha-hydroxylase catalyzes the synthesis of 7alpha-hydroxylated primary bile acids during neonatal period when cholesterol 7alpha-hydroxylase activity is absent in the *CYP7A* knockout mice (10). A cDNA with sequence identity of 35% to CYP7A was recently isolated from mouse hippocampus cDNA library (150). This is the second gene in the *CYP7* family and was designated as *Cyp7b*. The CYP7B is mainly expressed in the brain and very low mRNA levels were found in male rat liver and not detectable in female rat liver and mouse kidney. Subsequently, Martin *et al*.(18) transfected kidney 293/T cells with CYP7B expression plasmid and showed that 27-hydroxycholesterol was metabolized to cholest-5-ene, 3beta,7alpha,27-triol in these cells overexpressing CYP7B. They also demonstrated that cholesterol synthesis was not inhibited by 27-hydroxycholesterol in cells overexpressing CYP7B. The mouse brain Cyp7b has later been shown to catalyzes 7alpha-hydroxylation of neurosteroids dehydroepiandrosterone and pregnenolone (151). The brain *Cyp7b1* over-expressed in kidney 293 cells was shown to metabolize 25-hydroxycholesterol to cholest-5-ene-3 beta,7alpha,25-triol (17). High activity of oxysterol 7alpha-hydroxylase was detected in mouse, hamster, rabbit and pig livers. Antibody against a 15 amino acid peptide of brain cyp7b cross-reacted with a polypeptide in mouse liver microsomes. The levels of this immuno-reacted peptide were induced after 18 days of life and correlated with oxysterol 7alpha-hydroxylase activity and mRNA levels. Dietary cholesterol or colestipol (a bile acid sequestrant) did not induce oxysterol 7alpha-hydroxylase activity, mRNA and protein level in adult mouse liver. These results suggest that cyp7b is present in mouse liver. However, liver cyp7b isoform has not been isolated from liver microsomes. It is thus of interest to clone the liver oxysterol 7alpha-hydroxylase cDNA for studying the structure, function and regulation of this novel family of oxysterol 7alpha-hydroxylase in bile acid biosynthesis. It is likely that different members of the *CYP7* gene sub-family are catalyzing 7alpha-hydroxylation of 27-hydroxycholesterol, 3beta-hydroxy-5-cholestenoic acid and 3beta-hydroxy-5-cholenoic acid in the liver and kidney. This enzyme may be important not only in bile acid synthesis but also in regulation of oxysterol synthesis which are potent regulators of cholesterol metabolism and homeostasis.

4.4. Sterol 12alpha-hydroxylase

Sterol 12alpha-hydroxylase is required for the synthesis of cholic acid in bile acid biosynthesis pathway. The level of expression of this enzyme activity in liver microsomes may play a role in regulation the ratio of cholic acid to chenodeoxycholic acid in different species (12). The activity is high in rabbit which produce predominantly cholic acid and is low in guinea pig which has CDCA as the major bile acids. Since chenodeoxycholic acid is a more potent inhibitor of bile acid synthesis than cholic acid, the ratio of these two primary bile acids in gallbrader may be important in development of cholesterol gallstone. Bile acids inhibit and cholestyramine stimulates sterol 12alpha-hydroxylase activity. The increase of bile acid synthesis, the pool of bile acids and the ratio of cholic acid to CDCA in diabetes mellitus may be due to the stimulation of 12alpha-hydroxylase activity (152). Recent study revealed that sterol 12alpha-hydroxylase activity was increased two-fold in livers of patients treated with cholestyramine or undergone ileal resection (153). The increase of the ratio of CA to CDCA in these patients is likely due to a compensatory increase of the sterol 12alpha-hydroxylase

activity. Bernhardsson *et al*. (154) first demonstrated that sterol 12alpha-hydroxylase activity could be reconstituted from a crude cytochrome P450 fraction and NADPH-cytochrome P450 reductase solubilized from rat liver microsomes. However, this enzyme activity was only partially inhibited by carbonmonoxide. Sterol 12alpha-hydroxylase was purified from rabbit liver microsomes (155). It was demonstrated in a reconstituted system that cytochrome b5 was required for full enzymatic activity in addition to cytochrome P450 and NADPH-cytochrome P450 reductase. Starvation increases the activity several fold in the rabbits. Streptozotocin also stimulates enzyme activity. It was reported that this enzyme activity also was inhibited by bile acids in human liver (153). The rabbit cDNA encoding sterol 12alpha-hydroxylase has been cloned recently (156). It encodes a polypeptide of 500 amino acid residues of cytochrome P450. The amino acid sequence showed a 39% similarity with human prostacyclin synthase (CYP8) and 31% similarity with rabbit cholesterol 7alpha-hydroxylase. This cytochrome P450 protein was designated as CYP12. Transfection of COS cells with this cDNA expressed sterol 12alpha-hydroxylase activity toward 7alpha-hydroxy-4-cholesten-3-one. Northern blot showed that the mRNA was exclusively expressed in the liver. Fasting of rats and mice led to a several fold increase in both enzyme activity and mRNA levels. However, starvation of rabbits had little effect on enzyme activity and mRNA levels. Post-translational mechanisms may regulate CYP12 expression. The *CYP12* gene has not been cloned yet.

5. CONCLUSION AND FUTURE PERSPECTIVE

Rapid progress in understanding molecular mechanisms of regulation of major enzymes in bile acid synthesis in the last decade will lead to identification of more inborn errors in bile acid synthesis and gene therapy for treatment of metabolic diseases associated with imbalance of cholesterol and bile acids metabolisms. Recent knockout of the *CYP7A* gene in mouse provided experimental evidence which supports the concept that bile acids are important in neonatal development, vitamin absorption and survival. It also revealed that alternative pathways exist to compensate bile acid synthesis when the major pathway of bile acid synthesis is blocked. Overexpression of cholesterol 7alpha-hydroxylase in mice lacking LDL receptor decreases the rate of LDL cholesterol appearance in plasma (157) and support the idea that overexpression of this enzyme activity may be an effective strategy for lowering plasma LDL concentration. Regulation of the *CYP27*, *CYP7B* and *CYP12* genes will be the focus of research when these genes are further characterized. The *CYP27* knockout mouse have been obtained and will be a useful animal model for studying CTX. Purification of liver microsomal CYP7B and cloning of its gene will provide important tools for further studying the alternative pathway and roles of oxysterols in regulation of bile acid and cholesterol synthesis and atherosclerosis. Double knockout of *CYP7A/CYP7B* or *CYP7A/CYP27* in mice would be useful for studying bile acid biosynthesis. These genes are attractive targets for designing lipid lowering drugs. Massive screening of drugs targeting to regulation of the *CYP7A* gene has been undertaken by several major drug companies and will be applied to other three genes in the pathways.

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7. REFERENCES

1. Myant, N. B., & K. A. Mitropoulos: Cholesterol 7alpha-hydroxylase. *J. Lipid Res.* 18, 135-153 (1977)

2. Kozarsky, K. F., M. H. Donahee, A. Rigotti, S. N. Iqbal, E. R. Edelman, & M. Krieger: Overexpression of the HDL receptor SR-BI alters plasma HDL and bile cholesterol levels. *Nature* 387, 414-417 (1997)

3. Rigotti, A., B. L. Trigatti, M. Penman, H. Rayburn, J. Herz, & M. Krieger: A targeted mutation in the murine gene encoding the high density lipoprotein (HDL) receptor scavenger receptor class B type I reveals its key role in HDL metabolism. *Proc. Natl. Acad. Sci. USA* 94, 12610-12615 (1997)

4. Bjorkhem, I. : Mechanism of degradation of the steroid side chain in the formation of bile acids. *J. Lipid Res.* 33, 455-471 (1992)

5. Axelson, M., & J. Sjovall: Potential bile acid precursors in plasma-possible indicators of biosynthetic pathways to cholic and chenodeoxycholic acids in man. *J. Steroid Biochem.* 36, 631-640 (1990)

6. Javitt, N. B., R. Pfeffer, E. Kok, S. Burstein, B. I. Cohen, & K. Budai: Bile acid synthesis in cell culture. *J. Biol. Chem.* 264,10384-10387 (1989)

7. Princen, H. M. G., P. Meijer, B. G. Wolthers, R. J. Vonk, & F. Kuipers: Cyclosporin A blocks bile acid synthesis in cultured hepatocytes by specific inhibition of chenodeoxycholic acid synthesis. *Biochem. J.* 275, 501-505 (1991)

8. Vlahcevic, Z. R., R. T. Stravitz, D. M. Heuman, P. B. Hylemon, & W. M. Pandak: Quantitative estimations of the contribution of different bile acid pathways to total bile acid synthesis in the rat. *Gastroenterology* 113, 1949-1957 (1997)

9. Lund, E., O. Andersson, J. Zhang, A. Babiker, G. Ahlborg, U. Diczfalusy, K. Einarsson, J. Sjovall, & I. Bjorkhem: Importance of a novel oxidative mechanism for elimination of intracellular cholesterol in humans. *Arter. Thromb. Vasc. Biol.* 16, 208-212 (1997)

10. Ishibashi, S., M. Schwartz, P. K. Frykman, J. Hertz, & D. W. Russell: Disruption of cholesterol 7alpha-hydroxylase gene in mice:I. Postnatal lethality reversed by bile acid and vitamin supplementation. *J. Biol. Chem.* 271, 18017-18023 (1996)

11. Javitt, N. B. Bile acid synthesis from cholesterol: regulatory and auxiliary pathways: *FASEB J.* 8, 1308-1311 (1994)

12. Bjorkhem, I.: Mechanism of bile acid biosynthesis in mammalian liver. In: Steroid and bile acid . Eds: Danielsson, H. and Sjovall, J. Elsevier Sci. Pub. The Netherlands, 231-277 (1985)

13. Russell, D. W., and K. D. R. Setchell: Bile acid biosynthesis. *Biochem.* 31, 4737-4749 (1992)

14. Shoda, J., A. Toll, M. Axelson, F. Pieper, K. Wikvall, & J. Sjovall: Formation of 7alpha- and 7beta-Hydroxylated bile acid precursors from 27-hydroxycholesterol in human liver microsomes and mitochondria. *Hepatology* 17, 395-403 (1993)

15. Bjorkhem, I., B. Nyberg, & K. Einarsson: 7alpha-hydroxylation of 27- hydroxycholesterol in human liver microsomes. *Biochemica et Biophysica Acta* 1128, 73-76 (1992)

16. Martin, K. O., K. Budai, & N. B. Javitt: Cholesterol and 27-hydroxycholesterol 7alpha-hydroxylation:evidence for two different enzymes. *J. Lipid Res.* 34, 581-588 (1993)

17. Schwarz, M., E. G. Lund, R. Lathe, I. Bjorkhem, & D. W. Russell: Identification and characterization of a mouse oxysterol 7alpha-hydroxylase cDNA. *J. Biol. Chem.* 272, 23995-24001 (1997)

18. Martin, K. O., A. B. Reiss, R. Lathe, & N. B. Javitt: 7alpha-hydroxylation of 27-hydroxycholesterol:biologic role in the regulation of cholesterol synthesis. *J. Lipid Res.* 38, 1053-1058 (1997)

19. Chiang, J. Y. L., & Z. R. Vlahcevic: The regulation of cholesterol conversion to bile acids. In: Adv. Mol. Cell Biol., Ed: Jefcoate, C. JAI press, Inc., Greenwich, London. 269-316 (1996)

20. Vlahcevic, Z. R., P. B. Hylemon, & J. Y. L. Chiang: Hepatic cholesterol metabolism. In The Liver: Biology and Pathology. Eds: Aries, I., Boyer, J., Fausto, N., Jakoby, W., Schachter, D. &Shafritz, D..Raven Press, Ltd., N. Y. 379-389 (1994)

21. Vlahcevic, Z. R., W. M. Pandak, D. M. Heuman, & P. B. Hylemon: Function and regulation of hydroxylases involved in the bile acid biosynthesis pathways. Seminars in liver disease 12, 403-419 (1992)

22. Vlahcevic, Z. R., D. M. Heuman, & P. B. Hylemon. Regulation of Bile Acid Synthesis: *Hepatology* 13, 590-600 (1991)

23. Ogishima, T., S. Deguchi, & K. Okuda: Purification and characterization of cholesterol 7alpha-hydroxylase from rat liver microsomes. *J. Biol. Chem.* 262, 7646-7650 (1987)

24. Chiang, J. Y. L., W. F. Miller, & G. M. Lin: Regulation of cholesterol 7alpha-hydroxylase in the liver:Purification of cholesterol 7alpha-hydroxylase and the immunochemical evidence for the induction of cholesterol 7alpha-hydroxylase by cholestyramine and circadian rhythm. *J.Biol.Chem.* 265, 3889-3897 (1990)

25. Li, Y. C., D. P. Wang, & J. Y. L. Chiang: Regulation of cholesterol 7alpha-hydroxylase in the liver: cDNA cloning, sequencing and regulation of cholesterol 7alpha-hydroxylase mRNA. *J. Biol. Chem.* 265, 12012-12019 (1990)

26. Jelinek, D. F., S. Andersson, C. A. Slaughter, & D. W. Russell: Cloning and regulation of cholesterol 7alpha-hydroxylase, the rate-limiting enzyme in bile acid biosynthesis. *J. Biol. Chem.* 265, 8190-8197 (1990)

27. Noshiro, M., M. Nishimoto, K. Morohashi, & K. Okuda: Molecular cloning of cDNA for cholesterol 7alpha-hydroxylase from rat liver microsomes: nucleotide sequence and exoression. *FEBS Lett.* 257, 97-100 (1989)

28. Karam, W. G., & J. Y. L. Chiang: Polymorphisms of human cholesterol 7alpha-hydroxylase. *Biochem. Biophys. Res. Commun.* 185, 588-595 (1992)

29. Crestani, M., G. Galli, & J. Y. L. Chiang: Genomic cloning,sequencing and analysis of hamster cholesterol 7alpha-hydroxylase gene (CYP7). *Arch. Biochem. Biophys.* 306, 451-460 (1993)

30. Cohen, J. C., J. J. Cali, D. F. Jelinek, M. Mehrabian, R. S. Sparkes, A. J. Lusis, D. W. Russell, & H. H. Hobbs: Cloning of the human cholesterol 7alpha-hydroxylase gene (CYP7) and localization to chromosome 8q11-q12. *Genomics* 14, 153-161 (1992)

31. Machleder, D., B. Ivandic, C. Welch, L. Castellani, K. Reue, & A. J. Lusis: Complex genetic control of HDL levels in mice in response to an atherogenic diet. *J. Clin. Invest.* 99, 1406-1419 (1997)

32. Nelson, D. R., T. Kamataki, D. J. Waxman, F. P. Guengerich, R. W. Estabrook, R. Feyereisen, F. J. Gonzalez, M. J. Coon, I. C. Gunsalus, O. Gotoh, K. Okuda, & D. W. Nebert: The P450 superfamily: update on new sequences, gene mapping, accession number, early trivial names of enzymes, and nomenclature. *DNA & Cell Biol.* 12, 1-51 (1993)

33. Chiang, J., Y,L., T. P. Yang, & D. P. Wang : Cloning and 5?-flanking sequence of a rat cholesterol 7alpha-

hydroxylase gene. *Biochim. Biophys. Acta.* 1132, 337-339 (1992)

34. Jelinek, D., &D. W. Russell: Structure of the rat gene encoding cholesterol 7alpha-hydroxylase. *Biochemistry* 29, 7781-7785 (1990)

35. Wang, D. P., & J. Y. L. Chiang: Structure and nucleotide sequences of the human cholesterol 7alpha-hydroxylase gene (CYP7). *Genomics* 20, 320-323 (1994)

36. Molowa, D. T., W. S. Chen, G. M. Cimis, & C. P. Tan: Transcriptional regulation of the human cholesterol 7alpha-hydroxylase gene. *Biochemistry* 31, 2539-2544 (1992)

37. Thompson, J. F., M. E. Lira, D. B. Lloyd, L. S. Hayes, S. Williams, & L. Elsenboss: Cholesterol 7alpha-hydroxylase promoter separated from cyclophilin pseudogene by Alu sequence. *Biochim. Biophys. Acta.* 1168, 239-242 (1993)

38. Poorman, J. A., R. A. Buck, S. A. Smith, M. L. Overturf, & D. S. Loose-Mitchell: Bile acid excretion and cholesterol 7alpha-hydroxylase expression in hypercholesterolemia-resistant rabbits. *J. Lipid Res.* 34, 1675-1685 (1993)

39. Tzung, K., K. Ishimura-Oka, S. Kihara, K. Oka, & L. Chan: Structure of the Mouse Cholesterol 7alpha-Hydroxylase Gene. *Genomics* 21, 244-247 (1994)

40. Schwarz, M., E. G. Lund, K. D. R. Setchell, H. J. Kayden, J. E. Zerwekh, I. Bjorkhem, J. Hertz, & D. W. Russell: Disruption of cholesterol 7alpha-hydroxylase gene in mice:II. bile acid deficiency is overcome by induction of oxysterol 7alpha-hydroxylase. *J. Biol. Chem.* 271, 18024-18031 (1996)

41. Heuman, D. M., P. B. Hylemon, & Z. R. Vlahcevic: Regulation of bile acid synthesis. III Correlation between biliary bile salt hydrophobicity index and the activities of enzymes regulating cholesterol and bile acid synthesis in the rat. *J. Lipid Res.* 30, 1160-1171 (1989)

42. Shefer, S., L. Nguyen, G. Salen, A. K. Batta, D. Brooker, F. G. Zaki, I. Rani, & G. S. Tint: Feedback regulation of bile acid synthesis in the rat:Differing effects of taurocholate and tauroursocholate. *J. Clin. Invest.* 85, 1191-1198 (1990)

43. Xu, G., G. Salen, A. K. Batta, S. Shefer, L. B. Nguyen, W. Niemann, T. S. Chen, R. Arora-Mirchandani, G. C. Ness, & G. S. Tint: Glycocholic acid and glycodeoxycholic acid but not glycoursocholic acid inhibit bile acid synthesis in the rabbit. *Gastroenterology* 102, 1717-1723 (1992)

44. Bertolotti, M., N. Abate, P. Loria, M. Dilengite, F. Carubbi, A. Pinetti, A. Digrisolo, & N. Carulli: Regulation of bile acid synthesis in humans: Effect of treatment with bile acids, cholestyramine, or simvastatin on cholesterol 7alpha-hydroxylation rates in vivo. *Hepatology* 14, 830-837 (1991)

45. Stange, E. F., J. Scheibner, & H. Ditschuneit: Role of primary and secondary bile acids as feedback inhibitors of bile acid synthesis in the rat in vivo. *J. Clin. Invest.* 84, 173-180 (1989)

46. Hall, R., E. Kok, & N. B. Javitt: Bile acid synthesis:down-regulation by monohydroxy bile acids. *FASEB J.* 2, 152-156 (1988)

47. Scheibner, J., M. Fuchs, M. Schiemann, & E. F. Stange: Deoxycholate and cholate modulate the source of cholesterol substrate for bile acid synthesis in the rat. *Hepatology* 21, 529-538 (1995)

48. Twisk, J., M. F. M. Hoekman, L. M. Muller, T. Iida, T. Tamaru, A. Uzerman, W. H. Mager, & H. M. G. Princen: Structural aspects of bile acids involved in the regulation of cholesterol 7alpha-hydroxylase. *Eur. J. Biochem.* 228, 596-604 (1995)

49. Pandak, W. M., Z. R. Vlahcevic, J. Y. L. Chiang, D. M. Heuman, & P. B. Hylemon: Bile acid synthesis: VI Regulation of cholesterol 7alpha-hydroxylase by taurocholate and mevalonate. *J. Lipid Res.* 33, 659-668 (1992)

50. Pandak, W. M., Y. C. Li, J. Y. L. Chiang, E. J. Studer, E. C. Gurley, D. M. Heuman, Z. R. Vlahcevic, & P. B. Hylemon: Regulation of cholesterol 7alpha-hydroxylase mRNA and transcriptional activity by taurocholate and cholesterol in the chronic biliary diverted rat. *J. Biol. Chem.* 266, 3416-3421 (1991)

51. Stravitz, R. T., P. B. Hylemon, D. M. Heuman, L. R. Hagey, C. D. Schteingart, H.-T. Ton-Nu, A. F. Hoffman, & Z. R. Vlahcevic: Transcriptional regulation of cholesterol 7alpha-hydroxylase mRNA by conjugated bile acids in primary cultures of rat hepatocytes. . *J. Biol. Chem.* 268, 13987-13993 (1993)

52. Twisk, J., E. M. Lehmann, & H. M. G. Princen: Differential feedback regulation of cholesterol 7alpha-hydroxylase mRNA and transcriptional activity by rat bile acids in primary monolayer cultures of rat hepatocytes. *Biochemistry J.* 290, 685-691 (1993)

53. Crestani, M., W. G. Karam, & J. Y. L. Chiang: Effects of bile acids and steroid/thyroid hormones on the expression of cholesterol 7alpha-hydroxylase mRNA and the CYP7 gene in HepG2 cells. *Biochem. Biophys. Res. Commun.* 198, 546-553 (1994)

54. Taniguchi, T., J. Chen, & A. D. Cooper: Regulation of cholesterol 7alpha-hydroxylase gene expression in HepG2 cells. *J. Biol. Chem.* 269, 10071-10078 (1994)

55. Dueland, S., J. Reichen, G. T. Everson, & R. A. Davis: Regulation of cholesterol and bile acid homeostasis in bile-obstructed rats. *Biochem. J.* 280, 373-377 (1991)

56. Shefer, S., B. T. Kren, G. Salen, C. J. Steer, L. B. Nguyen, T. Chen, G. S. Tint, & A. K. Batta: Regulation of bile acid synthesis by deoxycholic acid in the rat: different effects on cholesterol 7alpha-hydroxylase and sterol 27-hydroxylase. *Hepatology* 22, 1215-1221 (1995)

57. Akerlund, J. E., & I. Bjorkhem: Studies on the regulation of cholesterol 7alpha-hydroxylase and HMG-CoA reductase in rat liver: effects of lymphatic drainage and ligation of the lymph duct. *J. Lipid Res.* 31, 2159-2166 (1990)

58. Pandak, W. M., D. M. Heuman, P. B. Hylemon, J. Y. L. Chiang, & Z. R. Vlahcevic: Failure of intravenous infusion of taurocholate to down-regulate cholesterol 7alpha-hydroxylase in rats with biliary fistulas. *Gastroenterology* 108, 533-544 (1995)

59. Spady, D. K., J. A. Cuthbert, M. N. Willard, & R. S. Meidell: Feedback regulation of hepatic 7alpha-hydroxylase expression by bile salts in the hamster. *J. Biol. Chem.* 271, 18623-18631 (1996)

60. Trawick, J. D., K. D. Lewis, S. Dueland, G. L. Moore, F. R. Simon, & R. A. Davis: Rat hepatoma L35 cells, a liver-differentiated cell line, display resistance to bile acid repression of cholesterol 7alpha-hydroxylase. *J. Lipid Res.* 37, 588-598 (1996)

61. Bjorkhem, I., E. Lund, & M. Rudling. Coordinate regulation of cholesterol 7alpha-hydroxylase and HMG-CoA reductase in the liver. In: Subcellular Biochemistry, Ed: Bittman, R. Plenum Press, N Y, 23-55 (1997)

62. Bjorkhem, I., G. Eggertsen, & U. Andersson.: On the mechanism of stimulation of cholesterol 7alpha-hydroxylase by dietary cholesterol. *Biochim. Biophys. Acta.* 1085**,** 329-335 (1991)

63. Rudel, L., C. Deckelman, M. Wilson, M. Scobey, & R. Anderson: Dietary Cholesterol and Downregulation of Cholesterol 7alpha-Hydroxylase and Cholesterol Absorption in African Green Monkeys. *J. Clin. Invest.* 93**,** 2463-2472 (1994)

64. Horton, J. D., J. A. Cuthbert, & D. K. Spady: Regulation of hepatic 7alpha-hydroxylase expression and response to dietary cholesterol in the rat and hamster. *J. Biol. Chem.* 270, 5381-5387 (1995)

65. Xu, G., G. Salen, S. Shefer, G. C. Ness, L. B. Nguyen, T. S. Parker, Z. Zhao, T. M. Donnelly, & G. S. Tint: Unexpected inhibition of cholesterol 7alpha-hydroxylase by cholesterol in New Zealand white and Watanabe heritable hyperlipidemic rabbits. *J. Clin. Invest.* 95, 1497-1504 (1995)

66. Duane, W. C. : Effects of lovastain and dietary cholesterol on sterol homeostasis in healthy human subjects. *J. Clin. Invest.* 92**,** 911-918 (1993)

67. Kern, F. : Effects of dietary cholesterol on cholesterol and bile acid homeostasis in patients with cholesterol gallstones. *J. Lipid. Res..* 93, 1186-1194 (1994)

68. Kern, F. : Normal plasma cholesterol in an 88-year-old man who eats 25 eggs a day. *New England J. Med.* 324, 896-899 (1991)

69. Straka, M. S., L. H. Junker, L. Zacarro, D. L. Zogg, S. Dueland, G. T. Everson, & R. A. Davis: Substrate stimulation of 7alpha-hydroxylase, an enzyme located in the cholesterol-poor endoplasmic reticulum. *J. Biol. Chem.* 265, 7145-7149 (1990)

70. Ramirez, M. I., D. Karaoglu, D. Haro, C. Barillas, R. Bashirzadeh, & G. Gil: Cholesterol and bile acid regulate cholesterol 7alpha-hydroxylase expression at the transcriptional level in culture and in transgenic mice. *Mol. Cell. Biol.* 14, 2809-2821 (1994)

71. Jones, M. P., W. M. Pandak, P. B. Hylemon, J. Y. L. Chiang, D. M. Heuman, & Z. R. Vlahcevic: Cholesterol 7alpha-hydroxylase: Evidence for transcriptional regulation by cholesterol and/or metabolic products of cholesterol in the rat. *J. Lipid Res.* 34, 885-892 (1993)

72. Sundseth, S. S., & D. J. Waxman: Hepatic P-450 cholesterol 7alpha-hydroxylase. Regulation in vivo at the protein and mRNA level in response to mevalonate, diurnal rhythm, and bile acid feedback. *J. Biol. Chem.* 265, 15090-15095 (1990)

73. Vlahcevic, Z. R., W. M. Pandak, P. B. Hylemon, & D. M. Heuman: Role of newly synthesized cholesterol or its metabolites on the regulation of bile acid biosynthesis after short-term biliary diversion in the rat. *Hepatology* 18, 660-668 (1993)

74. Bjorkhem, I. : Effects of mevinolin in rat liver. Evidence for a lack of coupling between synthesis of HMG-CoA reductase and cholesterol 7alpha-hydroxylase activity. *Biochim. Biophys. Acta* 877, 43-49 (1986)

75. Ness, G. C., Z. Zhao, & R. K. Keller: Effect of squalene synthase inhibition on the expression of hepatic cholesterol biosynthetic enzyme, LDL receptor, and cholesterol 7alpha-hydroxylase. *Arch. Biochem. Biophys.* 311, 277-285 (1994)

76. Pandak, W. M., Z. R. Vlahcevic, D. M. Heuman, & P. B. Hylemon: Regulation of bile acid synthesis. V. Inhibition of conversion of 7-dehydrocholesterol to cholesterol is associated with down-regulation of cholesterol 7alpha-hydroxylase activity and inhibition of bile acid synthesis. *J. Lipid Res.* 31, 2149-2158 (1990)

77. Doerner, K. C., E. C. Gurley, Z. R. Vlahcevic, & P. B. Hylemon: Regulation of cholesterol 7alpha-hydroxylase expression by sterols in primary hepatocyte cultures. *J. Lipid Res.* 36, 168-177 (1995)

78. Dueland, S., J. Drisko, L. Graf, D. Machleder, A. J. Lusis, & R. A. Davis: Effect of dietary cholesterol and taurocholate on cholesterol 7alpha-hydroxylase and hepatic LDL receptors in inbred mice. *J. Lipid Res.* 34, 923-931 (1993)

79. Spady, D. K., & J. A. Cuthbert: Regulation of hepatic sterol metabolism in the rat. Parallel regulation of activity and mRNA for 7alpha-hydroxylase but not HMG-CoA reductase or LDL receptor. *J. Biol. Chem.* 267, 5584-5591 (1992)

80. Ness, G. C., L. C. Pendleton, & Z. Zhao: Thyroid hormone rapidly increases cholesterol 7alpha-hydroxylase mRNA levels in hypophysectomized rats. *biochim. Biophys. Acta* 1214, 229-233 (1994)

81. Ness, G. C., L. C. Pendleton, Y. C. Li, & J. Y. L. Chiang: Effect of thyroid hormone on hepatic cholesterol 7alpha-hydroxylase, LDL receptor, HMG-CoA reductase, farnesyl pyrophosphate synthetase and apolipoprotein A-I mRNA levels in hypophysectomized rats. *Biochem. Biophys. Res.Commun.* 172, 1150-1156 (1990)

82. Hylemon, P. B., E. C. Gurley, R. T. Stravitz, J. S. Litz, W. M. Pandak, J. Y. L. Chiang, & Z. R. Vlahcevic: Hormonal regulation of cholesterol 7alpha-hydroxylase mRNA levels and transcriptional activity in primary rat hepatocyte cultures. *J. Biol. Chem.* 267, 16866-16871 (1992)

83. Pandak, W. M., D. M. Heuman, K. Redford, R. T. Stravitz, J. Y. L. Chiang, P. B. Hylemon, & Z. R. Vlahcevic: Hormonal regulation of cholesterol 7alpha-hydroxylase specific activity, mRNA levels and transcriptional activity in vivo in the rat. *J. Lipid Res.* 38, 2483-2491 (1997)

84. Princen, H. M. G., P. Meijer, B. Hofstee: Dexamethasone regulates bile acid synthesis in monolayer cultures of rat hepatocytes by induction of cholesterol 7alpha-hydroxylase. *Biochem. J.* 262, 341-348 (1989)

85. Leighton, J. K., S. Dueland, M. S. Straka, J. Trawick, & R. A. Davis: Activation of the silent endogenous cholesterol 7alpha-hydroxylase gene in rat hepatoma cells: a new complementation group having resistance to 25-hydroxycholesterol. *Mol. Cell. Biol.* 11, 2049-2056 (1991)

86. Crestani, M., D. Stroup, & J. Y. L. Chiang: Hormonal regulation of the cholesterol 7alpha-hydroxylase gene (CYP7). *J. Lipid. Res.* 36, 2419-2432 (1995)

87. Wang, D. P., D. Stroup, M. Marrapodi, M. Crestani, G. Galli, & J. Y. L. Chiang: Transcriptional regulation of the human cholesterol 7a-hydroxylase gene (CYP7A) in HepG2 cells. *J. Lipid Res.* 37, 1831-1841 (1996)

88. Zaret, K., C. M. DiPersio, D. A. Jackson, W. J. Montigny, & D. L. Weinstat: Conditional enhancement of liver-specific gene transcription. *Proc. Natl. Acad. Sci. USA* 85, 9076-9080 (1988)

89. Twisk, J., M. F. M. Hoekman, E. M. Lehmann, P. Meijer, W. H. Mager, & H. M. G. Princen: Insulin suppresses bile acid synthesis in cultured rat hepatocytes by down-regulation of cholesterol 7alpha-hydroxylase and sterol 27-hydroxylase gene transcription. *Hepatology* 21, 501-510 (1995)

90. Bennion, L. J., & S. M. Grundy: Effects of diabetes mellitus on cholesterol metabolism in man. *N. Engl. J. Med.* 296**,** 1365-1371 (1977)

91. Subbiah, M. T. R., & R. L. Yunker. Cholesterol 7alpha-hydroxylase of rat liver: an insulin sensitive enzyme: *Biochem. Biophys. Res. Commun.* 124, 896-902 (1984)

92. Crestani, M., A. Sadeghpour, D. Stroup, G. Galli, & J. Y. L. Chiang: The opposing effects of retinoic acid and phorbol esters converge to a common response element in the promoter of the rat cholesterol 7alpha-hydroxylase gene (CYP7A). *Biochem. Biophys. Res. Commun.* 225, 585-592 (1996)

93. Rudling, M., P. Parini, & B. Angelin: Growth hormones and bile acid synthesis:key role for the activity of hepatic microsomal cholesterol 7a-hydroxylase in the rat. *J. Clin. Invest.* 99, 2239-2245 (1997)

94. Berkowitz, C. M., C. S. Shen, B. M. Bilir, E. Guibert, & J. J. Gumucio: Different hepatocytes express the cholesterol 7alpha-hydroxylase gene during its circadian modulation in vivo. *Hepatology* 21, 1658-1667 (1995)

95. Lavery, D. J., & U. Schibler: Circadian transcription of the cholesterol 7alpha-hydroxylase gene may involve the liver-enriched bZIP protein DBP. *Genes and Devel.* 7, 1871-1884 (1993)

96. Falvey, E., F. Fleury-Olela, & U. Schibler: The rat hepatic leukemia factor (HLF) gene encodes two transcriptional activators with distinct circadian rhythms,

tissue distributions and target preferrences. *EMBO J.* 14, 4307-4317 (1995)

97. Lee, Y.-H., J. A. Alberta, F. J. Gonzalez, & D. J. Waxman: Multiple, functional DBP sites on the promoter of the cholesterol 7alpha-hydroxylase P450 gene, CYP7. *J. Biol. Chem.* 269, 14681-14689 (1994)

98. Ogawa, H., & Y. Anasi: Diurnal rhythms of rat liver serine dehydrogenase, D-site binding protein, and 3-hydroxy-3-methylglutaryl coenzyme A reductase mRNA levels are altered by destruction of the suprachiasmatic nucleus of the hypothalamus. *Arch. Biochem. Biophys.* 321, 115-122 (1995)

99. Feingold, K. R., D. K. Spady, A. S. Pollock, A. H. Moser, & C. Grunfeld: Endotoxin, TNF, and IL-1 decrease cholesterol 7alpha-hydroxylase mRNA levels and activity. *J. Lipid Res.* 37, 223-228 (1996)

100. Feingold, K. R., A. S. Pollock, A. H. Moser, J. K. Shigenaga, & C. Grunfeld: Discordant regulation of proteins of cholesterol metabolism during the acute phase response. *J. Lipid Res.* 36, 1474-1482 (1995)

101. Nakano, K., K. Chijiiwa, S. Okammoto, H. Yamashita, S. Kuroki, & M. Tanaka: Hepatic cholesterol 7alpha-hydroxylase activity and serum 7alpha-hydroxy-cholesterol level during liver regeneration after partial hepatectomy in rats. *Eur. Surg. Res.* 27, 389-395 (1995)

102. Cheema, S. K., D. Cikaluk, & L. B. Agellon: Dietary fats modulate the regulatory potential of dietary cholesterol on cholesterol 7alpha-hydroxylase gene expression. *J. Lipid Res.* 38, 315-323 (1997)

103. Stahlberg, D., E. Reihner, M. Rudling, L. Berglund, K. Einarsson, & B. Angelin: Influence of bezafibrate on hepatic cholesterol metabolism in gallstone patients:reduced activity of cholesterol 7alpha-hydroxylase. *Hepatology* 21, 1025-1030 (1994)

104. Bertolotti, M., M. Concari, P. Loria, N. Agate, A. Pinetti, M. E. Guicciardi, & N. Carulli: Effects of different phenotypes of hyperlipoproteinemia and of treatment with fibric acid derivatives on the rates of cholesterol 7alpha-hydroxylation in humans. *Arter. Throm. and Vas. Biol.*15, 1064-1069 (1995)

105. Trawick, J. D., S.-L. Wang, D. Bell, & R. A. Davis: Transcriptional induction of cholesterol 7alpha-hydroxylase by dexamethasone in L35 hepatoma cells requires sulfhydryl reducing agents. *J. Biol. Chem.* 272, 3099-3102 (1997)

106. Sudjana-Sugiaman, E., G. Eggertsen, & I. Bjorkhem: Stimulation of HMG-CoA reductase as a consequence of phenobarbital-induced primary stimmulation of cholesterol 7alpha-hydroxylase in rat liver. *J. Lipid Res.*. 35, 319-327 (1994)

107. Sudjana-Sugiaman, E., g. Eggertsen, P. Sjoblom, Y.-r. Macda, K. I. Okuda, & I. Bjorkhem: Presence of cholesterol 7alpha-hydroxylase enzyme protein in COS-cells leads to increased HMG-CoA reductase activity. *Biochem. Biophys. Res. Commun.* 202, 896-901 (1994)

108. Chiang, J. Y. L., & D. Stroup: Identification and characterization of a putative bile acid responsive element in cholesterol 7alpha-hydroxylase gene promoter. *J. Biol. Chem.* 269, 17502-17507 (1994)

109. Stroup, D., M. Crestani, & J. Y. L. Chiang: Orphan Receptors chicken Ovalbumin Upstream Promoter Transcription Factor II (COUP-TFII) and Retinoid X Receptor (RXR) Activate and Bind the Rat Cholesterol 7alpha-hydroxylase Gene (CYP7A). *J. Biol. Chem.* 272, 9833-9839 (1997)

110. Xanthopoulos, G., & J. Mirkovitch: Gene regulation in rodent hepatocytes during development, differentiation and disease. *Eur. J. Biochem*. 216, 353-360 (1993)

111. Johnson, P. F. : Transcriptional Activators in Hepatocytes. *Cell Growth and Differientation* 1, 47-51 (1990)

112. Cooper, A. D., J. Chen, M. J. Botelho-Yetkinler, Y. Cao, T. Taniguchi, & B. Levy-Wilson: Characterization of hepatic-specific regulatory elements in the promoterregion of the human cholesterol 7alpha-hydroxylase gene. *J. Biol. Chem.* 272, 3444-3452 (1997)

113. Hoekman, M. F. M., J. M. J. Rientjes, J. Twisk, R. J. Planta, H. M. G. Princen, & W. H. Mager: Transcriptional regulation of the gene encoding cholesterol 7alpha-hydroxylase in the rat. *Gene* 130, 217-223 (1993)

114. Stroup, D., M. Crestani, & J. Y. L. Chiang. Identification of a bile acid response element in the cholesterol 7alpha-hydroxylase gene (CYP7A). *Am. J. Physiol.* 273: G508-G517 (1997)

115. Setchell, K. D. R., C. M. P. Rodrigues, C. Clerici, A. Solinas, A. Morelli, C. Gartung, & J. Boyer: Bile acid concentrations in human and rat liver tissue and in hepatocyte nuclei. *Gastroenterology* 112, 226-235 (1997)

116. Bocos, C., M. Gottlicher, K. Gearing, C. Banner, E. Enmark, M. Teboul, A. Crickmore, & J. Gustafsson: Fatty acid activation of peroxisome proliferator-activated receptor (PPAR). *J. Steroid Biochem. Molec. Biology* 53, 467-473 (1995)

117. Devchand, P. R., H. Keller, J. M. Peters, M. Vazquez, F. J. Gonzalez, & W. Wahli: The PPARalpha-leukotriene

B4 pathway to inflammation control. *Nature* 384, 39-43 (1996)

118. Forman, B. M., J. Chen, & R. M. Evans: Hypolipidemic drugs, polyunsaturated fatty acids,and eicosanoids are ligands for peroxisome proliferator-activated receptors α and δ. *Proc. Natl. Acad. Sci. USA* 94, 4312-4317 (1997)

119. Keller, H., C. Dreyer, J. Medin, A. Mahfoudi, K. Ozato, & W. Wahli: Fatty acids and retinoids control lipid metabolism through activation of peroxisome proliferator-activated receptor-retinoid X receptor heterodimers. *Proc. Natl. Acad. Sci., USA* 90, 2160-2164 (1993)

120. Stravitz, R. T., Z. R. Vlahcevic, E. C. Gurley, & P. B. Hylemons: Repression of cholesterol 7alpha-hydroxylase transcription by bile acids is mediated through protein kinase C in primary cultures of rat hepatocytes. *J. Lipid Res.* 36, 1359-1368 (1995)

121. Rao, Y.-P., R. T. Stravitz, Z. R. Vlahcevic, E. C. Gurley, J. J. Sando, & P. B. Hylemon: Activation of protein kinase Calpha and delta by bile acids: correlation with bile acid structure and diacylglycerol formation. *J. Lipid Res.* 38, 2446-2454 (1997)

122. Bjorkhem, I., O. Andersson, U. Diczfalusy, B. Sevastik, R.-j. Xiu, C. Duan, & E. Lund: Atherosclerosis and sterol 27-hydroxylase: evidence for a role of this enzyme in elimination of cholesterol from human macrophages. *Proc. Natl. Acad. Sci. USA* 91, 8592-8596 (1994)

123. Pikuleva, I. A., I. Bjorkhem, & M. R. Waterman: Expression, purification and enzymatic properties of recombinant human cytochrome P450c27 (CYP27). *Arch. Biochem. Biophys.* 343, 123-130 (1997)

124. Usui, E., M. Noshiro, Y. Ohyama, & K. Okuda: Unique property of liver mitochondrial P450 to catalyze the two physiologically important reactions involved in both cholesterol catabolism and vitamin D activation. *FEBS Lett* 274, 175-177 (1990)

125. Usui, E., M. Noshiro, Y. Ohyama, & K. Okuda: Molecular cloning of cDNA for vitamin D3 25-hydroxylase from rat liver mitochondria. *FEBS Lett.* 262, 135-138 (1990)

126. Lund, E., I. Bjorkhem, C. Furster, & K. Wikvall: 24, 25- and 27-hydroxylation of cholesterol by a purified preparation of 27-hydroxylase from pig liver. *Biochim. Biophys. Acta* 1166, 177-182 (1993)

127. Guo, Y.-D., S. Strugnell, D. W. Back, & G. Jones: Transfected human liver cytochrome P-450 hydroxylase vitamin D analogs at different side-chain positions. *Proc. Natl. Acad. Sci. USA* 90, 8668-8672 (1993)

128. Reiss, A. B., K. O. Martin, N. B. Javitt, D. W. Martin, E. A. Grossi, & A. C. Galloway: Sterol 27-hydroxylase: hign levels of activity in vascular endothelium. *J. Lipid Res*. 35, 1026-1030 (1994)

129. Reiss, A. B., K. O. Martin, D. E. Rojer, S. Iyer, E. A. Grossi, A. C. Galloway, & N. B. Javitt: Sterol 27-hydroxylase: expression in human arterial endothelium. *J. Lipid Res.* 38, 1254-1260 (1997)

130. Crisby, M., J. Nilsson, V. Kostulas, I. Bjorkhem, & U. Diczfalusy: Localization of sterol 27-hydroxylase immuno-reactivity in human atherosclerotic plaques. *Biochim. Biophys. Acta* 1344, 278-285 (1997)

131. Zhang, J., O. Larrson, & J. Sjovall: 7alpha-Hydroxylation of 25-hydroxycholesterol and 27-hydroxycholesterol in human fibroblasts. *Biochim Biophys Acta* 1995, 353-359 (1995)

132. Babiker, A., O. Andersson, E. Lund, R.-J. Xiu, S. Deeb, A. Reshef, E. Leitersdorf, U. Diczfalusy, & I. Bjorkhem: Elimination of cholesterol in macrophages and endothelial cells by sterol 27-hydroxylase mechanism. *J. Biol. Chem.* 272, 26253-26261 (1997)

133. Andersson, S., D. L. Davis, H. Dahlback, H. Jornvall, &D. W. Russell: Cloning, structure, and expression of the mitochondrial cytochrome P450 sterol 27-hydroxylase, a bile acid biosynthetic enzyme. *J. Biol. Chem.* 264, 8222-8229 (1989)

134. Cali, J. J., C.-L. Hsieh, U. Francke, & D. W. Russell: Mutations in the Bile Acid Biosynthetic Enzyme Sterol 27-Hydroxylase Underlie Cerebrotendinous Xanthomatosis. *J. Biol.Chem.* 266, 7779-7783 (1991)

135. Cali, J. J., & D. W. Russell. Characterization of Human Sterol 27-Hydroxylase: *J. Biol.Chem.* 266, 7774-7778 (1991)

136. Leitersdorf, E., A. Reshef, V. Meiner, R. Levitzki, S. P. Schwartz, E. J. Dann, N. Berkman, J. L. Cali, L. Klapholz, & V. M. Berginer: Frameshift and splice-junction mutations in the sterol 27-hydroxylase gene cause cerebrotendinous Xanthomatosis in Jews of Moroccan origin. *J Clin. Invest.* 91, 2488-2496 (1993)

137. Stravitz, R. T., Z. R. Vlahcevic, T. L. Russell, M. L. Heizer, N. G. Avadhani, & P. B. Hylemon: Regulation of sterol 27-hydroxylase and an alternative pathway of bile acid biosynthesis in primary cultures of rat hepatocytes. *J. Steroid Biochem. Molec. Biol.* 57, 337-347 (1996)

138. Vlahcevic, Z. R., S. K. Jairath, D. M. Heuman, R. T. Stravitz, P. B. Hylemon, N. G. Avadhani, & W. M. Pandak: Transcriptional regulation of hepatic sterol 27-hydroxylase by bile acids. *Am. J. Physiol.* 270, G646-G652 (1996)

139. Twisk, J., E. C. M. deWit, & H. M. G. Princen: Suppression of sterol 27-hydroxylase mRNA and transcriptional activity by bile acids in cultured rat hepatocytes. *Biochem. J.* 305, 505-511 (1995)

140. Dahlback-Sjoberg, H., I. Bjorkhem, & H. M. G. Princen: Selective inhibition of mitochondrial 27-hydroxylation of bile acid intermediates and 25-hydroxylation of vitamin D3 by cyclosporin A. *Biochem. J.* 293, 203-206 (1993)

141. Araya, Z., H. Sjoberg, & K. Wikvall: Different effects on the expression of CYP7 and CYP27 in rabbit liver by cholic acid and cholestyramine. *Biochem. Biophys. Res. Commun.* 216, 868-873 (1995)

142. Xu, G., G. Salen, S. Shefer, G. S. Tint, B. T. Kren, L. B. Nguyen, C. J. Steer, T. S. Chen, L. Salen & D. Greenblatt: Increased bile acid pool inhibits cholesterol 7alpha-hydroxylase in cholesterol fed new Zealand white rabbits. *Gastroenterology* 113, 1958-1965 (1996)

143. Verrips, A., G. C. H. Steenbergen-spanjers, J. A. F. M. Luyten, R. A. Wevers, J. H. J. Wokke, F. J. M. Gabreels, B. G. Wolthers, & L. P. W. J. van den Heuvel: Exon skipping in the sterol 27-hydroxylase gene leads to cerebrotendinous xanthomatosis. *Hum Genet* 100, 284-286 (1997)

144. Garuti, R., M. A. Croce, R. Tiozzo, M. T. Dotti, A. Federico, S. Bertolini, & S. Calandra: Four novel mutations of sterol 27-hydroxylase gene in Italian patients with cerebrotendinous xanthomatosis. *J. Lipid Res.* 38, 2322-2334 (1997)

145. Toll, A., J. Shoda, M. Axelson, J. Sjovall, & K. Wikvall: 7alpha-Hydroxylation of 26-hydroxycholesterol, 3beta-hydroxy-5-cholestenoic acid and 3beta-hydroxy-5-cholenoic acid by cytochrome P-450 in pig liver microsomes. *FEBS Lett* 296, 73-76 (1992)

146. Axelson, M., J. Shoda, J. Sjovall, A. Toll, & K. Wikvall. Cholesterol is converted to 7alpha-Hydroxy-3-oxo-4-cholestenoic acid in liver mitochondria. Evidence for a mitochondrial sterol 7alpha-hydroxylase: *J. Biol.Chem.* 267, 1701-1704 (1992)

147. Payne, D. W., C. Shackleton, H. Toms, I. Ben-Shloml, S. Kol, M. deMoura, J. F. Strauss, & E. Adashi: A novel nonhepatic hydroxycholesterol 7alpha-hydoxylase that is markedly stimulated by interleukin-1-β. J. Biol. Chem. 270, 18888-18896 (1995)

148. Zhang, J., Y. Akwa, E. E. Baulieu, & J. Sjovall. 7alpha-hydroxylation of 27-hydroxycholesterol in rat brain microsomes: Comptes Rendus de 1 Academie des Sciences-Serie Iii, Sciences de la Vie 318, 345-349 (1995)

149. Toll, A., K. Wikvall, E. Sudjana-Sugiaman, K. Kondo, & I. Bjorkman. 7alpha- hydroxylation of 25-hydroxycholesterol in liver microsomes: Evidence that the enzyme involved is different from cholesterol 7alpha-hydroxylase: Eur. J. Biochem. 224, 309-316 (1994)

150. Stapleton, G., M. Steel, M. Richardson, J. O. Mason, K. A. Rose, R. G. M. Morris, & R. Lathe: A novel cytochrome P450 expressed primarily in brain. J. Biol. chem. 270, 29739-29745 (1995)

151. Rose, K. A., G. Stapleton, K. Dott, M. P. Kieny, R. Best, M. Schwarz, D. W. Russell, I. Bjorkhem, J. Seckl, & R. Lathe: Cyp7b, a novel brain cytochrome P450, catalyzes the synthesis of neurosteroids 7alpha-hydroxy dehydroepiandrosterone and 7alpha-hydroxypregnenolone. Proc. Natl. Acad. Sci. 94, 4925-4930 (1997)

152. Kimura, K., Y. Ogura, & M. Ogura: Increased rate of cholic acid formation from 3alpha,7alpha-dihydroxy-5beta-cholestane in perfused livers from diabetic rats. Biochim. Biophys. Acta 963, 329-332 (1988)

153. Einarsson, K., J.-E. Akerlund, E. Reihner, & I. Bjorkhem: 12alpha-Hydroxylase activity in human liver and its relation to cholesterol 7alpha-hydroxylase activity. J. Lipid Res. 33, 1591-1595 (1992)

154. Bernhardsson, C., I. Bjorkhem, H. Danielsson, & H. Wikvall: 12alpha-Hydroxylation of 7alpha-hydroxy-cholesten-3-one by a reconstituted system from rat liver microsomes. Biochem. Biophys. Res. commun. 54, 1030-1038 (1993)

155. Ishida, H., M. Noshiro, K. Okuda, & M. J. Coon: Purification and characterization of 7alpha-hydroxy-4-cholesten-3-one 12alpha-hydroxylase. J. Biol. Chem. 267, 21319-21323 (1992)

156. Eggertsen, G., M. Olin, U. Andersson, H. Ishida, S. Kubota, U. Hellman, K.-I. Okuda, & I. Bjorkhem: Molecular cloning and expression of rabbit sterol 12alpha-hydroxylase. J. Biol. Chem. 271, 32269-32275 (1996)

157. Spady, D. K., J. A. Cuthbert, M. N. Willard, & R. S. Meidell: Overexpression of cholesterol 7alpha-hydroxylase (CYP7A) in mice lacking the low density lipoprotein (LDL) receptor gene. J. Biol. Chem. 273, 126-132 (1997)

Abbreviations: CA, cholic acid; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; UDCA, ursodeoxycholic acid; TCA, taurocholic acid; TCDCA, taurochenodeoxycholic acid;TDCA, taurodeoxycholic acid; TUDCA, tauroursodeoxycholic acid; CYP7A, cholesterol 7alpha-hydroxylase; CYP12, sterol 12alpha-hydroxylase CYP12; CYP27, sterol 27-hydroxylase; CYP7B, oxysterol 7alpha-hydroxylase; HMG-CoA reductase; LDL, low density lipoprotein; FP, footprint; HRE, hormone response elements; DR, direct repeat; nt, nucleotide; BAR, bile acid receptor; BARP, bile acid response protein; BARE; bile acid response element; PMA, phorbol 12-myristate 13-acetate

Keywords: Bile acid synthesis, Cytochrome P450, cholesterol 7alpha-hydroxylase, Sterol 27-hydroxylase, Sterol 12alpha-hydroxylase, Oxysterol 7alpha-hydroxylase, Gene regulation, Gene transcription

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