[Frontiers in Bioscience 13, 6276-6288, May 1, 2008]

Bile acids and gene regulation: from nuclear receptors to chromatin

Nico Mitro, Federica Gilardi, Cristina Godio, Elena Scotti, Emma De Fabiani, Donatella Caruso, Maurizio Crestani

Department of Pharmacological Sciences, University of Milan, via Balzaretti 9, 20133 Milan, Italy

TABLE OF CONTENTS

1. *Abstract*
2. *Introduction*
3. *Mechanisms of gene regulation by bile acids*
    3.1. Activation of gene transcription via FXR
    3.2. Transcriptional repression by bile acids
    3.3. Bile acids target chromatin modifying enzymes
4. *Perspectives*
5. *Acknowledgements*
6. *References*

1. ABSTRACT

Transcription regulation by bile acids is far more complicated than it appeared at first when several groups began their investigations in the early ’90. It has become clear now that bile acids regulate the transcription of genes involved in bile acid synthesis, transport and other metabolic pathways via multiple mechanisms that involve transcription factors, nuclear receptors, coregulators, chromatin and the related modifying enzyme complexes. At a first look this might seem surprising but if one considers the physical-chemical properties of these molecules it should be evident that, due to their detergent properties, bile acids may be harmful if they reach high concentrations in the liver and intestine. Therefore, living organisms have developed biochemical mechanisms that finely tune the concentration of bile acids according to the body needs and in response to environmental challenges. In this review, we will discuss the most recent evidences on the mechanisms through which bile acids regulate gene transcription, including the function of nuclear receptors and emphasizing the emerging role of chromatin and the associated modifying enzymes.

2. INTRODUCTION

The metabolic pathways leading to bile acids contributes substantially to the elimination of cholesterol (1), which can become harmful if it accumulates in the body. In fact, high levels of plasma cholesterol, in particular in the low-density lipoprotein (LDL) fraction, represent one of the major risk factors to develop atherosclerosis and cardiovascular disease (2). Two main metabolic pathways contribute to bile acid synthesis: the classical pathway, which begins with the addition of a hydroxyl group at position 7alpha of cholesterol, and the alternative pathway, which begins with the oxidation of the side-chain moiety of this molecule (3, 4). The first pathway is localized exclusively in the liver and the rate-limiting enzyme, cholesterol 7alpha-hydroxylase, controls this metabolic flux. The initial reactions of the latter pathway can occur in extrahepatic tissues where the enzymes sterol 27-hydroxylase (CYP27A) and oxysterol 7alpha-hydroxylase (CYP7B1) oxidize the side-chain and add a hydroxyl group at position 7alpha, respectively; the subsequent reactions take place in the liver. It is believed that the classical pathway is the major source of bile acids

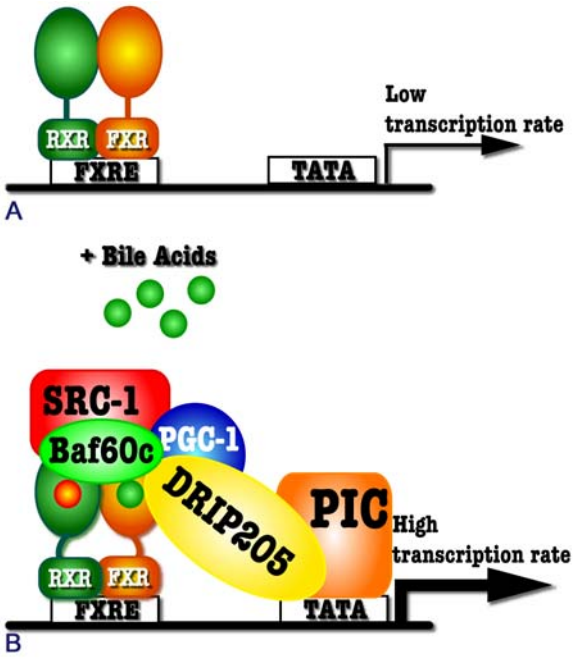

Figure 1. General mechanism of FXR-mediated transcriptional regulation. Under basal conditions (A) (i.e., low bile acid concentration) FXR is not activated and the transcription rate of target genes is low. Upon activation by bile acids (B) FXR undergoes conformational change and recruits coactivators such as steroid receptor coactivator-1 (SRC-1), PPAR gamma coactivator-1 (PGC-1), Baf60c (BRG1/Brm-associated factor of 60 kDa, subunit c) and DRIP205 a subunit of the vitamin D receptor interacting protein (DRIP)/thyroid hormone receptor associated protein (TRAP) coactivator complex. DRIP205 can contact proteins of the preinitiation complex (PIC). RXR: 9-*cis* retinoic acid receptor; FXRE: FXR responsive element; TATA: TATA box.

in the adult life, whereas the alternative pathway may increase only under particular conditions (4).

Given the relative importance and the quantitative contribution of the classical pathway to bile acid biosynthesis, it follows that the rate-limiting enzyme cholesterol 7alpha-hydroxylase is also an important checkpoint for cholesterol homeostasis. In fact, subjects carrying mutations in gene (*CYP7A1*) encoding cholesterol 7alpha-hydroxylase have increased levels of LDL-cholesterol and are more predisposed to vascular complications (5). Therefore, this enzyme is an ideal target for hypocholesterolemic drugs that could be used alone or in combination with other hypolipidemic agents to attain a more pronounced effect. This is important particularly for those patients at higher risk, such as the carriers of mutations in the LDL receptor, who accumulate extraordinarily high levels of cholesterol and die prematurely of myocardial infarction (6). Cholesterol 7alpha-hydroxylase is mostly regulated at the level of gene transcription by bile acids returning to the liver via the enterohepatic circulation but other extracellular stimuli such as hormones (7-9) and certain components of the diet (10, 11) affect the transcription of this gene as well. The comprehension of the mechanisms underlying the transcriptional repression by bile acids has become of great interest in recent years because it may lead to the design of novel treatments for hypercholesterolemia.

3. MECHANISMS OF GENE REGULATION BY BILE ACIDS

The discovery of farnesoid X receptor (FXR, NR1H4) as the physiological bile acid receptor (12-14) aided the definition of a molecular mechanism of bile acid-mediated transcriptional repression (15, 16). However, other mechanisms involving different members of the nuclear receptor gene superfamily also have been described, adding more complexity to the possible pathways through which bile acids regulate gene transcription (17-20). Therefore, to show how bile acids regulate gene transcription, at first we will briefly describe a few examples of transcriptional activation by bile acids through FXR and next we will focus on the mechanisms of transcriptional repression.

3.1. Activation of gene transcription via FXR

When FXR was first discovered it was named after its putative endogenous ligands, which initially were believed to be farnesol metabolites (21). However, the concentrations of farnesoids required to activate the receptor are higher than their physiological concentrations and the activation of FXR with one of the most active farnesoids, the juvenile hormone III, was much less effective than the most efficacious bile acids (14). Later on, however, three independent laboratories identified bile acids as the physiological ligands of FXR, which was indeed named bile acid receptor (BAR) (12-14). FXR/BAR is a class II nuclear receptor that binds to specific promoters as a heterodimer with the 9*cis*-retinoic acid receptor (RXR, NR2B1). FXR is a permissive partner therefore the target genes can be activated either by its ligands or by the RXR ligands. Gene activation occurs through a classical model as described with other nuclear receptors (Figure 1) and a number of different coactivators have been involved in the FXR-mediated activation of gene transcription (12-14, 22-26). By using the selected and amplified binding sequence imprinting technique it was found that the FXR/RXR heterodimer binds to the consensus containing inverted repeat of the sequence AGGTCA with a 1-base pair spacing (IR-1) with the highest affinity (27). However, FXR/RXR can bind to and activate through a variety of elements including direct repeats (DR) and IR elements with changes in the core half-site sequence, spacing nucleotide, and flanking nucleotides.

The crystal structure of FXR was solved in the presence of the synthetic ligand fexaramine showing that the ligand binding cavity of the FXR is largely hydrophobic (28). The definition of the crystal structure of FXR allowed to model different bile acids into the ligand binding cavity and to correlate their agonist, partial agonist or antagonist activity with the orientation adopted by critical alpha-helixes of this region of the receptor and with the

interaction of helix 12 (the activation function-2, AF-2, domain) with coactivators. Upon ligand binding the receptor recruits a coactivator complex, which consequently activate the basal transcriptional machinery. Among such coactivators DRIP205, a subunit of the vitamin D receptor interacting protein (DRIP)/thyroid hormone receptor associated protein (TRAP) coactivator complex, which interacts with several members of the nuclear receptor superfamily, was shown to interact with FXR in a ligand-dependent manner (23). The PPARgamma coactivator-1 (PGC-1) also interacts with FXR and activates the transcription of the *FXR* gene itself by interacting with PPARgamma and HNF-4alpha binding to its promoter (25).

One of the genes first described as a FXR target is the intestinal bile acid-binding protein (I-BABP) (12, 29). This is a soluble cytosolic protein that binds bile acids with high affinity and belongs to the fatty acid-binding protein (FABP) family. In the gut, its expression is restricted to the ileum, where it is thought to be involved in the active bile acid reabsorption and enterohepatic circulation. In the liver, FXR activates the gene encoding the bile salt excretory pump (BSEP, ABCB11) via an imperfect IR-1 element (30). ABCB11 is a critical protein for ATP-dependent transport of bile acids across the hepatocyte canalicular membrane and for generation of bile acid-dependent bile secretion and is considered rate-limiting for the enterohepatic circulation of bile acids. Moreover, liganded FXR also activates the transcription of the human multidrug resistance gene MDR3 encoding a P-glycoprotein that belongs to the ATP-binding cassette transporter family (ABCB4), a critical translocator for phospholipids across canalicular membranes of hepatocytes (31). Thus, FXR is an important regulator of the genes involved in the hepatic and intestinal transport of biliary components. A peculiar way through which FXR activates gene transcription occurs with the human uridine 5'-diphosphate-glucuronosyltransferase 2B4 (UGT2B4), an enzyme that converts hydrophobic bile acids into more hydrophilic glucuronide derivatives (32). Treatment of hepatocytes with FXR agonists results in the increase of UGT2B4 messenger RNA, protein and activity. A bile acid response element to which FXR binds was identified in the UGT2B4 promoter by site-directed mutagenesis, electrophoretic mobility shift and chromatin immunoprecipitation assays. However, RXR does not bind to this consensus sequence and its activation with ligands abolishes the induction of UGT2B4 expression and inhibits binding of FXR to this promoter, suggesting that RXR modulates FXR target gene activation. Since overexpression of UGT2B4 in HepG2 cells results in the attenuation of bile acid induction of the FXR target gene small heterodimer partner (SHP, NR0B2), the authors conclude that UGT2B4 gene induction by bile acids contributes to a feed-forward reduction of bile acid toxicity and to a decrease of the activity of these biological FXR activators (32).

One of the most peculiar genes regulated by ligand-activated FXR is *SHP*, which encodes an atypical nuclear receptor lacking the DNA binding domain. SHP does not bind to DNA consensus sequences but it can heterodimerize with other members of the nuclear receptor superfamily as well as some transcription factors and in general it acts as a transcriptional repressor. Typically, the promoter of *SHP* contains consensus sequences for FXR/RXR, therefore bile acids can upregulate its transcription (15, 16). We will see below that the bile acid-mediated induction of SHP is one of the mechanisms underlying the feedback regulation of *CYP7A1* gene transcription (15, 16).

Besides regulating genes of bile acid synthesis and transport, FXR also plays a very important role in lipid metabolism. A number of genes involved in lipid metabolism and transport has been identified such as the phospholipid transfer protein (PLTP) (33), which mediates the transfer of very low density lipoprotein (VLDL) phospholipids into high density lipoprotein (HDL); the apolipoprotein C-II (34), which stimulates the lipoprotein lipase (LPL) activity enhancing the catabolism of triglycerides; the nuclear receptor peroxisome proliferator-activated receptor alpha (PPARalpha) (35), which is an important regulator of fatty acid catabolism in the liver, to mention a few. The recent observations that bile acid-activated FXR decreases plasma triglycerides by multiple mechanisms are particularly interesting. The mechanistic explanations of this observation include the repression of apolipoprotein C-III (see below) (36) and the repression of sterol regulatory element binding protein-1c (SREBP-1c) (37, 38), a key transactivator of fatty acid biosynthetic enzymes (39), via the recruitment of PPAR gamma coactivator-1alpha (PGC-1alpha) to FXR itself (25) or the association of the transcriptional repressor SHP to liver X receptor (LXR, NR1H3), which positively regulates the transcription of SREBP-1c gene (39, 40). Activated FXR also increases the expression of complement *C3* (41), a precursor for acylation stimulating protein (ASP) produced by adipocytes following a high fat meal (42), which increases fatty acid uptake and triglyceride synthesis. This observation implies an unexpected link among bile acid-activated FXR, complement activation, lipid metabolism, and atherosclerosis. Finally, the relevance of FXR and the modulation of its transcriptional activity by bile acids and synthetic ligands in the vasculature have been highlighted recently. Microarray analysis revealed that FXR is expressed in a variety of normal and pathological human tissues (43). FXR is expressed at high levels in the vasculature and in a number of different metastatic cancers. Vascular smooth muscle cells exposed to FXR ligands undergo apoptosis in a manner that correlates with the ability of ligands to activate FXR. Therefore, FXR, besides being a regulator of bile acid and lipid homeostasis, has also functional relevance in the vasculature and may be considered a direct target for the treatment of proliferative and dyslipidemic diseases.

3.2. Transcriptional repression by bile acids

Bile acids repress the transcription not only of genes implicated in bile acid synthesis and transport but also of genes regulating lipid metabolism and transport. However, the mechanisms of bile acid-mediated gene repression are far more complicated than those of gene

activation and at present the relevance and contribution of different signaling pathways in the negative regulation is still debated. The paradigm of gene repression is represented by the feedback that bile acids exert on their own synthesis, through the genes encoding the main enzymes in the classical pathway, cholesterol 7alpha-hydroxylase (*CYP7A1*) and sterol 12alpha-hydroxylase (*CYP8B1*). *CYP7A1* gene was cloned in the early '90s (44-47) and the group of Chiang first described the negative bile acid response elements I and II (48, 49), located at nt –74/-55 and -149/-128, respectively. A few members of the nuclear receptor gene superfamily were identified among the factors that could bind to these two promoter regions, such as the chicken ovalbumin upstream promoter-transcription factor II (COUP-TFII, NR2F2) (50), hepatocyte nuclear factor-4alpha (HNF-4alpha, NR2A1) (51), liver receptor homolog-1/alpha1-fetoprotein transcription factor/*CYP7A1* promoter binding factor (LRH-1/FTF/CPF, NR5A2) (52). However, only after the discovery of FXR as the nuclear receptor for bile acids, it was possible to define a mechanism of transcriptional repression by bile acids. Two independent laboratories proposed an unusual mechanism whereby liganded FXR represses *CYP7A1* gene transcription indirectly by first activating the transcription of SHP, which in turn heterodimerizes with LRH-1/FTF/CPF and silences its transactivation potential (15, 16). However, *Cyp7a1* and *Cyp8b1* genes are still downregulated in *Shp* null mice fed a bile acid-enriched diet (53, 54). These observations proved that the FXR/SHP cascade explains only partially the feedback regulation of bile acids on *CYP7A1*. Incidentally, since the BARE of *CYP7A1* also contains a conserved binding site for HNF-4alpha, it was shown that this nuclear receptor mediates the repression by bile acids via a mitogen activated protein kinase cascade (17). Later on, it was also discovered that bile acids cause the dissociation of the coactivators cAMP Response Element Binding protein-Binding Protein (CBP) and PGC-1alpha from HNF-4alpha, which eventually decreases the amount of RNA polymerase II on the core promoter (18). Notably, it was demonstrated that the effect of bile acids on the transcriptional activity of HNF-4alpha is not mediated by FXR/SHP, because the selective ligand GW4064 does not affect the activity of HNF-4alpha in Gal4-based assays. This observation is somewhat conflicting with a previous paper where it was shown that SHP interacts with and represses the transcriptional activity of HNF-4alpha (55). However, this is still an unsettled issue because Goodwin *et al.* also showed that SHP does not interact with HNF-4alpha in a mammalian two-hybrid system (16). In any case, a more recent study also showed that SHP has a greater affinity for LRH-1 than for HNF-4alpha and other members of the nuclear receptor superfamily (56), thus suggesting that LRH-1 may be the primary target of SHP on the *CYP7A1* promoter. Therefore, based on the fact that the FXR/SHP pathway does not seem to affect the transcriptional activity of HNF-4alpha and on coimmunoprecipitation experiments with *in vitro* synthesized HNF-4alpha, De Fabiani *et al.* (18) hypothesized that the dissociation of CBP and PGC-1alpha from HNF-4alpha is a consequence of a posttranslational modification of the receptor that decreases its affinity for these coactivators. Interestingly, in this study it was also demonstrated for the first time that bile acids affect the transcription of the gene encoding the liver phosphoenolpyruvate carboxykinase (*PEPCK*), the key enzyme in hepatic gluconeogenesis, with a similar mechanism (18). Considering that in type 2 diabetes the hepatic glucose output is increased (57), this discovery is intriguing because it may open new possibilities to modulate glucose metabolism in this disease.

Other mechanisms underlying the transcriptional repression of *CYP7A1* have been described. Gupta *et al.* (19) provided evidence that bile acids down-regulate *CYP7A1* transcription via activation of the c-Jun N-terminal kinase (JNK) pathway, which increases the transcription of SHP. Additionally, transcriptional repression by bile acids in the liver is achieved through the FXR-mediated induction of fibroblast growth factor 19 (FGF19), a growth factor that signals through the FGFR4 receptor tyrosine kinase and represses *CYP7A1* gene through a JNK-dependent pathway (58). Interestingly, in contrast with the original paper first describing the FGF19-mediated repression of the human *CYP7A1* gene transcription, it was shown that FGF15, the mouse ortholog of FGF19, is expressed and induced in the small intestine but not in the liver and it was proposed to function as an enterohepatic signal regulating bile acid homeostasis (59). Interestingly, FGF15 also regulates gallbladder filling in response to bile acids traversing the small intestine, the major site of FGF15 production (60). More recently, it has been demonstrated that FGF15/19 requires beta-Klotho, a 130 kDa protein that acts as a co-receptor of FGFR4 (61). beta-Klotho is required for the specific action of FGF15/19 in the liver and beta-Klotho deficient mice display a phenotype similar to that of FGF15 KO or FGFR4 KO mice (59, 62, 63), which are characterized by higher CYP7A1 expression and decreased gallbladder size (64). Studies in human subjects demonstrated that the intestinal flux of bile acids occurring after a meal regulates the plasma levels of FGF19, which in turn decreases bile acid production via the repression of *CYP7A1* gene transcription (65). The levels of FGF19 fluctuate during the day with peaks 90-120 minutes following the postprandial increase of bile acids in the blood. Collectively, all these studies clearly show that FGF15/19, FGFR4 and beta-Klotho are key components in the signaling that regulate bile acid metabolism through the integrated action of the gut-liver axis. In addition, FGF15/19 may represent the long searched "intestinal" factor that was hypothesized when *CYP7A1* gene resulted resistant to feedback repression in animals with bile fistula injected intravenously with bile acids, as opposed to animals intraduodenally infused with bile acids, whose CYP7A1 enzyme activity and mRNA levels decreased significantly (66).

The expression levels of HNF-4alpha and LRH-1 are also important in the regulation of bile acid synthesis. It was shown, in fact, that bile acids decrease HNF-4alpha gene transcription and protein levels and at the same time enhance the expression of LRH-1 (20, 67, 68). The functional consequence is that LRH-1 increases the

occupancy of the BARE of *CYP7A1* and *CYP8B1* by competing with HNF-4alpha for binding to these two promoter and eventually causing the decrease of gene transcription (20, 67, 68).

More recently, Sanyal *et al.* (69) have found that GPS2, a subunit of a corepressor complex, is a differential coregulator of CYP7A1 and CYP8B1 expression. GPS2 physically interacts with SHP, LRH-1, HNF4alpha and FXR but with different recruitment strategies that lead to differential transcriptional outcomes. These authors also observed species-specific differences in the regulation of bile acid biosynthesis as the human *CYP8B1* is a direct FXR target gene and inferred that their findings may have therapeutic implications in diseases linked to bile acid disorders.

Finally, it was shown that the xenobiotic sensor pregnane X receptor (PXR, NR1I2) also represses the transcription of *CYP7A1* and *PEPCK* genes by interfering with HNF-4alpha (70, 71). Treatment of hepatic cells with rifampicin, a ligand of PXR, induces the dissociation of PGC-1alpha from HNF-4alpha bound to the *CYP7A1* and *CYP8A1* promoters and at the same time its recruitment to PXR itself. Thus, the cross-talk between PXR and HNF-4alpha regulates the expression of these two important genes in bile acid synthesis by competing for the coactivator PGC-1alpha. It should be noted that the secondary bile acid, lithocholic acid (LCA), is also a ligand of PXR (72, 73), therefore toxic bile acids can signal to the nucleus via this xenobiotic sensor. Since PXR is mostly expressed in the liver and, to a lesser extent, in the intestine and it activates the transcription of the *CYP3A4* gene, a cytochrome P450 involved in the detoxification of xenobiotics and several endogenous molecules, it is conceivable that its activation by LCA is an adaptive response that allows to cope with potentially toxic bile acids that may accumulate in cholestatic liver diseases. In the intestine, LCA also activates the Vitamin D Receptor (VDR, NR1I1) (74) at concentrations that are lower than those required to activate PXR in the liver. LCA-liganded VDR transactivates *CYP3A4*, therefore this nuclear receptor has been proposed as an intestinal bile acid sensor that mediates the detoxifying response to LCA in the intestine and protects against colon cancer (74).

As discussed previously, bile acids regulate genes involved in other metabolic pathways. We have already mentioned that one example is *PEPCK* gene, which is repressed by bile acids as a consequence of the dissociation of the coactivators CBP and PGC-1alpha from HNF-4alpha (18), a strong activator bound to accessory factor 1 binding site (AF1) of the glucocorticoid responsive unit of this promoter (75). In this study, the authors demonstrate that this regulation is FXR-independent because the GW4064 does not affect PEPCK mRNA levels in cell culture despite the increase of SHP mRNA. In two independent studies, it was shown that SHP regulates *PEPCK* gene transcription. In one case, by repressing the glucocorticoid-stimulated transcription of *PEPCK* (76), in the other by interacting with and decreasing the transcription activity of the nuclear receptor HNF-4alpha (77). SHP represses the transcription of the other gluconeogenic gene encoding glucose-6-phosphatase by interacting with the forkhead transcription factor Foxo1 (77). Therefore, these results, along with the observations of De Fabiani and coworkers (18), indicate that bile acids regulate the transcription of gluconeogenic genes in an FXR/SHP-dependent and –independent fashion.

Bile acids also repress the transcription of the apolipoprotein C-III and A-I genes (36, 78). Apolipoprotein C-III inhibits the activity of lipoprotein lipase, an important regulator of serum triglyceride catabolism, whereas the apolipoprotein A-I is an important component of high density lipoproteins (HDL), whose levels are inversely correlated to the risk of coronary artery disease (79). Negative FXR responsive elements were identified in these two gene promoters. Liganded FXR binds as a monomer or homodimer to the C site of the apoA-I promoter (78) whereas it binds as a heterodimer with RXR to the apoC-III promoter, where it displaces HNF-4alpha, which is a strong activator of apoC-III transcription (36). The repressive effect of FXR/RXR is not simply due to a competitive mechanism because the bile acid-mediated repression is still observed in HNF-4alpha knock-out mice (36). The molecular mechanism of FXR-mediated repression of these two genes is not well understood, however the authors suggest that in these promoter contexts FXR may recruit corepressor complexes. In chromatin immunoprecipitation (ChIP) experiments we observed that the bile acid-induced binding of FXR/RXR to the apoC-III promoter is accompanied by the deacetylation of histone 3 and 4 and by the decrease of RNA polymerase II associated to the core promoter (N. Mitro, C. Godio and M. Crestani, unpublished results), thus suggesting that bile acids may recruit histone deacetylases (HDACs) and possibly chromatin remodeling complexes to this genome region.

3.3. Bile acids target chromatin-modifying enzyme complexes

We have discussed the role of FXR/SHP/LRH-1-dependent and HNF-4alpha-dependent pathways of gene repression, however it remains to clarify the detailed mechanistic basis of the HNF-4alpha-mediated repression of gene transcription and its interplay with LRH-1. Most importantly, a key issue is to understand why bile acids repress gene transcription via multiple mechanisms and what is the relative importance of HNF-4alpha and FXR/SHP in this regulatory network. The most recent studies on the effects of bile acids on chromatin structure in gene regulation have shed new light and provide a more detailed explanation of this phenomenon. The study of Kemper *et al.* (80, 81) established the link between chromatin structure and gene repression by showing that bile acids cause the recruitment of the ATP-dependent mSin3A-Swi/Snf chromatin remodeling complex on *CYP7A1* promoter through direct interaction with SHP, which is also associated to HDAC1. All this leads to chromatin remodeling and gene repression. Moreover, it was also shown that SHP interacts with the G9a methyltransferase and it associates with underacetylated and lysine 9-methylated histone H3 (81, 82), a typical epigenetic mark of transcriptionally inactive chromatin (83). The results of these two laboratories suggest a

multistep mechanism of transcriptional repression whereby SHP competes with coactivators for interactions with nuclear receptors (55, 84), and then it sequentially recruits HDAC complexes and the G9a methyltransferase to specific promoters, which deacetylate nucleosomes and methylates lysine 9 of deacetylated histone H3, respectively. Since SHP can also form a stable repressive complex on underacetylated histone 3, it might be speculated that it can interpret the histone code in selected chromatin regions. It should be mentioned, though, that in mice overexpressing SHP in the liver, HDAC1 is recruited only on the *Shp* and *Cyp8b1* promoters and not on *Cyp7a1* promoter (85). This is in contrast to the observations made in human liver cell lines (80, 86) and may reflect subtle species differences in the mechanisms of regulation of genes involved in bile acid synthesis.

The role of HNF-4alpha in the transcriptional repression of *CYP7A1* was further clarified by performing the kinetic analysis of bile acid repression. Mitro *et al.* (86) found that CYP7A1 mRNA levels in HepG2 cells exposed to chenodeoxycholic acid (CDCA) decrease rapidly, whereas the FXR ligand GW4064 decreases CYP7A1 mRNA only after several hours. Moreover, since both CDCA and GW4064 increase SHP mRNA levels after several hours it was inferred that FXR/SHP mediates a long-term regulation, while other mechanisms may be responsible for a rapid effect that occurs in a shorter time. In keeping with these results, by performing a detailed analysis of factors recruited to *CYP7A1* promoter in response to CDCA treatment, it was shown that a corepressor complex, comprising the silencing mediator of retinoid and thyroid receptors-alpha (SMRTalpha), the Nuclear-Corepressor (N-CoR), HDAC3 and 7, associates rapidly with the promoter region of *CYP7A1*. This results in deacetylation of histone H3 and H4 and in the decreased amount and elongation activity of RNA polymerase II on this promoter. More intriguingly, these authors discovered that bile acids stimulate the rapid translocation of HDAC7 from the cytoplasm to the nucleus, a prerequisite for the repressive action of HDAC7 and other class II HDACs on gene transcription (86). This effect is FXR/SHP-independent since the synthetic agonist GW4064 does not change the intracellular localization of HDAC7. Based on the fact that small interfering RNA (siRNA) oligonucleotides directed against HDAC7, but not siRNA directed against other HDACs, and that the inhibition of its nuclear translocation with the phosphatase inhibitor calyculin A antagonizes the negative repression of bile acids on CYP7A1 mRNA levels, the authors propose that the translocation of HDAC7 to the nucleus may be the key event triggering the repression of *CYP7A1* gene transcription. One possible mechanism explaining the action of HDAC7 on *CYP7A1* gene transcription is the interaction with the nuclear receptor HNF-4alpha and its subsequent deacetylation, which would inactivate the receptor activity according to a mechanism previously shown by Soutoglou *et al.* (87). However, more direct evidences are necessary to prove that HNF-4alpha undergoes acetylation/deacetylation cycles when liver cells are exposed to bile acids.

These observations suggest that the administration of HDAC inhibitors could prevent the negative feedback of bile acids on *CYP7A1* gene transcription and it could also increase the catabolic conversion of cholesterol to bile acids with a consequent decrease of plasma cholesterol. Indeed, administration of two structurally distinct HDAC inhibitors, valproic acid (VPA) and trichostatin A (TSA) (88, 89), increases the mRNA levels of Cyp7a1 in LDL receptor deficient mice (*Ldl-r*$^{-/-}$) and at the same time decreases plasma total and LDL cholesterol (86). These results describe new aspects of an important mechanism of gene regulation and open new therapeutical perspectives for the treatment of familial hypercholesterolemia (FH, OMIM # 143890), a genetic disease caused by mutations of LDL-R that leads to accelerated atherosclerosis and early myocardial infarctions (6). Besides prospecting a possible new therapeutical strategy for patients that do not respond to currently available treatments such as statins, HDAC inhibitors may be used in the future for treating some patients affected by complex dyslipidemic disorders that usually do not respond well to treatments with other hypolipidemic agents.

Although these results provided a clear demonstration that bile acids affect *CYP7A1* gene transcription via chronologically distinct mechanisms, they also raise new questions that need to be addressed in the future. For example, it is not clear how the repressive complex containing HDAC3, HDAC7 and SMRTalpha can remain associated to the *CYP7A1* promoter after HNF-4alpha dissociates from it. It is conceivable that the HDAC/SMRT-containing complex is stabilized on the *CYP7A1* promoter by the interaction with bile acid-induced SHP, which targets LRH-1. In this scenario, HNF-4alpha would work as a platform for the early recruitment of the repressive complex, which deacetylates HNF-4alpha itself and histone H3 in the *CYP7A1* promoter and commits the transcriptional machinery to repression. After the initial exposure to CDCA the repressive complex could be stabilized via interaction with other factors binding to this promoter. All this allows achieving a rapid response to toxic concentrations of bile acids, to stabilize a repressive complex and to remodel chromatin to a more compacted and less accessible state. On the other hand, SHP may be very important to protect hepatic cells from excessive bile acid concentrations under certain patho-physiological conditions. Although *Shp* null mice fed bile acid-containing diet seem to be resistant to liver toxicity (90), recent evidences also indicate that SHP plays a protective role against Nur77 (NR4A1)-induced apoptosis of liver cells that may occur as a consequence of viral infection, alcoholic and autoimmune hepatitis (91). Therefore, considering these apparently contradictory results, more studies will be necessary to clarify the role of SHP in protecting the liver from toxic stimuli.

Another issue is to understand how the mRNA levels of CYP7A1 can decrease so rapidly after the exposure to bile acids. Results from ChIP experiments with specific antibodies against phosphoserine 2 and 5 of the CTD or the large subunit of RNA polymerase II indicate that bile acids decrease rapidly the phosphorylation of

serine 2 (N. Mitro, C. Godio and M. Crestani, unpublished results). Because serine 2 phosphorylation is largely associated to transcriptional elongation (92, and references therein), it is possible that the inhibition of the elongation activity of RNA polymerase II is the primary consequence of the recruitment of the corepressor complex on the *CYP7A1* gene promoter upon treatment of liver cells with bile acids. It is important to remember that the CYP7A1 mRNA half-life is very short because the 3'-untranslated region contains motifs that are typically present in short-lived mRNAs (93). The dephosphorylation of serine 2 at the CTD of RNA polymerase II implies the intervention of protein phosphatases in response to bile acids. Indeed, the opposite actions of CTD-protein kinases, such as cyclin-dependent kinases and mitogen-activated protein kinases, and of CTD-phosphatases, such as the TFIIF-dependent CTD phosphatase (FCP1), regulate the phosphorylation state of the large subunit of RNA polymerase II CTD (92, and references therein).

What is the advantage of such a complex system for negatively regulating *CYP7A1* gene transcription? HNF-4alpha and HDAC7, which associates to it when hepatic cells are exposed to bile acids, seem key actors in the plot. Although it was shown that fatty acids and their Coenzyme A thioesters bind to the ligand binding domain of HNF-4alpha (94, 95), the transcriptional activity of this receptor can be modulated also by posttranslational modifications such as acetylation/deacetylation of lysine residues in the N-terminal region (87), a short term regulatory system that allows to respond quickly to extracellular stimuli such as bile acids. This appears to be one of the early events that marks the local chromatin structure and commits the RNA polymerase II machinery to the repression of *CYP7A1* transcription. One should bear in mind that bile acids are detergents that may be harmful if they reach critically high concentrations in the liver, thus under particular circumstances as liver cholestasis, it is important to respond promptly to the elevation of bile acid concentration, to avoid cell damage. As discussed above, different tissues have different strategies to cope with high concentrations of bile acids to get rid of toxic molecules. Therefore, HNF-4alpha seems well suited to this purpose being a molecular switch that can respond rapidly to environmental changes. From an evolutionary point of view, since HNF-4 is considered a very old member of the nuclear receptor superfamily and phylogenetical analysis in various genomes suggests that ancestors of nuclear receptors are most likely orphan transcription factors (96), it is tempting to speculate that one of the mechanisms for bile acid-mediated transcriptional repression of *CYP7A1* gene that appeared early during the evolution may involve HNF-4. It is possible that the regulatory loop of bile acid repression mediated by HNF-4 be an evolutionary memory of an ancestor mechanism, which evolved further when NRs became ligand dependent transcription factors in cordates and added other layers of regulation on top of HNF-4. At this regard, it is noteworthy that the analysis of the activity of the FXR gene from the marine skate, *Leucoraja erinacea*, a vertebrate lineage that diverged over 400 million years ago, seems to suggest that FXR is an ancient nuclear receptor that may have acquired ligand specificity for bile acids later in evolution by deletion of a sequence from its LBD (97). If this intriguing hypothesis is confirmed, this may represent an example of evolutionary adaptation of protein function as new ligands have appeared in upper vertebrates.

To summarize, we propose that the transcriptional repression of *CYP7A1*, and possibly of other genes involved in bile acid synthesis, is a complex and multistep process that occurs through the following events (Figure 2): i) bile acids stimulate the translocation of HDAC7 from the cytoplasm to the nucleus; ii) once inside the nucleus HDAC7 associates with the *CYP7A1* promoter probably by interacting with transcription factors bound to this promoter along with a corepressor complex containing also SMRTalpha and HDAC3; iii) the HDAC complex deacetylates local chromatin regions; iv) coactivators dissociates from HNF-4alpha and from the *CYP7A1* promoter; v) RNA polymerase II arrests the elongation of mRNA because the serine 2 of the CTD is dephosphorylated; vi) after the dissociation of HNF-4alpha the corepressor complex is maintained on the *CYP7A1* promoter because in the meantime the repressor SHP has been induced and recruited to this promoter through the interaction with LRH-1; vii) the mSin3A-Swi/Snf-Brm ATP-dependent chromatin remodeling complex remodels nucleosomes, after the histones in the promoter of *CYP7A1* are sequentially deacetylated by HDACs and then methylated by G9a methylase, keeping the transcription of this gene to low levels. In this model, it remains to explain how bile acids promote the translocation of HDAC7 from the cytoplasm to the nucleus and future studies should address this question. To add more complexity and flexibility to this regulation, LRH-1 expression increases upon bile acid treatment and competes with HNF-4alpha for binding to the *CYP7A1* promoter, leading to transcriptional repression. Lastly, bile acids decrease the expression of HNF-4alpha and facilitate the competition of LRH-1 for binding to the *CYP7A1* promoter.

4. PERSPECTIVES

Since the discovery of FXR as the physiological receptor for bile acids we have witnessed a dramatic increase of interest in the field. Bile acids have emerged as important signaling molecules that regulate different metabolic pathways and cellular functions. This has generated great attention in companies developing new synthetic molecules affecting the activity of FXR. Therefore, it is expected that the identification of selective modulators of FXR may provide new options to treat hypertriglyceridemia, hypercholesterolemia, gallstone disease and disorders in glucose metabolism.

Because bile acids activate multiple signaling pathways and can elicit numerous cellular responses, other targets may be used to design new molecules affecting lipid metabolism. This could be the case of HDACs, which are important transcriptional repressors of *CYP7A1* and may be targeted with appropriate inhibitors to lower plasma cholesterol by increasing its conversion to bile acids. However, the recent discoveries that bile acids can cause

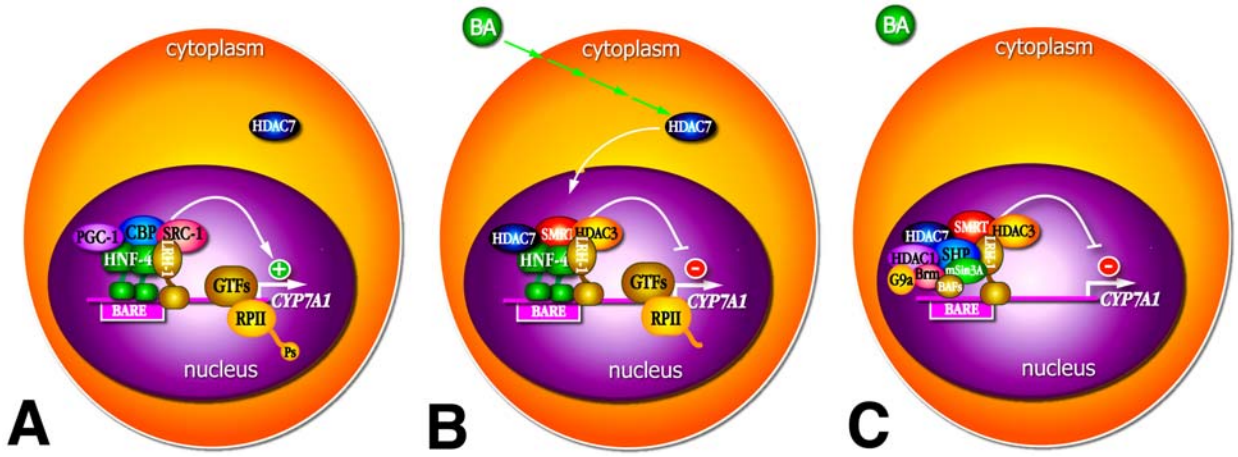

Figure 2. Multistep regulation of *CYP7A1* gene by bile acids. (A) When intrahepatic concentrations of bile acids are low HDAC7 is mainly localized in the cytoplasm and *CYP7A1* gene transcription is activated by HNF-4 and LRH-1 bound to the bile acid responsive element (BARE). These nuclear receptors recruit the coactivators PGC-1, CBP and SRC-1. General transcription factors (GTFs) are recruited to the *CYP7A1* promoter and RNA polymerase II (RPII) C-terminal domain (CTD) repeat is phosphorylated. (B) Upon increase of intrahepatic bile acid concentrations, HDAC7 translocates into the nucleus and assembles a corepressor complex containing HDAC7, HDAC3 and SMRT on HNF-4 bound to the BARE of *CYP7A1* promoter. At the same time, coactivators dissociate from the *CYP7A1* promoter and the CTD of RPII is dephosphorylated, causing the arrest of transcriptional elongation. (C) At a later stage, RPII dissociates from the promoter, the nuclear receptor SHP associates with LRH-1 and keeps the corepressor complex on *CYP7A1* promoter. HDAC1, the ATP-dependent chromatin remodeling complex including mSin3A, Brm and BAFs also are assembled and remodel nucleosomes of the *CYP7A1* promoter. The histone methyltransferase, G9a, methylates lysine 9 of histone H3, thus marking chromatin in the transcriptional inactive state.

chromatin remodeling at specific gene loci will require more studies to assess the suitability of molecules affecting HDAC activity and chromatin structure as possible hypolipidemic agents. Mammalian HDAC family comprises 18 members grouped into four classes of enzymes according to phylogenetic analysis and homology to yeast HDACs (98). The inhibitors available today are not selective for a specific HDAC, therefore it will be crucial to identify and validate the key HDAC in the feedback repression of *CYP7A1* gene transcription by bile acids, to assess the liver genes regulated by this HDAC and finally design a molecule specifically interfering with its activity. This strategy may minimize the possible side effects of HDAC inhibitors, limiting the effect to the desired target. It should also be considered that the field of chromatin modification pathways is emerging as new and promising area for basic research and it is likely to offer attractive perspectives of new therapeutics for cancer, neurodegenerative diseases and metabolic disorders. Although the deep comprehension of the histone code (99, 100) and how it is interpreted by chromatin modifying enzymes and other factors warrant more investigations, nonetheless this field holds promises to provide new options for the rational design of new drugs or strategies for different types of diseases.

5. ACKNOWLEDGEMENTS

We wish to thank Miss Elda Desiderio Pinto for administrative assistance. We apologize for the many important papers that we did not cite in this review because of space constraint. This work was made possible by grants from the European Commission (NORTh, QLG1-CT2001-01513; SOUTH, LSHM-CT-2006-037498), from the Italian Ministry of University and Scientific Research (MURST, COFIN 2002062991, 2002064429, 2004067491, 2005033023) and from Telethon (GGP04252).

6. REFERENCES

1. Russell, D. W.: The enzymes, regulation, and genetics of bile acid synthesis. *Annu. Rev. Biochem.*, 72, 137-74 (2003)

2. Ross, R.: Atherosclerosis -- An Inflammatory Disease. *N Engl. J. Med.*, 340, 115-126 (1999)

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

4. Javitt, N. B.: Cholesterol, hydroxycholesterols, and bile acids. *Biochem. Biophys. Res. Commun.*, 292, 1147-53. (2002)

5. Pullinger, C. R., C. Eng, G. Salen, S. Shefer, A. K. Batta, S. K. Erickson, A. Verhagen, C. R. Rivera, S. J. Mulvihill, M. J. Malloy & J. P. Kane: Human cholesterol 7alpha-hydroxylase (CYP7A1) deficiency has a hypercholesterolemic phenotype. *J. Clin. Invest.*, 110, 109-117 (2002)

6. Kwiterovich, P. O., Jr., D. S. Fredrickson & R. I. Levy: Familial hypercholesterolemia (one form of familial type II hyperlipoproteinemia). A study of its biochemical, genetic

and clinical presentation in childhood. *J. Clin. Invest.*, 53, 1237-49 (1974)

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

8. 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)

9. 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)

10. Alberti, S., G. Schuster, P. Parini, D. Feltkamp, U. Diczfalusy, M. Rudling, B. Angelin, I. Bjorkhem, S. Pettersson & J. Gustafsson: Hepatic cholesterol metabolism and resistance to dietary cholesterol in LXRbeta-deficient mice. *J. Clin. Invest.*, 107, 565-573. (2001)

11. Peet, D. J., S. D. Turley, W. Ma, B. A. Janowski, J.-M. A. Lobaccaro, R. E. Hammer & D. J. Mangelsdorf: Cholesterol and bile acid metabolism are impaired in mice lacking the nuclear oxysterol receptor LXRalpha. *Cell*, 93, 693-704 (1998)

12. Makishima, M., A. Y. Okamoto, J. J. Repa, H. Tu, R. M. Learned, A. Luk, M. V. Hull, K. D. Lustig, D. J. Mangelsdorf & B. Shan: Identification of a nuclear receptor for bile acids. *Science*, 284, 1362-1365 (1999)

13. Parks, D. J., S. G. Blanchard, R. K. Bledsoe, G. Chandra, T. G. Consler, S. A. Kliewer, J. B. Stimmel, T. M. Willson, A. M. Zavacki, D. D. Moore & J. M. Lehmann: Bile acids: natural ligands for an orphan nuclear receptor. *Science*, 284, 1365-1368 (1999)

14. Wang, H., J. Chen, K. Hollister, L. C. Sowers & B. M. Forman: Endogenous bile acids are ligands for the nuclear receptor FXR/BAR. *Mol. Cell*, 3, 543-553 (1999)

15. Lu, T. T., M. Makishima, J. J. Repa, K. Schoonjans, T. A. Kerr, J. Auwerx & D. J. Mangelsdorf: Molecular basis for feedback regulation of bile acid synthesis by nuclear receptors. *Mol. Cell*, 6, 507-515 (2000)

16. Goodwin, B., S. A. Jones, R. R. Price, M. A. Watson, D. D. McKee, L. B. Moore, C. Galardi, J. G. Wilson, M. C. Lewis, M. E. Roth, P. R. Maloney, T. M. Willson & S. A. Kliewer: A regulatory cascade of the nuclear receptors FXR, SHP-1, and LRH-1 represses bile acid biosynthesis. *Mol. Cell*, 6, 517-26 (2000)

17. De Fabiani, E., N. Mitro, A. C. Anzulovich, A. Pinelli, G. Galli & M. Crestani: The Negative Effects of Bile Acids and Tumor Necrosis Factor-alpha on the Transcription of Cholesterol 7alpha-Hydroxylase Gene (*CYP7A1*) Converge to Hepatocyte Nuclear Factor-4. A novel mechanism of feedback regulation of bile acid synthesis mediated by nuclear receptors. *J. Biol. Chem.*, 276, 30708-30716 (2001)

18. De Fabiani, E., N. Mitro, F. Gilardi, D. Caruso, G. Galli & M. Crestani: Coordinated Control of Cholesterol Catabolism to Bile Acids and of Gluconeogenesis via a Novel Mechanism of Transcription Regulation Linked to the Fasted-to-fed Cycle. *J. Biol. Chem.*, 278, 39124-39132 (2003)

19. Gupta, S., R. T. Stravitz, P. Dent & P. B. Hylemon: Down-regulation of Cholesterol 7alpha-Hydroxylase (*CYP7A1*) Gene Expression by Bile Acids in Primary Rat Hepatocytes Is Mediated by the c-Jun N-terminal Kinase Pathway. *J. Biol. Chem.*, 276, 15816-15822 (2001)

20. Zhang, M. & J. Y. L. Chiang: Transcriptional Regulation of the Human Sterol 12alpha-Hydroxylase Gene (CYP8B1). ROLES OF HEPATOCYTE NUCLEAR FACTOR 4alpha IN MEDIATING BILE ACID REPRESSION. *J. Biol. Chem.*, 276, 41690-41699 (2001)

21. Forman, B. M., E. Goode, J. Chen, A. E. Oro, D. J. Bradley, T. Perlmann, D. J. Noonan, L. T. Burka, T. McMorris, W. W. Lamph, R. M. Evans & C. Weinberger: Identification of a nuclear receptor that is activated by farnesol metabolites. *Cell*, 81, 687-693 (1995)

22. Lew, J.-L., A. Zhao, J. Yu, L. Huang, N. de Pedro, F. Pelaez, S. D. Wright & J. Cui: The Farnesoid X Receptor Controls Gene Expression in a Ligand- and Promoter-selective Fashion. *J. Biol. Chem.*, 279, 8856-8861 (2004)

23. Pineda Torra, I., L. P. Freedman & M. J. Garabedian: Identification of DRIP205 as a Coactivator for the Farnesoid X Receptor. *J. Biol. Chem.*, 279, 36184-36191 (2004)

24. Kanaya, E., T. Shiraki & H. Jingami: The nuclear bile acid receptor FXR is activated by PGC-1a in a ligand-dependent manner. *Biochem. J.*, 382, 913-921 (2004)

25. Zhang, Y., L. W. Castellani, C. J. Sinal, F. J. Gonzalez & P. A. Edwards: Peroxisome proliferator-activated receptor-gamma coactivator 1alpha (PGC-1alpha) regulates triglyceride metabolism by activation of the nuclear receptor FXR. *Genes Dev.*, 18, 157-169 (2004)

26. Debril, M.-B., L. Gelman, E. Fayard, J.-S. Annicotte, S. Rocchi & J. Auwerx: Transcription Factors and Nuclear Receptors Interact with the SWI/SNF Complex through the BAF60c Subunit. *J. Biol. Chem.*, 279, 16677-16686 (2004)

27. Laffitte, B. A., H. R. Kast, C. M. Nguyen, A. M. Zavacki, D. D. Moore & P. A. Edwards: Identification of the DNA Binding Specificity and Potential Target Genes for the Farnesoid X-activated Receptor. *J. Biol. Chem.*, 275, 10638-10647 (2000)

28. Downes, M., M. Verdecia, A. Roecker, R. Hughes, J. Hogenesch, H. Kast-Woelbern, M. Bowman, J. Ferrer, A. Anisfeld, P. Edwards, J. Rosenfeld, J. Alvarez, J. Noel, K. Nicolaou & R. Evans: A chemical, genetic, and structural analysis of the nuclear bile acid receptor FXR. *Mol. Cell*, 11, 1079-92 (2003)

29. Grober, J., I. Zaghini, H. Fujii, S. A. Jones, S. A. Kliewer, T. M. Willson, T. Ono & P. Besnard: Identification of a Bile Acid-responsive Element in the Human Ileal Bile Acid-binding Protein Gene. Involvement of the Farnesoid X Receptor/9-Cis-Retinoic Acid Receptor Heterodimer. *J. Biol. Chem.*, 274, 29749-29754 (1999)

30. Ananthanarayanan, M., N. Balasubramanian, M. Makishima, D. J. Mangelsdorf & F. J. Suchy: Human Bile Salt Export Pump Promoter Is Transactivated by the Farnesoid X Receptor/Bile Acid Receptor. *J. Biol. Chem.*, 276, 28857-28865 (2001)

31. Huang, L., A. Zhao, J.-L. Lew, T. Zhang, Y. Hrywna, J. R. Thompson, N. de Pedro, I. Royo, R. A. Blevins, F. Pelaez, S. D. Wright & J. Cui: Farnesoid X Receptor Activates Transcription of the Phospholipid Pump MDR3. *J. Biol. Chem.*, 278, 51085-51090 (2003)

32. Barbier, O., I. P. Torra, A. Sirvent, T. Claudel, C. Blanquart, D. Duran-Sandoval, F. Kuipers, V. Kosykh, J. C. Fruchart & B. Staels: FXR induces the UGT2B4 enzyme in hepatocytes: A potential mechanism of negative feedback control of FXR activity. *Gastroenterology*, 124, 1926-1940. (2003)

33. Urizar, N. L., D. H. Dowhan & D. D. Moore: The Farnesoid X-activated Receptor Mediates Bile Acid Activation of Phospholipid Transfer Protein Gene Expression. *J. Biol. Chem.*, 275, 39313-39317 (2000)

34. Kast, H. R., C. M. Nguyen, C. J. Sinal, S. A. Jones, B. A. Laffitte, K. Reue, F. J. Gonzalez, T. M. Willson & P. A. Edwards: Farnesoid X-Activated Receptor Induces Apolipoprotein C-II Transcription: a Molecular Mechanism Linking Plasma Triglyceride Levels to Bile Acids. *Mol. Endocrinol.*, 15, 1720-1728 (2001)

35. Pineda Torra, I., T. Claudel, C. Duval, V. Kosykh, J.-C. Fruchart & B. Staels: Bile Acids Induce the Expression of the Human Peroxisome Proliferator-Activated Receptor α Gene via Activation of the Farnesoid X Receptor. *Mol. Endocrinol.*, 17, 259-272 (2003)

36. Claudel, T., Y. Inoue, O. Barbier, D. Duran-Sandoval, V. Kosykh, J. Fruchart, J. Fruchart, F. Gonzalez & B. Staels: Farnesoid X receptor agonists suppress hepatic apolipoprotein CIII expression. *Gastroenterology*, 125, 544-555 (2003)

37. Zhang, Y., K. Ma, S. Song, M. B. Elam, G. A. Cook & E. A. Park: Peroxisomal Proliferator-activated Receptor-gamma Coactivator-1alpha (PGC-1alpha) Enhances the Thyroid Hormone Induction of Carnitine Palmitoyltransferase I (CPT-Ialpha). *J. Biol. Chem.*, 279, 53963-53971 (2004)

38. Watanabe, M., S. M. Houten, L. Wang, A. Moschetta, D. J. Mangelsdorf, R. A. Heyman, D. D. Moore & J. Auwerx: Bile acids lower triglyceride levels via a pathway involving FXR, SHP, and SREBP-1c. *J. Clin. Invest.*, 113, 1408-1418 (2004)

39. Repa, J. J., G. Liang, J. Ou, Y. Bashmakov, J. M. Lobaccaro, I. Shimomura, B. Shan, M. S. Brown, J. L. Goldstein & D. J. Mangelsdorf: Regulation of mouse sterol regulatory element-binding protein-1c gene (SREBP-1c) by oxysterol receptors, LXRalpha and LXRbeta. *Genes Dev.*, 14, 2819-2830. (2000)

40. Schultz, J. R., H. Tu, A. Luk, J. J. Repa, J. C. Medina, L. Li, S. Schwendner, S. Wang, M. Thoolen, D. J. Mangelsdorf, K. D. Lustig & B. Shan: Role of LXRs in control of lipogenesis. *Genes Dev.*, 14, 2831-2838. (2000)

41. Li, J., P. C. Pircher, I. G. Schulman & S. K. Westin: Regulation of Complement C3 Expression by the Bile Acid Receptor FXR. *J. Biol. Chem.*, 280, 7427-7434 (2005)

42. Cianflone, K., Z. Xia & L. Y. Chen: Critical review of acylation-stimulating protein physiology in humans and rodents. *Biochim. Biophys. Acta*, 1609, 127-143 (2003)

43. Bishop-Bailey, D., D. T. Walsh & T. D. Warner: Expression and activation of the farnesoid X receptor in the vasculature. *Proc. Natl. Acad. Sci. U.S.A.*, 101, 3668-3673 (2004)

44. Nishimoto, M., O. Gotoh, K. Okuda & M. Noshiro: Structural anlaysis of the gene encoding rat cholesterol 7alpha-hydroxylase, the key enzyme for bile acid biosynthesis. *J. Biol. Chem.*, 266, 6467-6471 (1991)

45. 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)

46. 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)

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

48. 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)

49. Stroup, D., M. Crestani & J. Y. Chiang: Identification of a bile acid response element in the cholesterol 7alpha-

hydroxylase gene (CYP7A). *Am. J. Physiol.*, 273, G508-G517. (1997)

50. 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)

51. Crestani, M., A. Sadeghpour, D. Stroup, G. Galli & J. Y. L. Chiang: Transcriptional activation of the cholesterol 7alpha-hydroxylase gene (*CYP7A*) by nuclear hormone receptors. *J. Lipid Res.*, 39, 2192-2200 (1998)

52. Nitta, M., S. Ku, C. Brown, A. Y. Okamoto & B. Shan: CPF: An orphan nuclear receptor that regulates liver-specific expression of the human cholesterol 7alpha-hydroxylase gene. *Proc. Natl. Acad. Sci. U S A*, 96, 6660-6665 (1999)

53. Kerr, T., S. Saeki, M. Schneider, K. Schaefer, S. Berdy, T. Redder, B. Shan, D. Russell & M. Schwarz: Loss of Nuclear Receptor SHP Impairs but Does Not Eliminate Negative Feedback Regulation of Bile Acid Synthesis. *Dev. Cell*, 2, 713-720 (2002)

54. Wang, L., Y. Lee, D. Bundman, Y. Han, S. Thevananther, C. Kim, S. Chua, P. Wei, R. Heyman, M. Karin & D. Moore: Redundant pathways for negative feedback regulation of bile acid production. *Dev. Cell*, 2, 721-731 (2002)

55. Lee, Y.-K., H. Dell, D. H. Dowhan, M. Hadzopoulou-Cladaras & D. D. Moore: The Orphan Nuclear Receptor SHP Inhibits Hepatocyte Nuclear Factor 4 and Retinoid X Receptor Transactivation: Two Mechanisms for Repression. *Mol. Cell. Biol.*, 20, 187-195 (2000)

56. Li, Y., M. Choi, K. Suino, A. Kovach, J. Daugherty, S. A. Kliewer & H. E. Xu: Structural and biochemical basis for selective repression of the orphan nuclear receptor liver receptor homolog 1 by small heterodimer partner. *Proc. Natl. Acad. Sci., U.S.A.*, 102, 9505-9510 (2005)

57. Consoli, A. & N. Nurjhan: Contribution of gluconeogenesis to overall glucose output in diabetic and nondiabetic men. *Ann. Med.*, 22, 191-195 (1990)

58. Holt, J. A., G. Luo, A. N. Billin, J. Bisi, Y. Y. McNeill, K. F. Kozarsky, M. Donahee, D. Y. Wang, T. A. Mansfield, S. A. Kliewer, B. Goodwin & S. A. Jones: Definition of a novel growth factor-dependent signal cascade for the suppression of bile acid biosynthesis. *Genes Dev.*, 17, 1581-1591 (2003)

59. Inagaki, T., M. Choi, A. Moschetta, L. Peng, C. L. Cummins, J. G. McDonald, G. Luo, S. A. Jones, B. Goodwin, J. A. Richardson, R. D. Gerard, J. J. Repa, D. J. Mangelsdorf & S. A. Kliewer: Fibroblast growth factor 15 functions as an enterohepatic signal to regulate bile acid homeostasis. *Cell Metab.*, 2, 217-225 (2005)

60. Choi, M., A. Moschetta, A. L. Bookout, L. Peng, M. Umetani, S. R. Holmstrom, K. Suino-Powell, H. E. Xu, J. A. Richardson, R. D. Gerard, D. J. Mangelsdorf & S. A. Kliewer: Identification of a hormonal basis for gallbladder filling. *Nat Med*, 12, 1253-1255 (2006)

61. Lin, B. C., M. Wang, C. Blackmore & L. R. Desnoyers: Liver-specific Activities of FGF19 Require Klotho beta. *J. Biol. Chem.*, 282, 27277-27284 (2007)

62. Yu, C., F. Wang, M. Kan, C. Jin, R. B. Jones, M. Weinstein, C.-X. Deng & W. L. McKeehan: Elevated Cholesterol Metabolism and Bile Acid Synthesis in Mice Lacking Membrane Tyrosine Kinase Receptor FGFR4. *J. Biol. Chem.*, 275, 15482-15489 (2000)

63. Yu, C., F. Wang, C. Jin, X. Huang & W. L. McKeehan: Independent Repression of Bile Acid Synthesis and Activation of c-Jun N-terminal Kinase (JNK) by Activated Hepatocyte Fibroblast Growth Factor Receptor 4 (FGFR4) and Bile Acids. *J. Biol. Chem.*, 280, 17707-17714 (2005)

64. Ito, S., T. Fujimori, A. Furuya, J. Satoh, Y. Nabeshima & Y.-i. Nabeshima: Impaired negative feedback suppression of bile acid synthesis in mice lacking betaKlotho. *J. Clin. Invest.*, 115, 2202-2208 (2005)

65. Lundasen, T., C. Galman, B. Angelin & M. Rudling: Circulating intestinal fibroblast growth factor 19 has a pronounced diurnal variation and modulates hepatic bile acid synthesis in man. *J. Int. Med.*, 260, 530-536 (2006)

66. 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)

67. del Castillo-Olivares, A., J. A. Campos, W. M. Pandak & G. Gil: The Role of α1-Fetoprotein Transcription Factor/LRH-1 in Bile Acid Biosynthesis: A KNOWN NUCLEAR RECEPTOR ACTIVATOR THAT CAN ACT AS A SUPPRESSOR OF BILE ACID BIOSYNTHESIS. *J. Biol. Chem.*, 279, 16813-16821 (2004)

68. Yang, Y., M. Zhang, G. Eggertsen & J. Y. Chiang: On the mechanism of bile acid inhibition of rat sterol 12alpha-hydroxylase gene (CYP8B1) transcription: roles of α-fetoprotein transcription factor and hepatocyte nuclear factor 4alpha. *Biochim. Biophys. Acta*, 1583, 63-73. (2002)

69. Sanyal, S., A. Bavner, A. Haroniti, L.-M. Nilsson, T. Lundasen, S. Rehnmark, M. R. Witt, C. Einarsson, I. Talianidis, J.-A. Gustafsson & E. Treuter: Involvement of corepressor complex subunit GPS2 in transcriptional pathways governing human bile acid biosynthesis. *Proc. Natl. Acad. Sci., U.S.A.*, 104, 15665-15670 (2007)

70. Bhalla, S., C. Ozalp, S. Fang, L. Xiang & J. K. Kemper: Ligand-activated Pregnane X Receptor Interferes with HNF-4 Signaling by Targeting a Common Coactivator

PGC-1alpha: Functional Implications in Hepatic Cholesterol and Glucose Metabolism. *J. Biol. Chem.*, 279, 45139-45147 (2004)

71. Li, T. & J. Y. L. Chiang: Mechanism of rifampicin and pregnane X receptor inhibition of human cholesterol 7a-hydroxylase gene transcription. *Am. J. Physiol. Gastrointest. Liver Physiol.*, 288, G74-G84 (2005)

72. Staudinger, J. L., B. Goodwin, S. A. Jones, D. Hawkins-Brown, K. I. MacKenzie, A. LaTour, Y. Liu, C. D. Klaassen, K. K. Brown, J. Reinhard, T. M. Willson, B. H. Koller & S. A. Kliewer: The nuclear receptor PXR is a lithocholic acid sensor that protects against liver toxicity. *Proc. Natl. Acad. Sci., U.S.A.*, 98, 3369-3374 (2001)

73. Xie, W., A. Radominska-Pandya, Y. Shi, C. M. Simon, M. C. Nelson, E. S. Ong, D. J. Waxman & R. M. Evans: An essential role for nuclear receptors SXR/PXR in detoxification of cholestatic bile acids. *Proc. Natl. Acad. Sci., U.S.A.*, 98, 3375-3380 (2001)

74. Makishima, M., T. T. Lu, W. Xie, G. K. Whitfield, H. Domoto, R. M. Evans, M. R. Haussler & D. J. Mangelsdorf: Vitamin D Receptor As an Intestinal Bile Acid Sensor. *Science*, 296, 1313-1316 (2002)

75. Hall, R. K., F. M. Sladek & D. K. Granner: The Orphan Receptors COUP-TF and HNF-4 Serve as Accessory Factors Required for Induction of Phosphoenolpyruvate Carboxykinase Gene Transcription by Glucocorticoids. *Proc. Natl. Acad. Sci., U.S.A.*, 92, 412-416 (1995)

76. Borgius, L. J., K. R. Steffensen, J.-A. Gustafsson & E. Treuter: Glucocorticoid Signaling Is Perturbed by the Atypical Orphan Receptor and Corepressor SHP. *J. Biol. Chem.*, 277, 49761-49766 (2002)

77. Yamagata, K., H. Daitoku, Y. Shimamoto, H. Matsuzaki, K. Hirota, J. Ishida & A. Fukamizu: Bile Acids Regulate Gluconeogenic Gene Expression via Small Heterodimer Partner-mediated Repression of Hepatocyte Nuclear Factor 4 and Foxo1. *J. Biol. Chem.*, 279, 23158-23165 (2004)

78. Claudel, T., E. Sturm, H. Duez, I. P. Torra, A. Sirvent, V. Kosykh, J.-C. Fruchart, J. Dallongeville, D. W. Hum, F. Kuipers & B. Staels: Bile acid-activated nuclear receptor FXR suppresses apolipoprotein A-I transcription via a negative FXR response element. *J. Clin. Invest.*, 109, 961-971 (2002)

79. Miller, G. J. & N. E. Miller: Plasma-high-density-lipoprotein concentration and development of ischaemic heart-disease. *Lancet*, 1, 16-9 (1975)

80. Kemper, J. K., H. Kim, J. Miao, S. Bhalla & Y. Bae: Role of an mSin3A-Swi/Snf Chromatin Remodeling Complex in the Feedback Repression of Bile Acid Biosynthesis by SHP. *Mol. Cell. Biol.*, 24, 7707-7719 (2004)

81. Fang, S., J. Miao, L. Xiang, B. Ponugoti, E. Treuter & J. K. Kemper: Coordinated Recruitment of Histone Methyltransferase G9a and Other Chromatin-Modifying Enzymes in SHP-Mediated Regulation of Hepatic Bile Acid Metabolism. *Mol. Cell. Biol.*, 27, 1407-1424 (2007)

82. Boulias, K. & I. Talianidis: Functional role of G9a-induced histone methylation in small heterodimer partner-mediated transcriptional repression. *Nucl. Acids Res.*, 32, 6096-6103 (2004)

83. Kouzarides, T.: Histone methylation in transcriptional control. *Curr. Opin. Genet. Dev.*, 12, 198-209 (2002)

84. Lee, Y.-K. & D. D. Moore: Dual Mechanisms for Repression of the Monomeric Orphan Receptor Liver Receptor Homologous Protein-1 by the Orphan Small Heterodimer Partner. *J. Biol. Chem.*, 277, 2463-2467 (2002)

85. Boulias, K., N. Katrakili, K. Bamberg, P. Underhill, A. Greenfield & I. Talianidis: Regulation of hepatic metabolic pathways by the orphan nuclear receptor SHP. *EMBO J.*, 24, 2624-2633 (2005)

86. Mitro, N., C. Godio, E. D. Fabiani, E. Scotti, A. Galmozzi, F. Gilardi, D. Caruso, A. B. V. Chacon & M. Crestani: Insights In The Regulation Of Cholesterol 7alpha-Hydroxylase Gene Reveal A Target For Modulating Bile Acid Synthesis. *Hepatology*, 46, in press (2007)

87. Soutoglou, E., N. Katrakili & I. Talianidis: Acetylation regulates transcription factor activity at multiple levels. *Mol. Cell*, 5, 745-751 (2000)

88. Gottlicher, M., S. Minucci, P. Zhu, O. H. Kramer, A. Schimpf, S. Giavara, J. P. Sleeman, F. Lo Coco, C. Nervi, P. G. Pelicci & T. Heinzel: Valproic acid defines a novel class of HDAC inhibitors inducing differentiation of transformed cells. *EMBO J.*, 20, 6969-6978 (2001)

89. Vigushin, D. M., S. Ali, P. E. Pace, N. Mirsaidi, K. Ito, I. Adcock & R. C. Coombes: Trichostatin A Is a Histone Deacetylase Inhibitor with Potent Antitumor Activity against Breast Cancer *in vivo*. *Clin Cancer Res*, 7, 971-976 (2001)

90. Wang, L., Y. Han, C.-S. Kim, Y.-K. Lee & D. D. Moore: Resistance of SHP-null Mice to Bile Acid-induced Liver Damage. *J. Biol. Chem.*, 278, 44475-44481 (2003)

91. Yeo, M. G., Y.-G. Yoo, H.-S. Choi, Y. K. Pak & M.-O. Lee: Negative Cross-Talk between Nur77 and Small Heterodimer Partner and Its Role in Apoptotic Cell Death of Hepatoma Cells. *Mol. Endocrinol.*, 19, 950-963 (2005)

92. Palancade, B. & O. Bensaude: Investigating RNA polymerase II carboxyl-terminal domain (CTD) phosphorylation. *Eur. J. Biochem.*, 270, 3859-3870 (2003)

93. Noshiro, M., M. Nishimoto & K. Okuda: Rat liver cholesterol 7alpha-hydroxylase. Pretranslational regulation

for circadian rhythm. *J. Biol. Chem.*, 265, 10036-10041 (1990)

94. Petrescu, A. D., R. Hertz, J. Bar-Tana, F. Schroeder & A. B. Kier: Ligand Specificity and Conformational Dependence of the Hepatic Nuclear Factor-4alpha (HNF-4alpha). *J. Biol. Chem.*, 277, 23988-23999 (2002)

95. Duda, K., Y.-I. Chi & S. E. Shoelson: Structural Basis for HNF-4a Activation by Ligand and Coactivator Binding. *J. Biol. Chem.*, 279, 23311-23316 (2004)

96. Auwerx, J., J. Drouin & V. Laudet: Recepteurs a la Provencale: EMBO Workshop on the Biology of Nuclear Receptors. *EMBO Reports*, 4, 1122-1126 (2003)

97. Cai, S.-Y., L. Xiong, C. G. Wray, N. Ballatori & J. L. Boyer: The farnesoid X receptor FXRalpha/NR1H4 acquired ligand specificity for bile salts late in vertebrate evolution. *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, 293, R1400-1409 (2007)

98. Yang, X.-J. & S. Gregoire: Class II Histone Deacetylases: from Sequence to Function, Regulation, and Clinical Implication. *Mol. Cell. Biol.*, 25, 2873-2884 (2005)

99. Strahl, B. D. & C. D. Allis: The language of covalent histone modifications. *Nature*, 403, 41-5. (2000)

100. Jenuwein, T. & C. D. Allis: Translating the Histone Code. *Science*, 293, 1074-1080 (2001)

Key Words: Bile Acids, Liver, Nuclear Receptors, Coregulators, Chromatin, Review

Send correspondence to: Maurizio Crestani, Ph.D., Department of Pharmacological Sciences, University of Milan, 20133 Milan, Italy, Tel: 39 02 50318393/1, Fax: 39 02 50318391, E-mail: Maurizio.Crestani@unimi.it