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Chapter 9.

Enterohepatic bile acid transporters and transcriptional control of their expression

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9.1. Overview of the enterohepatic circulation of bile acids

Bile acids are amphipathic physiological detergents that play essential roles in promoting absorption, excretion, and transport of cholesterol, lipids, lipophilic nutrients, and other hydrophobic compounds in the liver and the intestine [1]. The two primary bile acids in humans are cholic acid (CA) and chenodeoxycholic acid (CDCA). In the intestinal bacterial flora these can be converted to secondary bile acids, deoxycholic acid (DCA) and lithocholic acid (LCA), respectively.

Bile acids are synthesized from cholesterol in the liver and stored in the gall bladder, from which they are postprandially released into the small intestine. From the small intestine the bile acids are recycled back to the liver via portal blood. A bile acid molecule may shuttle up to a dozen times between the liver and the intestine during one day, each time crossing the membrane domains of hepatocytes and enterocytes. This enterohepatic circulation of bile acids is highly efficient in healthy human individuals: less than 10% of the total bile acid pool escapes ileal reabsorption, and thus enters the colon. In the colon, secondary bile acids are formed, which may be either passively absorbed into colonocytes, or lost through fecal excretion. This small loss into feces is compensated for by hepatic de novo synthesis of bile acids from cholesterol, catalyzed by cascades of cytochrome P450 (CYP) enzymes located in endoplasmic reticulum, in mitochondria, or in cytosol [2]. Thus, conversion of cholesterol into bile acids also provides an important route for elimination of cholesterol from the human body.

Disruption of the biliary secretion of bile acids may result in cholestasis, where intrahepatic and systemic accumulation of bile acids causes cytotoxicity. In the liver this may eventually progress to fibrosis and cirrhosis.
9.2. The chief transporters in the enterohepatic circulation of bile acids

Enterohepatic circulation of bile acids is mediated by specific transporters, most of which are integral plasma membrane proteins, and are expressed in hepatocytes and enterocytes in a polarized manner [3]. The chief transporters involved in bile acid cycling and bile formation are presented in Figure 9-1, and discussed in more detail below.

Following their synthesis, bile acids are excreted from hepatocytes into bile canaliculi. This represents a major driving force for generation of bile flow and is a rate-limiting step in overall bile acid transport. The efflux of monovalent bile acids from hepatocytes occurs mainly via the canalicular bile salt export pump (BSEP, ABCB11), which belongs to the superfamily of ATP-binding cassette (ABC) transporters [4]. The ABC transporters couple the energy released from ATP hydrolysis to their transport activity [5]. Most eukaryotic ABC proteins share several evolutionarily conserved domains, and typically contain two hydrophobic transmembrane domains, each of which spans the membrane six times. While BSEP is responsible for the efflux of monovalent bile acids from hepatocytes into bile, the multidrug resistance-associated protein 2 (MRP2, ABCC2) exports divalent and sulphated and/or glucuronidated bile acids, as well as other conjugated anions such as chemotherapeutic agents and antibiotics, into bile [6]. MRP2 is also a member of the ABC transporter family that is located at the canalicular membrane of hepatocytes.

In addition to BSEP and MRP2, there are other transporters localized at the canalicular membrane of hepatocytes that are involved in bile formation. Whereas bile acids are the most prominent (60-70%) solid component of bile, hepatic bile also contains phospholipids, notably phosphatidylcholine, and cholesterol. Phospholipids are translocated from the inner to
the outer leaflet of the canalicular membranes of hepatocytes by yet another ABC transporter, namely the multidrug resistance protein 3 (MDR3, \( \text{ABCB}4 \)) [5]. The transport system for cholesterol at the canalicular membrane of hepatocytes is the protein heterodimer ABCG5/ABCG8 [7]. The efflux of lipids mediated by MDR3 and ABCG5/ABCG8 promotes the formation of mixed micelles containing bile acids, cholesterol, and phospholipids. This process not only solubilizes cholesterol, but also helps to protect cholangiocytes against the harmful detergent effects of bile acids in the biliary tree.

After their passage to the intestinal lumen, bile acids are efficiently taken up into ileocytes via the apical sodium-dependent bile acid transporter (ASBT, \( SLC10A2 \)) [8]. Characteristic of the SLC10 family of transporters [9], ASBT contains seven transmembrane domains and its bile acid uptake activity is electrogenically coupled with cotransport of sodium.

It has been proposed that transcellular shuttling of bile acids from the apical membrane domain of enterocytes to the basolateral membrane may be facilitated by the small cytosolic protein called the ileal bile acid-binding protein (I-BABP) [10,11]. It has been suggested that I-BABP physically interacts with ASBT [12], although the functional significance of this interaction remains unclear.

At the basolateral membrane domain of ileal enterocytes, bile acids are extruded into portal blood by the heterodimeric organic solute transporter OST\( \alpha \)/OST\( \beta \) [13]. The OST\( \alpha \) protein is predicted to contain seven membrane-spanning domains, whereas the smaller subunit OST\( \beta \) has a single transmembrane domain. Coexpression of both OST\( \alpha \) and OST\( \beta \) polypeptides is required for correct localization of the heterodimer at the cell membrane and for transport activity. Consistent with its role as the intestinal bile acid efflux transporter,
distribution of the OSTα/OSTβ heterodimer along the intestine closely mirrors that of the bile acid uptake system ASBT.

Finally, to complete the enterohepatic circulation, bile acids are extracted from the portal blood circulation by the liver. The sodium-taurocholate cotransporting polypeptide (NTCP, SLC10A1) at the sinusoidal membrane of hepatocytes is the chief uptake system for bile acids from portal blood into the parenchymal cells of the liver [14]. NTCP belongs to the same sodium-dependent SLC10 transporter family as ASBT, and is similarly likely to contain seven transmembrane domains and a cytoplasmic carboxy-terminus [9,15]. The amino acid identity between the human NTCP and ASBT is approximately 35 %.

While NTCP is responsible for the sodium-dependent uptake of bile acids into hepatocytes, certain members of the organic anion transporter (OATP, SLCO) family, notably OATP1B1 (gene symbol SLCO1B1; previously known as OATP-C/OATP2/SLC21A6), may also contribute to bile acid extraction from the portal blood at the basolateral membrane of hepatocytes in a sodium-independent manner [16].

Under normal physiological conditions, only negligible amounts of bile acids are effluxed back to portal blood at the basolateral hepatocyte membrane. However, in cholestatic states, the expression of the two basolateral bile acid overflow systems of the ABC transporter family, multidrug resistance-associated proteins 3 (MRP3, ABCC3) and 4 (MRP4, ABCC4), is increased [17-19]. Thus, in cholestasis, MRP3 and MRP4 may transport substantial amounts of bile acids from hepatocytes back into the systemic circulation for subsequent renal excretion. In addition to MRP3 and MRP4, OSTα and OSTβ are also expressed at the basolateral membranes of hepatocytes in humans [20]. It is thus conceivable that OSTα/OSTβ may also contribute to alternative bile acid efflux during cholestasis.
Similarly to I-BABP in enterocytes, proteins that are putatively involved in intracellular trafficking of bile acids from the basolateral to the canalicular membrane have been identified in hepatocytes [21,22]. One such intracellular protein capable of binding bile acids with a high affinity in the human liver is the hepatic bile acid-binding protein (HBAB), which may thus assist in the rapid transcellular vectorial transport of bile acids in hepatocytes [23].

9.3. Enterohepatic bile acid transporters in liver disease

Chronic cholestatic liver diseases, such as primary biliary cirrhosis and primary sclerosing cholangitis, are characterized by an impairment of bile formation or of bile flow. Altered expression or function of bile acid transporters can be either a cause or a consequence of cholestasis, thus leading to hepatotoxicity due to accumulation of bile acids and cholephilic toxins in hepatocytes. Amongst the genes encoding transporters that are involved in bile acid transport or bile formation there are several that have been identified or proposed as disease genes in the pathogenesis of cholestasis.

Progressive familial intrahepatic cholestasis, type 2 (PFIC2) is caused by mutations in the \( ABCB11 \) gene, which encodes BSEP [24,25]. These mutations in the \( ABCB11 \) gene lead to a rapidly progressive hepatic dysfunction in early infancy. In such patients the biliary bile salt levels can be reduced to less than 1 % of normal subjects. In a recent case report, specific \( ABCB11 \) mutations were identified in an adolescent cholestatic patient, which correlated with reduced BSEP protein expression in vivo and decreased bile acid transport activity in vitro [26]. Another case report suggested that heterozygous BSEP deficiency may predispose to transient neonatal cholestasis [27]. Furthermore, defective or altered function or expression of
BSEP may contribute to certain types of drug-induced cholestasis [28], and may be associated with intrahepatic cholestasis of pregnancy [29].

Defective MDR3 expression has also been associated with the inherited liver disease PFIC, namely the type 3 [30,31]. PFIC3 is characterized by high bile acid concentrations and elevated \( \gamma \)-glutamyl transpeptidase activity in serum. Several PFIC3-associated mutations in the \( ABCB4 \) gene may lead to either absent or severely decreased MDR3 expression at the canalicular membrane of hepatocytes. Similarly to BSEP, there is increasing evidence suggesting that deficiency of impaired activity of MDR3 may be involved in cholestasis induced by drugs, such as oral contraceptives [32,33].

Inherited mutations in the \( ABCC2 \) gene encoding the canalicular transporter MRP2 are linked to the Dubin-Johnson syndrome, characterized by reduced efflux of conjugated bilirubin into bile [34-37]. Some of these mutations have been reported to result in an absence of the MRP2 protein from the canalicular membrane of hepatocytes. In contrast to the PFIC syndromes, hepatic function is preserved in the Dubin-Johnson syndrome.

Mutations in the \( SLC10A2 \) gene encoding ASBT have been identified that can cause primary bile acid malabsorption, a rare disorder of the intestine characterized by congenital diarrhoea, steatorrhea, and reduced plasma cholesterol levels [38]. The ASBT variants carrying these mutations exhibit severely reduced bile acid transport activity \textit{in vitro}.

No mutations in the \( SLC10A1 \) gene encoding NTCP leading to clinically manifest defects in hepatic bile acid uptake have been characterized thus far. However, a recent study identified ethnicity-dependent single nucleotide polymorphisms in the \( SLC10A1 \) gene that were associated with a considerable decrease in transport function \textit{in vitro} [39] Thus, genetic heterogeneity in the \( SLC10A1 \) gene may play a role in the etiology of hypercholanemia. Furthermore, certain human diseases, such as advanced stage primary biliary cirrhosis [40]
and cholestatic alcoholic hepatitis [41], are associated with reduced NTCP expression. However, this change in NTCP expression may be a consequence of cholestatic liver injury, rather than a cause of it.

9.4. Control of bile acid transport and metabolism

In addition to their role as physiological detergents, bile acids possess crucial regulatory properties, which allow them to control their own transport and metabolism within the enterohepatic circulation through multiple feedforward and feedback mechanisms. Hepatocytes and enterocytes possess numerous signalling pathways that are activated or modulated by bile acids, and ultimately serve to maintain intracellular concentrations of potentially toxic bile acids at a constant level.

An important mechanism towards controlling bile acid levels within cells is to adjust the cellular uptake or efflux of bile acids by regulating the expression and/or activity of uptake and efflux proteins, as will be discussed in detail below. It should be noted, however, that additional mechanisms are also operational in preventing intracellular bile acid concentrations from reaching toxic levels. One such mechanism is to regulate the de novo synthesis of bile acids according to the existing intracellular bile acid content. To reduce bile acid synthesis, the expression levels of the key CYP enzymes involved in de novo bile acid synthesis, namely CYP7A1, CYP8B1, and CYP27A1 are suppressed [42]. Furthermore, expression levels of several phase II enzymes that, in addition to their role in drug detoxification, may also convert bile acids into less toxic and more hydrophilic derivatives, are induced in response to elevated levels of bile acids [43]. These metabolizing enzymes
include uridine 5'-diphosphate-glucuronosyltransferase 2B4 (UGT2B4) and dehydroepiandrosterone sulfotransferase (SULT2A1).

In this review we focus on the mechanisms that regulate the expression of bile acid transporters at the transcriptional level. However, it should be remembered that the activity of bile acid transporters is also known to be regulated at other levels, particularly through post-translational protein modification and protein-protein interactions [44,45]. The relative importance of transcriptional and post-translational events in controlling bile acid transport activity in either normal physiology or pathophysiology remains largely unelucidated. Both are likely to be highly important. It seems likely that the mechanisms involving modification at the protein level could elicit the most rapid changes in transporter activity, whereas transcriptional changes may be responsible for more intermediate- and longer-term regulation of transport.

9.5. Nuclear receptors as transcriptional regulators of bile acid homeostasis

Nuclear and steroid receptors form a large family of transcriptional regulators, with over one hundred members in all metazoan organisms, and almost fifty members in humans [46]. Most nuclear/steroid receptors share a conserved overall structural design: a ligand-independent activation function at the amino-terminus, a central conserved DNA-binding domain, and a carboxy-terminal region containing regions mediating ligand-binding, dimerization, and ligand-dependent transactivation. Most nuclear/steroid receptors bind to their DNA response elements as either hetero- or homodimers, which is reflected in their preferred DNA-binding motifs typically containing two hexameric half sites. These hexamers, the general consensus
sequence for which is AGGTCA, can be arranged as direct (DR), inverted (IR), or everted (ER) repeats, separated by a variable and receptor-specific number of base pairs.

The full transcriptional activity of most, but not all, nuclear/steroid receptors depends on a physical interaction by an agonist with their ligand-binding pocket. These ligands are typically small lipophilic molecules, such as hormones, fatty acids, oxysterols, or bile acids. Their binding induces a conformational shift in the carboxy-termini of the receptors, allowing their interaction with transcriptional coactivators [47]. These coactivators may act by modifying histones or other promoter-associated proteins, or by altering local chromatin structure, in a way that increases the rate of transcriptional initiation. Conversely, in the absence of an agonistic ligand, or when bound to an antagonistic ligand, the carboxy-termini of nuclear/steroid receptors associate with transcriptional corepressors that render the proximal promoters less permissive for transcription. The dependence of the transcriptional activity of most nuclear and steroid receptors on specific ligands allows them to monitor intracellular environment, and to elicit rapid transcriptional responses to changes in the concentrations of specific compounds.

9.5.1. FXR - the master regulator of bile acid transport and metabolism

The chief sensor of intracellular bile acid levels and the main executor of bile acid-induced transcriptional programmes is a nuclear receptor, namely the farnesoid X receptor (FXR) [48]. Bile acids directly interact with the ligand-binding domain of FXR. In transactivation and coactivator recruitment assays, CDCA is the most efficient FXR activator, followed by DCA and CA [49-51]. LCA alone can weakly activate FXR; however, it strongly antagonizes
CDCA-mediated stimulation of FXR [52]. This apparent antagonism of FXR function may contribute to LCA-induced cholestasis.

Bile acids are not the only ligands that interact with FXR directly. Recently, it has been suggested that the oxysterol 22(R)-hydroxycholesterol, an intermediate in the synthesis of bile acids and steroid hormones, can also directly interact with the ligand-binding pocket of FXR and mediate gene activation [53]. Traditionally, oxysterols have been considered to be agonistic ligands for another member of the nuclear receptor family, the liver X receptor (LXR), which functions as a chief regulator of cholesterol homeostasis. Furthermore, androsterone, a testosterone metabolite, can directly interact with the FXR ligand-binding domain, and enhance transcriptional activity of FXR through coactivator recruitment [54]. It is possible that different ligands induce different conformational states of FXR, leading to distinct patterns of gene regulation [55]. In this review we only discuss the significance of bile acids as FXR ligands and activators of FXR-induced transcriptional programmes.

In accordance with its function as a bile acid receptor, FXR is abundantly expressed in the tissues exposed to bile acids: liver, intestine, and kidneys. The consensus DNA-binding motif for FXR is in the format of an inverted repeat-1 (IR-1, inverted hexameric repeat separated by one base pair) [56], to which it binds as a heterodimer with another nuclear receptor, the retinoid X receptor (RXR). However, other configurations, such as IR-0 and ER-8, may allow binding of FXR in the context of specific promoters [57,58].

Supporting its physiological role in controlling bile acid homeostasis, FXR-null mice exhibit a phenotype similar to that of individuals suffering from Byler disease, an inherited cholestatic liver disorder [59]. Upon feeding with cholic acid, these mice lacking FXR exhibit severely increased hepatotoxicity, when compared to the wild-type counterparts. In further
support of the importance of FXR in human bile acid homeostasis, certain hereditary forms of cholestasis are associated with decreased FXR activity [60].

Encouraging reports showing hepatoprotective effects by synthetic FXR agonists in rodent models of cholestasis [61,62] suggest that specific FXR ligands could prove to be useful in the treatment of cholestatic liver diseases also in humans. One major concern regarding FXR-based therapy is that this nuclear receptor is involved in other metabolic processes in addition to bile acid homeostasis. Thus, its activation even with specific ligands may affect these unrelated processes in an undesired manner. For example, negative feedback suppression of bile acid synthesizing enzymes by bile acids is mediated by a complex cascade involving FXR, leading to reduced conversion of cholesterol to bile acids. In addition to controlling bile acid transporter and synthesis genes, FXR is crucially involved in glucose homeostasis via regulation of the genes encoding gluconeogenic enzymes [63,64]. In addition, FXR activated by bile acids has been recently shown to be an important mediator of liver regeneration [65]. It was hypothesized that FXR activation by increased level of bile acids would be a signal of reduced hepatic capacity and function. Thus, until the liver reaches the sufficient number of new hepatocytes to cope with the bile acid levels, FXR continues to trigger their proliferation.

9.5.2. The role of PXR and VDR as bile acid sensors

FXR is not the only nuclear receptor that can use bile acids as ligands that modulate transcriptional activity. In addition, the pregnane X receptor (PXR) and the vitamin D receptor (VDR) can mediate transcriptional responses to certain bile acids. PXR, together with its xenosensor partner constitutive androstane receptor (CAR), is a nuclear receptor that
typically utilizes drugs and xenobiotics as its ligands [66]. In response to these ligands, PXR induces the expression of genes encoding proteins involved in drug detoxification and elimination pathways. In addition to xenobiotics, certain bile acids, such as the highly toxic LCA, can serve as agonistic ligands for PXR [67,68]. Indeed, activation of PXR can protect mouse livers against LCA-mediated injury [67]. Double knock-out mice lacking both FXR and PXR exhibit more severe disturbances of bile acid metabolism than mice lacking only one of the nuclear receptors, demonstrating that both contribute to bile acid homeostasis [69]. PXR is a master regulator of the gene encoding the CYP3A4 enzyme [70], which, in addition to its role in detoxifying xenobiotics, also metabolizes bile acids to less toxic and more easily excreted derivatives. Thus, by being both activators of the CYP3A4 gene and substrates of the CYP3A4 enzyme, bile acids can initiate a hepatoprotective feedforward loop via PXR. Similarly to PXR, VDR can utilize the toxic secondary bile acid LCA as an agonistic ligand [71]. While VDR is only weakly expressed in hepatocytes, it is abundantly present in enterocytes. It is thus likely to play a more prominent role in the intestine in response to LCA. In addition to PXR, VDR can also transactivate the CYP3A4 gene. Thus, VDR activated by elevated levels of LCA in the intestine may, in a feedforward manner, contribute to enhanced expression of the CYP3A4 enzyme, which is capable of detoxifying LCA.

Common to all three nuclear receptors, FXR, PXR, VDR, that can utilize bile acids as ligands, is that they all bind to their respective DNA response elements as heterodimers with the nuclear receptor RXR, with very few exceptions. Positive or negative effects on the transcriptional activity of RXR-containing heterodimers by the RXR ligand 9-cis-retinoic acid appear to depend on the exact promoter context [72,73].

9.5.3. The bile acid-induced transcriptional repressor SHP
FXR can also negatively regulate the rate of transcription from specific promoters. In rare cases, FXR is known to repress its target genes, such as those encoding the human apolipoprotein A-I [74] and human apolipoprotein C-III [75], through direct binding to the promoter. However, FXR more commonly mediates negative transcriptional responses to elevated levels of bile acids indirectly, through inducing the expression of its target gene encoding the transcriptional repressor called small heterodimer partner, SHP. SHP is an atypical member of the nuclear receptor family: it does not contain a DNA-binding domain, and does not depend on a ligand for its activity. Instead, it directly interacts with a variety of DNA-bound transcriptional activators, interfering with their transcriptional activity. While SHP can suppress the activity of transcription factors from certain other families, it most commonly targets other nuclear receptors and steroid receptors (reviewed in [76]). Via the SHP pathway bile acids can influence the activities of a wider range of transcription factors than only those nuclear receptors that are directly modulated by them.

Several mechanisms have been suggested for SHP-mediated suppression of transactivator proteins. SHP may compete with transcriptional coactivators over the same or overlapping interaction surface on transactivators that are bound to the promoter elements [77]. Alternatively, SHP may interfere with the binding of transactivators to their DNA response elements [78]. Additionally, SHP may recruit transcriptional corepressors to its target promoters, thus contributing to the reduced transcriptional rate of a given promoter [79].

The importance of SHP in the control of bile acid homeostasis is indicated by the fact that SHP-null mice exhibit an imbalance in bile acid metabolism and abnormal responses when challenged with diets rich in bile acids [80,81].
9.6. FXR-dependent mechanisms that regulate human bile acid transporter genes

We will next discuss the FXR-dependent effects of bile acids on the expression of transporter genes in hepatocytes and enterocytes. These concepts are summarized in Figure 9-2.

It should be emphasized that while we focus on FXR-dependent effects by bile acids in this review, bile acids do also elicit signalling pathways that lead to changes in specific gene expression, which are independent of FXR or other nuclear receptors. These alternative bile acid-stimulated pathways include signalling through mitogen-activated protein kinases [82,83] and through the G-protein-coupled receptor TGR5 [84]. Parallel bile acid-stimulated signalling pathways may ensure that the desired transcriptional responses are achieved.

9.6.1. Positive feedforward control of bile acid efflux systems by bile acids

In response to bile acids FXR induces BSEP expression via direct interaction of FXR-RXR heterodimers with an IR-1 element located in the proximal promoter of the ABCB11 gene [85-87]. Thus, excessive levels of bile acids stimulate hepatocanalicular clearance of bile acids. The FXR-RXR binding element is conserved between the human and rodent ABCB11/Abcb11 promoters, supporting its functional importance. In agreement with FXR being a crucial activator of the ABCB11 gene, the BSEP expression is reduced in mice lacking the FXR gene [59].

The ABCC2 gene encoding the MRP2 transporter provides an illustrative example of the complex interactions between the metabolic nuclear receptors. Both the human and rodent ABCC2/Abcc2 promoters can be activated by either FXR, PXR, or CAR in the presence of
their respective ligands or activators [58]. Interestingly, each of these three nuclear receptors can interact with the same atypical ER-8 element present in the regulatory region of the ABCC2 gene. The relative significance or the degree of redundancy between the three transcription factors in the regulation of MRP2 expression is not yet clear.

FXR also transactivates the ABCB4 gene encoding MDR3, the phospholipid transporter at the canalicular membrane of hepatocytes [85]. Thus, bile acids may, in a coordinated manner and via activation of FXR, induce the excretion of both bile acids (BSEP, MRP2) and phospholipids (MDR3) into bile. In agreement with this, FXR-null mice are prone to developing cholesterol gallstones, caused by the deficiency in the excretion of bile acids and phospholipids [59].

Expression of the genes encoding the alternative bile acid export pumps at the basolateral hepatocyte membranes, MRP3 (ABCC3) and MRP4 (ABCC4), appears to be induced in cholestasis in an FXR-independent manner, at least in bile duct-ligated or bile acid-fed mice [86,87]. It may be that the other bile acid-responsive nuclear receptor PXR is responsible for the bile acid-mediated induction of the ABCC3 and ABCC4 genes, or can at least compensate in the absence of FXR [88]. In agreement with the cholestatic mouse models, it has been reported that in human cholestatic liver disease, expression of MRP3 and MRP4 is similarly elevated [40,89,90].

In analogy to the ABCB11 gene encoding the hepatic efflux system BSEP, the two genes encoding the heterodimeric efflux system in the intestine, OSTα/OSTβ are induced by bile acids through direct binding of FXR-RXR heterodimers to the two human OST promoters [91,92]. While the OSTβ promoter appears to have a single IR-1-like binding site for FXR-RXR, the human OSTα promoter contains two adjacent IR-1-like FXR response elements,
both of which are functional and required for full response to bile acids. Physiological evidence in support of the induction of OST expression by bile acids is provided by a recent study showing that both mRNA and protein levels of OSTα and OSTβ are increased in cholestatic liver tissue of primary biliary cirrhosis patients [93]. In further agreement with the proposed role of FXR in inducing the OST genes, their baseline expression is reduced and their bile acid-mediated induction is abolished in FXR-null mice [92-94]. Accordingly, the location and sequence of the IR-1-like FXR response elements are largely conserved in both human and mouse OSTα and OSTβ genes.

Bile acid-activated FXR also stimulates expression of the gene encoding the intracellular bile acid transporter I-BABP in the ileum [95]. Supporting this, I-BABP expression is dramatically reduced in FXR-deficient mice [59]. FXR response elements of the IR-1 type have been identified in both the human and rodent I-BABP/IBabp promoters. The bile acid-activated FXR can thus upregulate the expression of both the membrane-bound bile acid uptake system OSTα/OSTβ and the intracellular bile acid transporter I-BABP in a coordinated manner within enterocytes.

9.6.2. Negative feedback control of bile acid uptake systems by bile acids

In rodent models of cholestasis, such as bile duct ligation or bile acid feeding, expression of the hepatic bile acid uptake system Ntcp is suppressed at both the protein and mRNA level [96,97]. Furthermore, certain human cholestatic states, such as advanced stage primary biliary cirrhosis [40] and cholestatic alcoholic hepatitis [41], are similarly associated with reduced NTCP expression. It has been recently suggested that the mechanism of down-regulation of
human NTCP expression in response to bile acids involves the FXR-SHP cascade. FXR-induced SHP targets the transactivator glucocorticoid receptor (GR), which interacts with its response element just upstream of the transcription initiation site on the human *SLC10A1* promoter [98].

FXR also suppresses transcription of the *SLCO1B1* gene encoding the sodium-independent bile acid transporter OATP1B1 at the basolateral membrane of human hepatocytes [99]. FXR-mediated repression of the *SLCO1B1* promoter takes place through a multistep regulatory cascade, which involves bile acid-mediated repression of the gene encoding the liver-enriched homeodomain factor hepatocyte nuclear factor-1α (HNF-1α). HNF-1α, in turn, is a strong DNA-binding transactivator of the *OATP1B1* promoter. The regulatory region of the *HNF-1α* gene contains a consensus DNA-binding response element for the nuclear receptor HNF-4α, the transcriptional activity of which can be targeted by negative interference by FXR-induced SHP.

Similarly to NTCP, treatment of cultured cells with bile acids suppresses the expression of the intestinal bile acid uptake transporter ASBT, another member of the SLC10 transporter family in humans [100]. In accordance with this, *SLC10A2* gene expression is reduced in patients with obstructive cholestasis, when compared to healthy controls [101]. Two transactivators of the human *SLC10A2* promoter, namely the GR [98,102] and the nuclear receptor heterodimer, retinoic acid receptor (RAR)-RXR [100], have been suggested as targets for negative interference by the bile acid-induced transcriptional repressor SHP. It remains to be determined whether these two transactivators are simultaneously targeted by SHP to ensure efficient downregulation of the *SLC10A2* promoter by bile acids, or whether the two pathways are targeted under different circumstances. Interestingly, while both the
SLC10A1 (NTCP) and SLC10A2 (ASBT) genes are transactivated by GR, the relative locations and configurations of the GR response elements within the two promoters are not conserved.

9.7. Crosstalk between the transcriptional control of bile acid and drug transporters

Transport and metabolism of exogenous compounds, such as drugs, nutrients, and environmental xenobiotics, bear many similarities to those of endogenous bile acids. Indeed, drugs may undergo enterohepatic circulation similarly to bile acids. Several transporters recognize both bile acids and drugs as substrates. For example, although their significance for overall bile acid transport in normal physiology remains uncertain, the two transporters of the human OATP1B subfamily, OATP1B1 and OATP1B3, can transport bile acids, in addition to drugs such as methotrexate and rifampicin [103]. The transcriptional regulatory circuits controlling the expression of drug transporter genes often contain feedforward and feedback loops, reminiscent of the mechanisms outlined for regulation of bile acid transport above (reviewed in [76]). It is becoming increasingly apparent that, in addition to reprogramming of genes involved in bile acid homeostasis, changes in intracellular concentrations of bile acids also influence the expression levels of hepatic and intestinal drug transporters. Thus, changes in intracellular bile acid levels may affect the efficiency of drug extraction and elimination.

HNF-4α is a liver-enriched nuclear receptor with a critical role in maintaining the hepatic pattern of gene expression [104]. It binds to the DNA response elements on its target promoters, preferably of the DR-1 or DR-2 configuration, as homodimers. The ligand-binding domain of HNF-4α has been suggested to be constitutively bound by endogenous fatty acids [105,106], thus its activity may not be readily modulated by exogenous ligands. In addition to
its previous roles in regulation of glucose and cholesterol metabolism, HNF-4α has recently emerged as a regulator of hepatic drug transport in humans. The genes encoding two major hepatic basolateral drug transporters in humans, namely organic anion transporter 2 (OAT2; SLC22A7) [107] and organic cation transporter 1 (OCT1; SLC22A1) [108], are transactivated by HNF-4α. OAT2 mediates uptake of drugs such as salicylates and cephalosporins from sinusoidal blood, whereas OCT1 transports dopamine, metformin, and verapamil, amongst other substrates.

In the regulatory regions of the human SLC22A7 gene, HNF-4α-mediated transactivation is mediated by a single DR-1 element. The human SLC22A1 promoter, in turn, contains two adjacent lower-affinity DR-2 elements, both of which are required for maximal transactivation by HNF-4α. In both promoter contexts, the bile acid-induced transcriptional repressor SHP interferes with transactivation by HNF-4α. It thus appears that in conditions of elevated intracellular bile acid concentrations, the expression of two major drug uptake systems at the basolateral hepatic membrane is reduced. This could limit the amount of xenobiotics that enter the hepatocyte for subsequent metabolism, when intracellular levels of toxic bile acids are already elevated. Also, the possibility of decreased hepatic extraction of drugs that are substrates of OAT2 and OCT1 should be taken into account, when such drugs are administered to patients suffering from cholestasis.

9.8. Concluding remarks

Enterohepatic bile acid transporters are crucial in maintaining bile acid homeostasis in healthy individuals. Their importance for hepatic and intestinal function is emphasized by numerous
recent demonstrations that either hereditary or acquired disturbances in the activity and/or expression of bile acid transporters are associated with cholestatic disease conditions. Bile acids regulate the expression of transporters that mediate the enterohepatic circulation of both bile acids and drugs via complex feedforward and feedback mechanisms. The main orchestrator of these transcriptional circuits is FXR, which, in response to bile acids, controls the expression levels of bile acid transporters at all membrane domains of enterohepatic circulation.

Increased knowledge of the transcriptional mechanisms governing the changes in bile acid transporter gene expression has progressed our understanding of the pathophysiology of cholestatic liver diseases. Additionally, this information is likely to provide us with novel tools for designing therapeutic strategies to combat diseases of the liver and the intestine.
References


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