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# Role of Nuclear Receptors in the Adaptive Response to Bile Acids and Cholestasis: Pathogenetic and Therapeutic Considerations

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**Abstract:** Cholestasis results in intrahepatic accumulation of cytotoxic bile acids which cause liver injury ultimately leading to biliary fibrosis and cirrhosis. Cholestatic liver damage is counteracted by a variety of intrinsic hepatoprotective mechanisms. Such defense mechanisms include repression of hepatic bile acid uptake and de novo bile acid synthesis. Furthermore, phase I and II bile acid detoxification is induced rendering bile acids more hydrophilic. In addition to "orthograde" export via canalicular export systems, these compounds are also excreted via basolateral "alternative" export systems into the systemic circulation followed by renal elimination. Passive glomerular filtration of hydrophilic bile acids, active renal tubular secretion, and repression of tubular bile acid reabsorption facilitate renal bile acid elimination during cholestasis. The underlying molecular mechanisms are mediated mainly at a transcriptional level via a complex network involving nuclear receptors and other transcription factors. So far, the farnesoid X receptor FXR, pregnane X receptor PXR, and vitamin D receptor VDR have been identified as nuclear receptors for bile acids. However, the intrinsic adaptive response to bile acids cannot fully prevent liver injury in cholestasis. Therefore, additional therapeutic strategies such as targeted activation of nuclear receptors are needed to enhance the hepatic defense against toxic bile acids.

**Keywords:** Bile acid transport; bile acid detoxification; bile acid synthesis; transcription factors; nuclear (orphan) receptors

## 1. General Aspects of Bile Acid Transport and Metabolism

**(A) Bile Acid Transport. (i) Hepatic Bile Acid Transport.** Hepatobiliary transport systems are essential for normal bile formation and hepatic elimination of various endo- and xenobiotics including bile acids, bilirubin, cholesterol, phospholipids, hormones, and drugs.[1−3] The liver comprises a broad range of specific uptake and export systems for various biliary compounds, as shown in Figure 1.

Bile is primarily formed by canalicular excretion of bile acids and non bile acid organic anions via ATP-binding cassette (ABC) transporters. Monovalent bile acids such as

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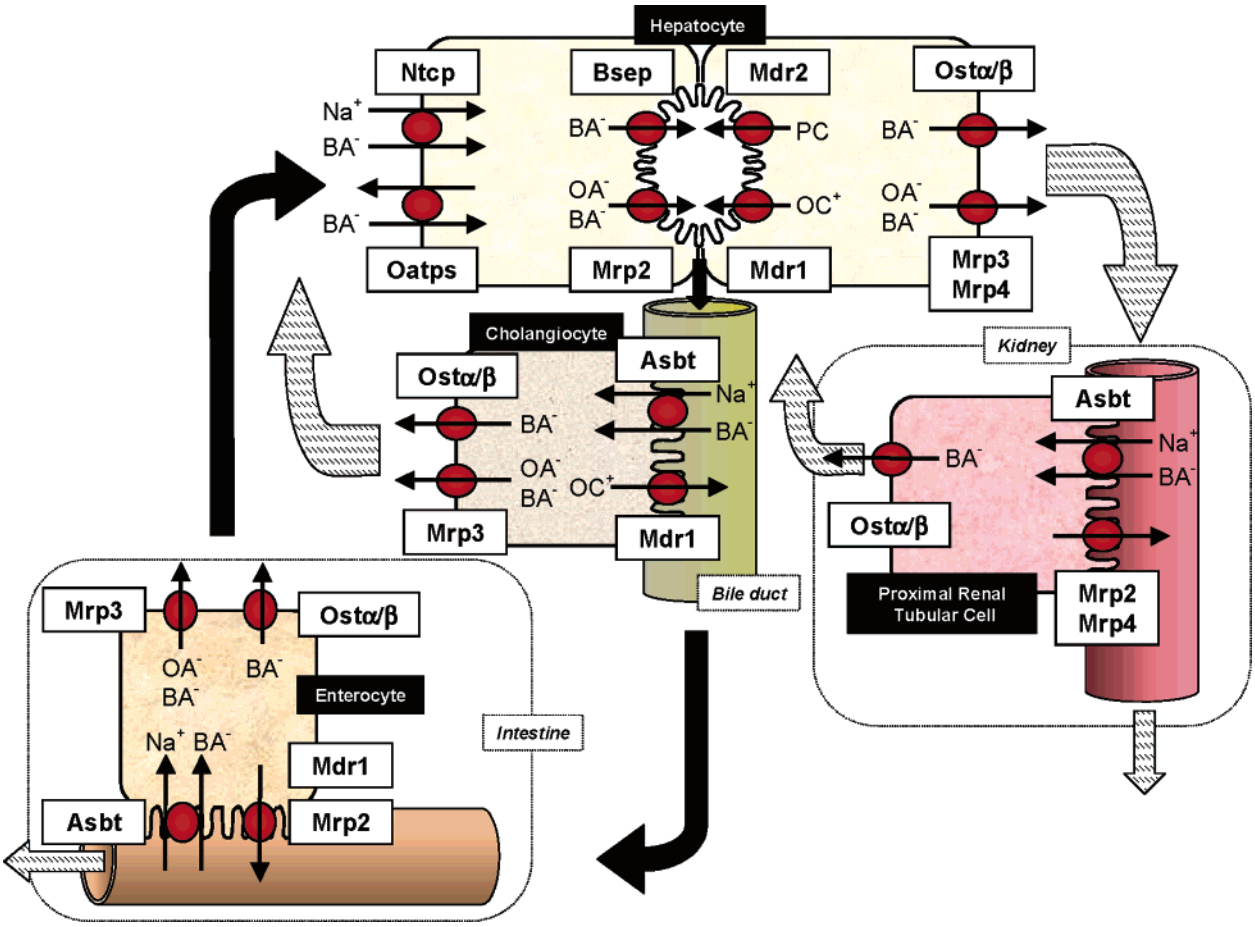


***Figure 1.*** Hepatobiliary transport systems in liver, kidney, and intestine. Bile acids ($BA^-$) are taken up by the $Na^+$/taurocholate cotransporter (Ntcp) and organic anion transporting proteins (Oatps) at the basolateral membrane of hepatocytes. Monovalent $BA^-$ are excreted by the canalicular bile salt export pump (Bsep), and divalent BAs and organic anions ($OA^-$) are exported by the canalicular conjugate export pump (Mrp2). The phospholipid export pump (Mdr2) mediates excretion of phosphatidylcholine (PC), which forms mixed micelles together with $BA^-$ and cholesterol in bile. Cationic drugs ($OC^+$) are excreted by the multidrug export pump (Mdr1). At the basolateral membrane of hepatocytes, Mrp3 and Mrp4 and the recently identified heteromeric organic solute transporter Ostα/$\beta$ provide an alternative excretion route for $BA^-$ and other $OA^-$ into the systemic circulation. $BA^-$ secreted into bile are reabsorbed in the terminal ileum via apical $Na^+$-dependent bile salt transporter (Asbt) and effluxed by Ostα/$\beta$ and Mrp3. Similar mechanisms for bile acid reabsorption exist in cholangiocytes and proximal renal tubules thus limiting bile acid loss via feces and urine. Mrp2 is also present in the apical membrane of enterocytes and—together with Mrp4—at proximal renal tubules. Mdr1 is also present in intestine and bile duct epithelial cells.

the glycine- or taurine-amidates of cholic acid (CA), chenodeoxycholic acid (CDCA), and ursodeoxycholic acid (UDCA) are excreted into the bile canaliculus via the bile salt export pump BSEP/Bsep (ABCB11/Abcb11).[4,5] Divalent bile acids with two negative charges such as sulfated tauro- or glycolithocholate are transported by multidrug resistance associated protein MRP2/Mrp2 (ABCC2/Abcc2).[6,7] MRP2/Mrp2 also mediates the excretion of a broad range of other non bile acid organic anions, mostly conjugates with glutathione, glucuronate, and sulfate formed by phase II conjugation in the hepatocyte,[7−10] and of reduced glutathione (GSH), a major determinant of bile acid-independent bile flow.[3] Canalicular bile is then modified by absorptive and

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secretory processes by the bile duct epithelium. Bile acids excreted into bile undergo an intensive enterohepatic circulation. They are reabsorbed in the intestine, taken up again by the liver, and re-excreted into bile, repeating this cycle several times before being eliminated with the feces. Hepatic uptake of bile acids occurs via the $Na^+$/taurocholate cotransporter (NTCP/Ntcp; SLC10A1/Slc10a1) or via members of the organic anion transporter (OATP/Oatp; SLCO/Slco) superfamiliy.[3]

Hepatocellular bile acid efflux via the basolateral membrane may become an important alternative spillover route for accumulating bile acids and bilirubin during bile acid overload under cholestatic conditions. Most alternative basolateral export transport systems belong to the MRP/Mrp family. MRP3/Mrp3 (ABCC3/Abcc3) and MRP4/Mrp4 (ABCC4/Abcc4) are capable of transporting bile acids and are constitutively expressed only at very low levels at the basolateral hepatocyte membrane.[11−16] They can be induced however under cholestatic conditions.[17−20] MRP3/Mrp3 transports divalent bile acids such as sulfated taurolithocholic acid and taurochenodeoxycholic acid with high affinity.[12,13,21] In addition, Mrp3 can also transport monovalent bile acids such as tauro- and glycocholic acid,[12,13,21] while human MRP3 only transports glycocholate with low affinity.[11,22] MRP4 mediates, among other substrates, the cotransport of bile acids with GSH. It has a high affinity for glycine and taurine-amidated CDCA, CA, and UDCA as well as unconjugated CA in the presence of physiological concentrations of GSH.[15,16] Furthermore, transport inhibition studies suggested that sulfated bile acids might also be substrates for MRP4 (14). In addition to the ABC transporter family, the recently identified organic solute transporter Ost$\alpha$/Ost$\beta$ represents a novel candidate basolateral bile salt export system in the liver. Both subunits (alpha and beta) of this heteromeric transporter are required for transport of various bile acids and their conjugates.[23−25] Rodent Ost$\alpha$/Ost$\beta$ is highly expressed in kidney, in intestine, and to lower extents in liver, where it localizes to the basolateral membrane of cholangiocytes and hepatocytes.[24,25] In humans, OST$\alpha$/OST$\beta$ is highly expressed in liver and localizes to both hepatocytes and cholangiocytes.[26] It is attractive to speculate that this recently characterized basolateral transporter may also participate in alternative bile acid export during cholestasis.

**(ii) Cholangiocellular Bile Acid Transport.** Cholangiocytes play an important role in bile secretion and contain several transport systems for absorptive and secretory

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processes[27,28] (Figure 1). Unconjugated bile acids may passively enter cholangiocytes, while amidated bile acids are reabsorbed in a sodium-dependent manner by the apical bile acid transporter Asbt (*Slc10a2*). Asbt is also responsible for bile acid uptake in the terminal ileum, where this transporter was originally discovered.[29,30] Mrp3 and tAsbt, an alternatively spliced and truncated form of Asbt, were proposed to mediate basolateral export of bile acids from cholangiocytes.[31−33] The key basolateral bile acid transporter in cholangiocytes, however, seems to be the recently identified Ostα/Ostβ, representing the basolateral counterpart to Asbt.[26]

**(iii) Intestinal Bile Acid Transport.** To complete enterohepatic circulation, an efficient reabsorption of bile acids in the intestine with subsequent delivery to portal circulation is required. Unconjugated bile acids are partly absorbed by passive diffusion, whereas most of the amidated bile acids are taken up into enterocytes by Asbt (*Slc10a2*) in the terminal ileum[34,35] (Figure 1). Information about intracellular bile acid transport in enterocytes is limited. The ileal bile acid binding protein (I-Babp) is able to bind bile acids and has been suggested to mediate transcellular bile acid movement.[36−39] Until recently it was speculated that either t-Asbt[33] or Mrp3[22,40] may represent the pendant to Asbt for basolateral bile acid export. Current findings, however, suggest that Ostα/Ostβ represents the major basolateral bile acid export mechanism in enterocytes[25] (Figure 1).

**(iv) Renal Bile Acid Transport.** Bile acids that escape first pass clearance by the liver or are actively excreted by hepatocytes into sinusoidal blood are filtered at the glomerulus from plasma into urine.[41] Thereafter, bile acids are reabsorbed by ASBT/Asbt localized to the apical membrane of proximal renal tubular cells and probably in turn excreted into systemic circulation by basolateral Ostα/Ostβ[26,42,43] (Figure 1). Under cholestatic conditions, however, renal excretion of bile acids may become a major alternative elimination route.[44] This may be attributed to increased passive glomerular filtration due to elevated serum bile acid levels and reduced tubular bile acid reabsorption via repressed Asbt.[45] Passive glomerular filtration of bile acids might also be aided by active tubular secretion. Candidate transporters for export of sulfated and glucuronidated bile acids are Mrp2 and Mrp4, which are both localized to the apical tubular membrane.[20,46,47] However, the relative con-


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tribution of active tubular excretion to passive glomerular filtration still remains to be determined.

**(B) Bile Acid Formation and Metabolism.** In the liver, cholesterol is converted to bile acids by a pathway consisting of a cascade of 15 reactions (reviewed in ref 48). In brief, the main bile acid biosynthetic (classic or neutral) pathway is initiated by CYP7A1, mediating the rate-limiting step in bile formation.[49] The alternative (or acidic) pathway is initiated by sterol 27-hydroxylase (CYP27A1).[50] CA and CDCA are the primary bile acids found in human bile. In humans, the classical pathway produces CA and CDCA in roughly equal amounts, whereas the acidic pathway produces mainly CDCA.[51,52] In the neutral pathway, CYP8B1 is involved in the synthesis of CA and controls the ratio of CA to CDCA. The bile acids synthesized in the liver are excreted into bile as conjugates with glycine or taurine. During enterohepatic circulation, bile acids are subjected to deamidation and 7α-dehydroxylation by intestinal microorganisms, yielding the secondary bile acids deoxycholic acid (DCA) and lithocholic acid (LCA). Most of the bile acids excreted into intestine are reabsorbed. Upon returning to the liver with the portal blood, unconjugated bile acids are reconjugated with glycine or taurine, followed in a few instances by sulfation and glucuronidation. Ketonic bile acids can be reduced, and bile acids can be hydroxylated.[53]

**(C) Regulation of Bile Acid Transport and Metabolism by Nuclear Receptors.** Bile acid homeostasis is the result of a balance between bile acid uptake, efflux, and biosynthesis. Maintenance of this balance is essential, since most bile acids are cytotoxic. When their concentrations reach abnormally high levels, they can cause liver injury, eventually leading to fibrosis and cirrhosis.[54] This homeostasis is the result of coordinated feedback and feedforward regulation of genes involved in bile acid synthesis, detoxification, and transport. Liver-enriched transcription factors (e.g., hepatocyte nuclear factors, HNF) and nuclear receptors (NRs) play a key role in the transcriptional regulation of hepatobiliary transport systems and of enzymes involved in bile acid metabolism.[55−59] The structural organization of NRs is similar despite variations in ligand affinity. In general, these proteins contain an amino-terminal ligand-independent transactivation domain, a core DNA-binding domain, a hinge region (providing protein flexibility to allow simultaneous receptor dimerization and DNA binding), and a large carboxy-terminal region (containing the ligand-binding domain, dimerization interface, and a ligand-dependent activation function).[60,61] Upon ligand binding, NRs undergo a conformational change that coordinately dissociates corepressors and facilitates recruitment of coactivator proteins to enable transcriptional activation.[62−64] NRs bind to their DNA response elements in a sequence-specific manner either as monomers, as homodimers, or as heterodimers.[61] The interested reader is referred to detailed reviews on NR function/structure and NR coactivators/corepressors which are beyond the scope of the current review.[61,62,65] Binding of biliary constituents (e.g., bile acids, bilirubin), lipid metabolites (e.g., oxysterols), and xenobiotics (e.g., drugs) to NRs facilitates the positive feedforward and negative feedback regulation of hepatic transport and metabolism of these compounds under physiologic and pathologic conditions.[55]

The farnesoid X receptor (FXR; NR1H4) was the first nuclear bile salt receptor to be identified.[66,67] Other NRs such as the pregnane X receptor (PXR; NR1I2) and vitamin D

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receptor (VDR; NR1I1) are also activated by hydrophobic, toxic bile acids such as LCA and its metabolite 3-keto-LCA, in addition to their classic hormonal and xenobiotic ligands.[68−70] The liver X receptor alpha (LXRα; NR1H3) is activated by oxysterols but also by 6α-hydroxy bile acids and their analogues.[60,71] Another xenobiotic receptor, constitutive androstane receptor (CAR; NR1I3), has recently been shown to be activated by bilirubin,[72,73] and a role of CAR for sensing bile acids has been postulated.[74] Thus, a picture is emerging where endobiotic and xenobiotic compounds share hepatic transport and metabolic systems and their regulatory NRs (Table 1). This "crosstalk" between endo- and xenobiotics at the NR level may have important implications for the therapeutic modulation of bile acid transport and metabolism.

## 2. Role of Nuclear Receptors in the Adaptive Transporter and Metabolic Response to Bile Acids

**(A) Overview on Bile Acid Transport and Metabolism during Cholestasis.** Cholestasis may result either from a functional defect in bile formation at the level of the hepatocyte or from an impairment in bile secretion and flow at the bile duct level and results in the intrahepatic and systemic accumulation of cholephiles such as bile acids.[1−3,75] Reduced expression and function of transport systems play a key role in the pathogenesis of cholestasis and can result in or maintain cholestasis.[1,3,57,75−77] Transport defects can be hereditary due to genetic defects or acquired as a result of cholestatic injury (e.g., inflammation, drugs, biliary obstruction). While transporter changes in hereditary cholestasis are primary, most alterations in acquired cholestasis are secondary effects which are mainly mediated by accumulating bile acids and proinflammatory cytokines. It should be kept in mind that not all of the encountered changes in transporter expression are "procholestatic" and "negative" from a teleological point of view. While some of these alterations contribute to cholestasis, other changes may represent compensatory ("anticholestatic") defense reactions which provide alternative excretory routes for accumulating cholephiles. Reducing basolateral bile acid uptake and simultaneously increasing basolateral bile acid excretion may be considered major hepatic defense mechanisms counteracting bile acid accumulation within hepatocytes. Moreover, bile acid hydroxylation and conjugation with sulfate or glucuronidate increases water solubility and reduces toxicity. After basolateral excretion these conjugates can subsequently be eliminated via the kidney.[78] In general, the hepatic clearance of bile acids can be divided into four phases that include the following: phase 0, hepatic uptake; phase I, hydroxylation; phase II, conjugation; and phase III, excretion (Figure 2). Under cholestatic conditions, when intrahepatic and systemic bile acid levels are high, a complex machinery of coordinated adaptive mechanisms is activated to counteract cholestatic liver injury. Adaptive mechanisms in response to cholestasis not only are restricted to the liver but also occur in kidney, intestine, and bile duct epithelia. Bile acid reabsorption in the intestine is reduced due to altered transporter expression whereas, in proximal renal tubular cells, putative bile acid export systems are induced in addition to reduced tubular bile acid reabsorption resulting in enhanced urinary bile acid excretion. In obstructed bile ducts, bile acid reuptake and delivery to the liver for subsequent detoxification is increased (Figure 2).

Most of our knowledge on alterations of hepatobiliary transport systems and adaptive mechanisms during cholestasis is derived from experimental animal models. These models include common bile duct ligation (CBDL, model

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***Table 1.*** Nuclear Receptors Mediating the Adaptive Response to Bile Acids[a]

| nuclear receptor | ligands | target genes | expected effects/functions |
|---|---|---|---|
| FXR (NR1H4) | CDCA, DCA, LCA, CA, possibly UDCA (weak ligand) synthetic: GW4064, 6α-ethyl-CDCA, fexaramines | *SHP*, *BSEP/Bsep*, *I-BABP*, *MRP2/Mrp2*, *OATP1B3*, *OSTα/β/Ostα/β*, *Sult2a1*, *CYP3A4*, *UGT2B4*, *UGT2B7* | induction of canalicular and alternative basolateral bile acid excretion<br>induction of phase I and II bile acid detoxification systems |
| SHP (NR0B2) | | *CYP7A1/Cyp7a1*, *CYP8B1/Cyp8b1*, *CYP27A1*, *Ntcp*, *ASBT/Asbt* | repression of bile acid synthesis and basolateral bile acid uptake |
| PXR (NR1I2) | rifampicin in humans, PCN in rodents, phenobarbital, dexamethasone, LCA, statins, St. John's wort, clotrimazole, possible UDCA | *MRP2/Mrp2*, *MRP3*, *Oatp1a4*, *MDR1* *CYP3A4*, *SULT2A1/Sult2a1*, (indirectly) *CYP7A1* *UGT1A1* | induction of canalicular and alternative basolateral bile acid excretion<br>induction of phase I and II bile acid and bilirubin detoxification systems<br>indirect repression of CYP7A1 |
| CAR (NR1I3)[b] | bilirubin, phenobarbital, TCPOBOP, dimethoxycoumarin, xenobiotics, Yin Chin, CITCO in humans | *MRP2/Mrp2*, *Mrp3*, *MRP4/Mrp4*, *CYP2B*, *CYP3A4*, *Sult2a1*, *UGT1A1* | induction of canalicular and alternative basolateral bile acid excretion<br>induction of phase I and II bile acid and bilirubin detoxification systems |
| VDR (NR1I1) | 1α,25-dihydroxy vitamin $D_3$, LCA | *CYP3A4*, *Sult2a1*, *Mrp3* | induction of bile acid hydroxylation, sulfation and export via Mrp3 |
| RARα (NR2B1) | *all-trans*-retinoic acid | *Ntcp*, *Mrp2*, *ASBT* | induction of Ntcp, Mrp2, ASBT |
| HNF4α (NR2A1) | potentially fatty acids | *Cyp7a1*, *CYP8B1/Cyp8b1*, *CYP27A1*, *Ntcp*, *HNF1α* | reduced HNF4α binding or expression downregulates bile acid synthesis and basolateral bile acid uptake |
| FTF (LRH-1, NR5A2) | | *MRP3*, *CYP7A1*, *CYP8B1*, *Asbt* | induction of MRP3, downregulation of CYP8B1 |
| PPARα (NR1C1) | fatty acids, fibrates, statins, eicasaonoids, leukotrienes, NSAIDs, WY-14643 | *Mdr2*, *ASBT*, *CYP7A1*, *UGT2B4* | protection of bile duct epithelium via increased phospholipid secretion, downregulation of CYP7A1 and induction of UGT2B4 |
| GR (NR3C1) | glucocorticoids, potentially UDCA | *CAR*, *ASBT*, *NTCP*, potentially *AE2*, *BSEP*, *MRP2* | beneficial effects in cholestatic jaundice ("steroid whitewash") may be mediated by direct transporter effects or indirect antiinflammatory effects |
| LXRα (NR1H3) | oxysterols, 6α hydroxy bile acids | *Cyp7a1*, *Abcg5/8*, *CYP3A4*, *SHP*, *LXR*, *LRH-1* | regulation of sterol transport, catabolism and elimination |

[a] CITCO, 6-(4-chlorophenyl)imidazo[2,1-*b*][1,3]thiazole-5-carbaldehyde *O*-(3,4-dichlorobenzyl)oxime; PCN, pregnenolone-16α-carbonitrile; TCPOBOP, 1,4-bis-[2-(3,5-dichloropyridyloxy)] benzene. [b] Please note that all substances listed for CAR are not truly ligands but activators of CAR.

for biliary duct obstruction), endotoxin challenge (sepsis-induced cholestasis), or administration of ethinylestradiol (cholestasis of pregnancy).[78] Bile acid feeding is commonly used to investigate direct effects of bile acids without (peri-) operative stress or induction of proinflammatory cytokines as observed during CBDL.[79] However, bile acid feeding cannot be considered a general model of cholestasis, since only bile acids but no other cholephiles accumulate and bile flow is maintained or even enhanced. The term "bile acid overload" for these models may be more appropriate. It has to be kept in mind that these models cannot unequivocally be applied to human cholestatic diseases. Species differences in transcriptional regulation, bile acid pool composition, and duration of cholestasis (days to weeks in animal models versus months to years in human diseases) make generalizations and extrapolations difficult. This review will focus on the effects of bile acids in experimental cholestasis (CBDL) and in models of bile acid overload as well as human cholestatic diseases. Endotoxin-induced cholestasis will not be discussed in this review, since the cytokines

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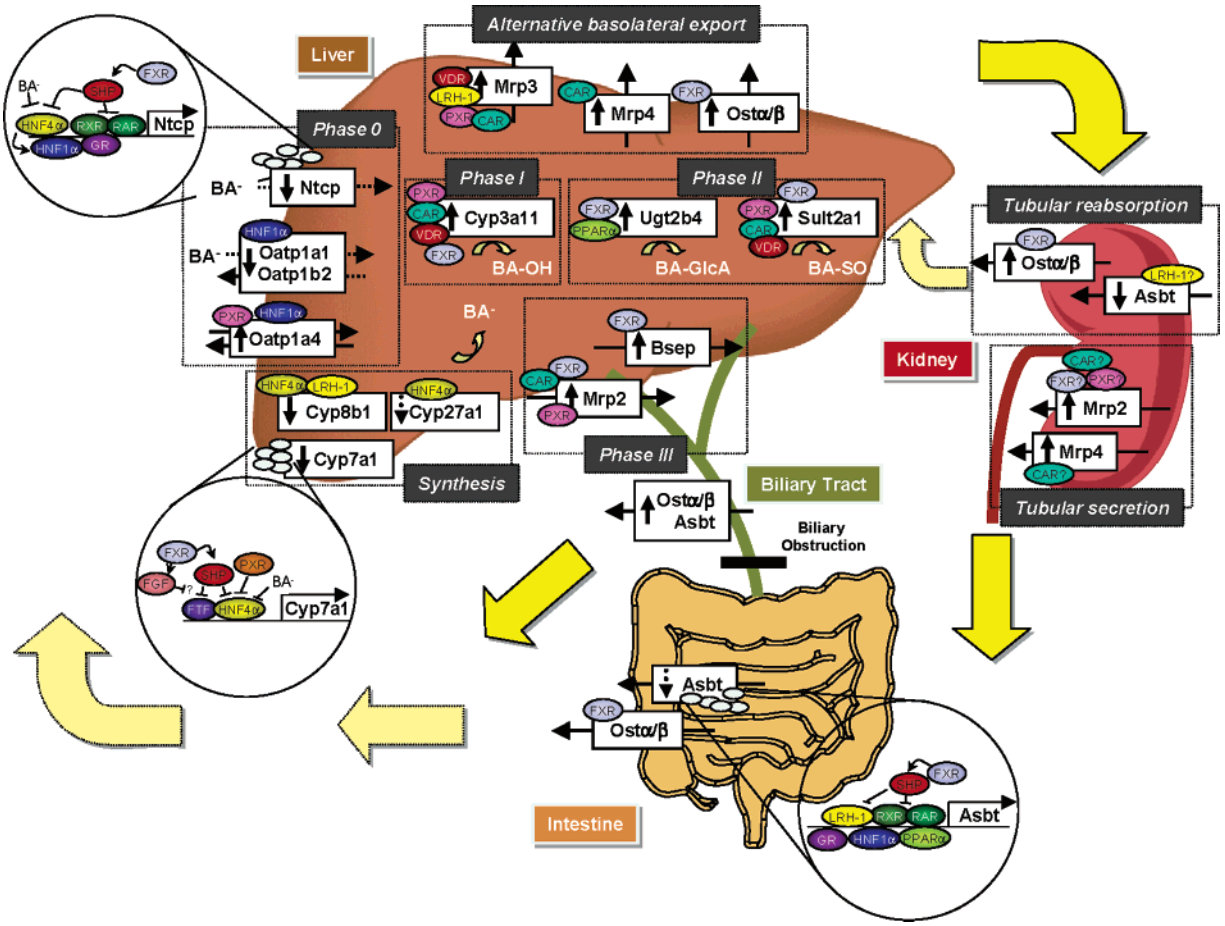


***Figure 2.*** Role of nuclear receptors for adaptive defense mechanisms against bile acid toxicity in liver, kidney, and intestine. Hepatocellular bile acid accumulation during cholestasis is limited by reducing basolateral (phase 0) bile acid uptake (downregulation of the uptake systems Ntcp, Oatp1a1, and Oatp1b2) and by induction of phase III "orthograde"/canalicular export pumps (Bsep, Mrp2) and ''retrograde"/alternative basolateral efflux pumps (Mrp3, Mrp4, Ostα/β). Furthermore, bile acid synthesis (Cyp7a1, Cyp27a1, and Cyp8b1) is repressed. Phase I hydroxylation (BA-OH) and phase II conjugation with sulfate (BA-$SO_4$) or glucuronidate (BA-GlcA) are increased during cholestasis and render bile acids better water soluble and less toxic. These conjugates are rapidly excreted by Mrps and probably also by Ostα/β into systemic circulation and are eliminated via the kidney. Reabsorption of bile acids in proximal renal tubuli is reduced as a result of downregulated Asbt; passive glomerular filtration of bile acids might be also assisted by active tubular excretion via induced Mrp2 and Mrp4. Expression of Asbt and Ostα/β in cholangiocytes is increased due to ductular proliferation and promotes bile acid reabsorption from obstructed bile ducts. Multiple nuclear receptors mediate this adaptive response: Bile acid activated FXR induces Bsep, Mrp2, Ostα/β, Cyp3a11, and Ugt2b4 and represses Ntcp, Cyp7a1, Cyp8b1, and Asbt indirectly via induction of SHP. PXR, CAR, and VDR induce expression of multiple export systems and phase I and II detoxification systems. At least in humans, PXR seems to be involved in repression of CYP7A1. Bile acids can activate FXR, PXR, and VDR, while CAR has not yet been demonstrated to be a bile acid receptor. CAR is activated by bilirubin, which is also retained during cholestasis. However, these endogenous changes are not sufficient to prevent cholestatic liver injury, and additional stimulation of specific nuclear receptors might further decrease liver toxicity. Please note that, for simplicity, regulation of human and rodent genes is not displayed separately. This scheme is focused on rodent genes; however, some of the displayed regulatory mechanisms have only been described in humans (see text for details). A detailed listing of nuclear receptors and their human and rodent targets is given in Table 1. With the exception of SHP and HNF4α, all nuclear receptors shown in this scheme function as heterodimers with RXR. For simplicity, however, RXR is not shown in the figure.

stimulated under this condition markedly repress nuclear receptors and thus overrule adaptive, secondary bile acid effects.[80−82]

**(B) Hepatic Bile Acid Uptake (Phase 0).** Limiting hepatic bile acid uptake during cholestasis may be considered a protective mechanism to reduce hepatocellular bile acid overload (Figure 2). Expression of the main basolateral bile acid uptake system NTCP/Ntcp is reduced in human cholestatic liver diseases[83−86] and in rodent models of cholestasis

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and bile acid overload.[87−94] Regulation of NTCP/Ntcp by bile acids is complex and differs considerably among humans, mice, and rats.[95] An important activator of the rat *Ntcp* gene is the nuclear receptor heterodimer formed by retinoid X receptor alpha:retinoic acid receptor alpha (RXRα:RARα).[96−98] It has been proposed that induction of a nuclear repressor, short heterodimer partner (SHP/NR0B2), by bile acid activated FXR interferes with RXRα:RARα mediated activation of the rat *Ntcp* promoter.[99] Other transactivators mediating repressive bile acid effects on rodent *Ntcp* are the NR HNF4α[95,100] and the liver-enriched transcription factor HNF1α,[97,101] which is highly dependent upon HNF4α.[102−105] Bile acids can directly repress *HNF4*α transcription.[102,106,107]


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Moreover, bile acid induced SHP inhibits the transcriptional activity of HNF4α.[102,108] Thus, RXRα:RARα and HNF4α are both targets for SHP-mediated repression of the rat *Ntcp* promoter (Figure 2). However, the role of SHP in *NTCP/Ntcp* regulation has recently been questioned, since SHP had no effect on *NTCP/Ntcp* promoter activity[95] and *Ntcp* downregulation in bile acid fed SHP knockout mice was maintained.[109] Potential SHP-independent mechanisms involve activation of the c-Jun N-terminal kinase signaling pathway by bile acids,[109] which leads to RXRα phosphorylation and subsequently to reduced binding of RXRα:RARα to the rat *Ntcp* promoter.[98] Nevertheless, FXR seems to play a major role in *Ntcp* regulation at least in mice, since CA-fed and bile duct ligated FXR knockout mice fail to downregulate *Ntcp*.[18] The human *NTCP* promoter does not contain the rat RXRα:RARα and HNF4α response elements.[95] SHP acts independent of RXRα:RARα and suppresses glucocorticoid receptor mediated activation of human *NTCP*,[110] which might account for *NTCP* downregulation in human cholestatic liver diseases.[83−86]

The organic solute transporter OATP1B1 (gene symbol *SLC01B1*, formerly known as OATP-C) is the predominant sodium-independent bile acid uptake system in humans.[111] Similar to NTCP/Ntcp, repression of *OATP1B1* in cholestatic liver diseases might also be mediated by FXR.[84−86,112] FXR activates a multistep regulatory cascade involving SHP, HNF4α, and HNF1α, the latter being a strong activator of the *OATP1B1* promoter.[102] The human *HNF1*α gene contains a binding site for the nuclear receptor HNF4α,[102] and activity of HNF4α is negatively modulated by SHP.[102,108] Alternatively and without the involvement of FXR and SHP, bile acids can decrease HNF4α nuclear binding activity and can repress the HNF4α gene promoter, thus leading to downregulation of HNF4α-activated genes.[102,106,107]

OATP1B3 (*SLC01B3*, formerly known as OATP8) is a multispecific uptake system for organic anions, xenobiotics, and peptides,[113,114] but the data concerning bile acid transport remain controversial.[2] *OATP1B3* is transactivated by FXR.[115] The purpose of FXR-mediated induction of *OATP1B3* gene expression under cholestatic conditions when other uptake systems are repressed could be maintaining hepatic uptake of xenobiotics and peptides. This could preserve the liver's metabolic functions in xenobiotic disposal even during cholestasis. Alternatively, OATP1B3 could function as a basolateral export system, since OATPs act as anion exchangers, and bidirectional transport of bromosulfophthalein and taurocholate has been observed.[116−118] Oatp1a4/*Slco1a4* (formerly known as Oatp2/*Slc21a5*) is positively regulated by LCA in a PXR-mediated fashion.[69,119] The detailed mechanisms regulating human and rodent *OATP/Oatp* gene expression are reviewed elsewhere in detail.[57,120]

Taken together, accumulating bile acids induce a negative feedback inhibition of their uptake by activation of a complex regulatory cascade involving HNF1α, HNF4α, and RXRα:RARα via FXR/SHP-dependent and independent mechanisms.

**(C) Bile Acid Hydroxylation (Phase I).** Bile acid hydroxylation renders bile acid more hydrophilic, less toxic, and more amenable for urinary excretion as reflected by markedly elevated concentrations of (poly-)hydroxylated bile acids in urine of patients with cholestatic diseases.[121−125] Bile acids as well as several drugs and xenobiotics are metabo-

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lized by CYP3A4 converting them to more hydrophilic compounds that can be eliminated more easily from the body.[56,126,127] CYP3A4 represents an important pathway for bile acid detoxification by catalyzing bile acid hydroxylation at the 6α, 1$\beta$, and C22 positions.[126,127] Expression of CYP3A4 is regulated by PXR,[68,69,128,129] VDR,[70,130] CAR,[131−133] and FXR[134] (Figure 2), and the administration of ligands for these receptors such as xenobiotics, drugs, but also bile acids can induce CYP3A4 expression.[56,135] Thus, bile acids—being both activators and substrates of CYP3A4—can initiate a feedforward mechanism aimed at minimizing hepatocellular damage caused by bile acid toxicity. Administration of hydrophobic LCA leads to induction of Cyp3a11 (the rodent homologue of human CYP3A4) in wild-type but not in PXR knockout mice, and lack of Cyp3a11 induction is associated with increased LCA-induced liver injury.[68,69] CYP3A4 induction by LCA in the intestine is also mediated by VDR, which is more sensitive to this hydrophobic bile acid and its metabolites than other nuclear receptors.[70] FXR ligands (i.e., CDCA and GW4046) also induce CYP3A4 expression in a PXR-independent fashion.[134] Accumulating bile acids in mouse models of obstructive cholestasis also lead to a feedforward induction of Cyp3a11[136] and of CYP3A4 in humanized transgenic mice.[137] However, FXR is not required for upregulation of Cyp3a11 in bile acid challenged[17,18] and common bile duct ligated (CBDL) mice.[136] On the contrary, FXR-deficient CBDL mice have even higher levels of Cyp3a11 and increased bile acid hydroxylation rates.[136] Thus, bile acids can induce a feedforward mechanism leading to bile acid hydroxylation by activation of PXR, VDR, FXR, and possibly CAR; however, the relative contribution of each NR still remains to be determined.

**(D) Bile Acid Conjugation (Phase II).** Besides hydroxylation, conjugation of bile acids with sulfate or glucuronidate is an important mechanism of bile acid detoxification. Dehydroepiandrosterone-sulfotransferase (SULT2A1/Sult2a1) catalyzes sulfoconjugation of a broad range of endogenous compounds including bile acids.[138−141] Upon sulfation, SULT2A1 substrates become polar, water soluble, and less toxic. As such, sulfated LCA is less cytotoxic than LCA

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when exposed to cells or animals[142] and is more amenable for rapid excretion.[143] The protective role of Sult2a1 induction has been demonstrated in mice treated with LCA.[144] Bile acid sulfation occurs under cholestatic conditions as reflected by the appearance of sulfated bile acids in serum and urine of patients with cholestatic liver diseases.[122,145−147] Nuclear receptors involved in the regulation of *Sult2a1* expression include FXR, PXR, VDR, and CAR (Figure 2).[148−153] These receptors bind to the same inverse repeat (IR)-0 element within the rodent *Sult2a1* gene promoter. Therefore, it is attractive to speculate that bile acid activated NRs lead to a feedforward induction of Sult2a1 thus reducing liver toxicity. CAR seems to play a central role in regulating bile acid sulfation. As such, CAR transgenic mice are resistant against LCA toxicity which can be attributed to increased LCA sulfation.[151] Furthermore, CAR was proposed to orchestrate bile acid sulfation and subsequent basolateral export, since activation of CAR also leads to overexpression of the basolateral export pump Mrp4, which is capable of transporting steroid sulfates.[152]

Glucuronidated bile acids represent up to 8% of the bile acid pool in the plasma of cholestatic patients, whereas in urine the proportion of these metabolites may increase to 35% of total bile acids.[123,154,155] Glucuronide conjugation consists of the transfer of the glucuronosyl group from uridine diphosphate (UDP)-glucuronic acid to the acceptor molecule[156] rendering it better water-soluble. Glucuronidation of bile acids is catalyzed by the UDP-glucuronosyltransferases UGT2B4 and UGT2B7[156,157] and is an almost selective conjugation pathway for 6α-hydroxylated bile acids such as hyocholic acid and hyodeoxycholic acid[158,159] that are formed from LCA and CDCA by CYP3A4.[68,69] In humans, the combined hydroxylation/glucuronidation detoxification pathway can be stimulated by the PXR ligand rifampicin.[160] An important consequence of bile acid glucuronidation is the introduction of an additional negative charge in the molecule that allows their transport by conjugate export pumps including MRP2/Mp2 and MRP3/Mrp3.[6,161] Alternative basolateral excretion via MRP3 may


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explain the appearance of these more hydrophilic compounds in urine of patients with cholestasis.

Bile acids can induce human UGT2B4 via activation of FXR.[162] *UGT2B4* is the only gene described so far to be activated by FXR through binding of an FXR monomer to a single hexameric DNA motif. FXR can activate *UGT2B4* transcription without its common heterodimeric partner RXR; activation of RXR by its ligands in fact inhibits DNA binding by FXR. RXR ligands might promote the formation of FXR:RXR dimers, thus reducing the pool of monomeric FXR capable of binding to its response element within the *UGT2B4* promoter.[162] The *UGT2B4* gene promoter also contains a peroxisome proliferator activated receptor (PPAR) response element and is activated by the PPARα agonist fenofibrate.[163] Molecular evidence for a cross-talk between the FXR and PPARα transcriptional pathways is provided by identification of PPARα as an FXR target gene.[164] Thus, bile acids can induce UGT2B4 expression directly via activation of FXR and indirectly via FXR-dependent induction of PPARα, which then activates *UGT2B4* transcription (Figure 2).

In contrast to UGT2B4, UGT2B7 seems to be repressed by bile acids such as LCA and CDCA in vitro.[165] *UGT2B7* promoter transfection experiments in this study showed that LCA-activated FXR decreased *UGT2B7* promoter activity via a negative FXR response element. The role of bile acid challenge in vivo on UGT2B7 expression still remains to be determined.

Taken together, bile acids can induce their own phase I and II detoxification in a feedforward fashion by activating PXR, VDR, and FXR. CAR also plays a central role in regulation of bile acid metabolism (Figure 2). However, whether bile acids themselves or other substances retained during cholestasis (e.g., bilirubin) activate this particular NR still has to be determined.

**(E) Repression of Bile Acid Synthesis.** Repression of bile acid synthesis is part of the adaptive response to cholestasis. Transcriptional regulation of CYP7A1, mediating the rate-limiting step in bile acid synthesis, occurs via a bile acid response element (BARE) located within the proximal promoter region.[166,167] The *CYP7A1* BARE can bind monomeric FTF (fetoprotein transcription factor, also known as liver receptor homologue, LRH-1)[168−170] and homodimeric HNF4α.[171,172] Moreover, bile acids repress *CYP7A1* via activation of FXR and induction of SHP, which in turn negatively interacts with FTF and suppresses *CYP7A1* gene transcription[169,170] (Figure 2). However, multiple redundant pathways regulate *CYP7A1*, and SHP is not the only mediator of CYP7A1 repression as demonstrated in SHP knockout mice.[109,173] Bile acids can also impair HNF4α-mediated activation of the *Cyp7a1* promoter by blocking the recruitment of the coactivator PGC-1α (peroxisome proliferator activated receptor gamma coactivator) and CBP (cAMP response element binding protein-binding protein).[174] Furthermore, CDCA can directly decrease *HNF4α* promoter activity and gene expression.[102,106,107] Rifampicin-activated PXR interacts with HNF4α and reduces interaction of PGC-

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1α with HNF4α thus leading to inhibition of human *CYP7A1* gene transcription in vitro.[175] However, in recent in vivo studies rifampicin did not significantly reduce *CYP7A1* expression or bile acid synthesis.[176,177] Thus, the role of this PXR-dependent pathway in bile acid mediated CYP7A1 repression still remains to be determined. Another recent in vitro study with primary human hepatocytes showed that FXR induced expression of fibroblast growth factor (FGF) 19, a secreted protein that represses *CYP7A1* through a c-Jun N-terminal kinase (JNK) dependent pathway.[178] FGF19 selectively binds to FGF receptor 4 (FGFR4), a transmembrane receptor with tyrosine kinase activity.[179] FGF15, the mouse homologue of human FGF19, is induced in the small intestine in an FXR-dependent fashion and signals from intestine to liver to repress Cyp7a1 through a mechanism involving FGFR4.[180] These studies implicate an additional gut−liver signaling pathway in the regulation of bile acid homeostasis.

CYP27A1 (catalyzing the first step in the alternative pathway of bile acid synthesis) and CYP8B1 (controlling the ratio of CDCA to CA) harbor a binding site for HNF4α in their gene promoters.[106,107,181] In addition, the *CYP8B1* promoter also contains a binding site for LRH-1[182] (Figure 2). While bile acids can repress CYP8B1 via FXR/SHP and, similar to CYP7A1, via FXR-independent pathways, their suppressive effect on CYP27A1 is less potent. The detailed mechanisms regulating bile acid biosynthesis are reviewed elsewhere.[183]

Taken together, bile acids repress their own synthesis via multiple redundant pathways involving FXR/SHP, HNF4α, and LRH-1 (FTF) (Figure 2). Moreover, a gut−liver axis exists to control bile acid synthesis.

**(F) Bile Acid Excretion (Phase III). (i) Canalicular Bile Acid Excretion.** Bile acid export at the canalicular membrane of hepatocytes is mediated by BSEP/Bsep and by MRP2/Mrp2. Expression of these transporters must be tightly controlled to prevent bile acid accumulation within the hepatocyte. As such, genetic defects of *BSEP* cause severe liver disease in humans called progressive familial intrahepatic cholestasis (PFIC type 2), which leads to irreversible liver damage due to intrahepatic bile acid accumulation.[184] Bile acids can induce their own efflux into bile by increasing both Bsep and Mrp2 expression as observed in mice fed CA or UDCA.[93,185] FXR mediates induction of BSEP/Bsep expression in response to bile acids (Figure 2). Human, rat, and mouse *BSEP/Bsep* promoters are transcriptionally activated by FXR,[186−188] and bile acids increase BSEP expression in primary human hepatocytes or HepG2 cells with the same rank order of potency that activates FXR.[17] Moreover, baseline Bsep expression is reduced in FXR knockout mice, and Bsep induction by bile acids is absent.[17,185,189]

The rat *Mrp2* promoter contains a response element for RXRα:RARα, which mediates Mrp2 induction by rexi-

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noids.[96] FXR also binds with high affinity with RXRα to response elements in the rat *Mrp2* promoter that are shared with CAR and PXR[190] (Figure 2). Thus, bile acids as well as CAR and PXR ligands induce rodent Mrp2 expression,[74,185,190−195] and human MRP2 is induced by FXR and PXR ligands as well.[190] In line, Mrp2 induction by CA is absent in mice lacking PXR.[196] However, the role of FXR for regulation of murine Mrp2 is questioned by the fact that CA is still able to induce Mrp2 expression in FXR knockout mice.[185]

These findings suggest that, while regulation of canalicular bile acid excretion via BSEP/Bsep is directly linked to FXR, regulation of MRP2/Mrp2 is more complex and involves multiple NRs (i.e., FXR, PXR, and CAR) (Figure 2).

**(ii) Basolateral (Alternative) Bile Acid Excretion.** While bile acids are normally excreted into bile, alternative basolateral bile acid excretion into portal blood may become a major way for hepatic bile acid elimination during cholestasis. Alternative basolateral bile acid export is mediated by members of the MRP/Mrp family (e.g., MRP3/Mrp3 and MRP4/Mrp4) and the recently characterized heteromeric bile acid transporter, the organic solute transporter OSTα/OST*β*.[25] These export systems are normally expressed at very low levels at the basolateral membrane but are dramatically upregulated after bile acid feeding and in experimental cholestasis in rodents as well as in human cholestatic liver diseases.[17,18,31,40,85,86,93,197−200] Because MRP3/Mrp3, MRP4 and Ostα/Ost*β* are able to transport sulfated and glucuronidated bile acids that are eliminated into urine during cholestasis, the induction of these transporters may explain the shift toward renal excretion of bile acids as a major mechanism for bile acid elimination in patients with chronic, long-standing cholestasis.[44,122−124,201]

A putative bile acid response element has been identified in the promoter region of human MRP3 isolated from Caco-2 cells.[202] In this study, CDCA stimulated MRP3 mRNA 3-fold in a dose- and time-dependent manner. Bile acid response elements included two α-1 FTF-like elements.[202] More recently, a vitamin D receptor response element (VDRE) has been identified in the murine Mrp3 promoter mediating activation by 1,25-dihydroxy vitamin $D_3$ and LCA.[203] Treatment of mice in vivo with LCA or 1,25-dihydroxy vitamin $D_3$ resulted in Mrp3 induction in the colon whereas the lack of hepatic Mrp3 induction by VDR ligands was attributed to low VDR levels in the liver.[203]

Overexpression of both Mrp3 and Mrp4 after common bile duct ligation or bile acid feeding is independent of FXR,[17,19,185] and it has been speculated that the inducing effects of bile acids on Mrp3 and Mrp4 expression could be mediated by PXR.[17] Administration of PXR ligands resulted only in overexpression of MRP3/Mrp3[191,193,204,205] but not of Mrp4,[191,204] making a role for PXR in Mrp4 regulation less likely. CAR, however, seems to be a central regulator for both Mrp3 and Mrp4 expression, since CAR ligands induced

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Mrp3[119,191,192,204,206] and MRP4/Mrp4 expression.[152,191,204] Furthermore, promoter studies in CAR knockout mice demonstrated that CAR transcriptionally activates the *Mrp4* gene promoter.[152]

The recently identified bile acid transporter OST$\alpha$/$\beta$ is transactivated by FXR. Two functional FXR binding motifs were identified in the human *OST*$\alpha$ gene, and one in the *OST*$\beta$ gene (Figure 2). Moreover, baseline Ost$\alpha$/Ost$\beta$ expression was reduced in FXR knockout mice compared to their wild-type littermates, and induction of Ost$\alpha$/Ost$\beta$ after bile duct ligation, administration of an FXR ligand, and CA was abolished.[18,207,208]

Thus, a complex picture is emerging where multiple nuclear receptors (including FXR, PXR, VDR, and CAR) are required for coordination of adaptive basolateral bile acid efflux under bile acid load and cholestatic conditions (Figure 2). Bile acids regulate their efflux into bile and into portal blood. Basolateral excretion is an alternative way of bile acid elimination in order to protect hepatocytes from accumulation of toxic bile acids. Induction of this adaptive pathway together with increased phase I and II bile acid metabolism explains the shift toward renal bile acid elimination in patients with longstanding cholestasis.[122,145−147] While FXR, PXR, and VDR (at least in the intestine) are receptors for bile acids, activation of CAR by bile acids has not yet been demonstrated. Other substances accumulating during cholestasis such as bilirubin, which has been demonstrated to be a CAR activator,[72,73] might explain overexpression of CAR target genes during cholestasis.

**(G) Cholangiocellular, Intestinal, and Renal Bile Acid Transport.** Increased cholangiocellular Asbt expression in CBDL rats may primarily be attributed to bile duct proliferation,[45] which probably overrules any potential transcriptional effects on *Asbt* gene expression. Enhanced Asbt expression may facilitate removal of bile acids from stagnant bile in the biliary lumen during bile duct obstruction. The same applies for the basolateral export systems Mrp3 and Ost$\alpha$/Ost$\beta$, which are also overexpressed during CBDL due to bile duct proliferation.[31,208]

The vast majority of bile acids secreted into bile and intestine are reabsorbed and subsequently delivered to portal circulation. The data on regulation of ileal Asbt by bile acids are conflicting.[209−211] Some of the divergent results can be attributed to species differences, since the mouse but not the rat *Asbt* promoter harbors an LRH-1 binding site.[212] Negative feedback regulation of murine *Asbt* by bile acids is mediated by FXR via SHP-dependent repression of LRH-1 activation of the *Asbt* promoter.[212] Factors involved in the regulation of human *ASBT* include HNF1$\alpha$, PPAR$\alpha$, GR, and RXR$\alpha$:RAR$\alpha$.[213−215] Bile acids exert their negative effects on *ASBT* via an FXR- and SHP-dependent mechanism upon RXR$\alpha$:RAR$\alpha$ activation of *ASBT*.[213] Whether ileal bile acid absorption in cholestasis in humans is positively or negatively regulated remains controversial. Patients with primary biliary cirrhosis (PBC) had increased ileal bile acid absorption,[216] while a recent study demonstrated reduced ASBT expression in obstructive cholestasis.[217] Whether bile acids from the intestinal lumen (low levels in obstructive cholestasis)

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or from the systemic circulation (elevated levels in cholestasis) influence ASBT expression still remains to be determined.

Urinary bile acid elimination is increased in various human cholestatic liver diseases and in animal models of cholestasis.[45,122−124,136,145−147,155,201] This is achieved by reduced reabsorption and probably also by increased active excretion in proximal tubular epithelial cells. CBDL leads to Asbt repression in rat kidney;[45] however, the mechanisms regulating rat Asbt still remain to be determined. Bile acid feeding and CBDL lead to overexpression of apical Mrp2 and Mrp4 in kidney similarly to liver. Of note, renal Mrp4 is localized to the apical membrane, while in the liver, Mrp4 is localized to the basolateral membrane of hepatocytes.[20,47] One might speculate that active excretion of bile acids via these export pumps may facilitate urinary bile acid elimination. However, the role of these transporters in the renal elimination of bile acids remains unclear.

## 3. Therapeutic Implications

To date, treatment options for cholestatic diseases are limited. Multilevel approaches are needed to minimize bile acid induced liver injury. Treatment strategies should aim at stimulation of defective transporter expression and function and should support bile acid detoxification and alternative elimination. Understanding the regulation of bile acid transport and metabolism under physiological and cholestatic conditions is a prerequisite for the development of novel treatment options customized to the particular requirements of specific cholestatic disorders. Therapeutic strategies may therefore be aimed at NRs and their target genes that affect "orthograde" biliary excretory routes, bile acid phase I and II detoxification systems, but also ''retrograde" alternative/basolateral overflow and renal elimination systems that aid to clear these metabolites from plasma. This coordinated stimulation of hepatocellular detoxification and elimination of biliary constituents should ameliorate cholestatic liver injury.

UDCA stimulates the expression and function of hepatobiliary transporters and enzymes involved in bile acid synthesis and detoxification at multiple transcriptional and posttranscriptional levels.[218−220] While UDCA proves to be effective in the treatment of human cholestatic liver diseases, most of the knowledge on its mechanisms of action was obtained from experiments in rodents. As such, UDCA stimulates the overall gene expression of both canalicular (Mrp2, Bsep) and alternative basolateral efflux pumps (Mrp3, Mrp4) in mouse liver.[18,93,185,221,221] Moreover, UDCA also stimulates renal (Mrp2, Mrp4) and intestinal (Mrp2, Mrp3) efflux pumps in mice, changes that may coordinately result in an increased overall elimination capacity for potentially toxic biliary constituents from the body.[185] Induction of CYP3A4/Cyp3a11 in primary human hepatocytes and in mouse liver by UDCA has recently been demonstrated.[17,18] However, in vivo, UDCA is only a weak inducer of human CYP3A4, as indicated by the formation of 1$\beta$-hydroxy DCA and 4$\beta$-hydroxycholesterol,[220,222] in particular when compared to rifampicin.[176] In contrast to these studies in healthy humans, in patients with PBC, no significant effects of UDCA on CYP3A-dependent steroid metabolism could be found.[176,223] While having only moderate effects on CYP3A4 expression in otherwise healthy human gallstone patients, UDCA markedly enhanced expression of BSEP, MDR3, and MRP4.[176] Besides induction of bile acid export and detoxification, UDCA has also been reported to repress CYP7A1/Cyp7a1, the key enzyme in bile acid synthesis in vitro.[18,224,225] However, UDCA administration to healthy gallstone patients did not cause *CYP7A1* downregulation.[176] Although UDCA changes gene expression at a transcriptional level, no definite nuclear receptor has been elucidated. FXR, PXR, and the glucocorticoid receptor may mediate some of the UDCA actions,[17,224,226] but most of the transcriptional transporter effects are independent of FXR.[18,185]

Pharmaceutical compounds targeting FXR have been proposed as promising therapeutical approaches[227−230] since studies in rodents revealed that FXR is an important factor determining liver injury in cholestasis.[19,189] Expression of

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FXR is reduced in diverse rodent models of cholestasis, especially after endotoxin challenge or CBDL.[82,231] Moreover, hereditary forms of cholestasis are associated with reduced FXR expression, and mutations in FXR target genes such as *BSEP* lead to progressive familial intrahepatic cholestasis (PFIC type 2).[184,232] Experiments with synthetic FXR agonists in cholestatic rodent models have been promising and resulted in reduced biochemical and histomorphological markers of liver injury in some models.[227,228,230] Reduced basolateral bile acid uptake via repression of Ntcp and Oatp 1 and 4, increased canalicular bile acid secretion via Bsep and Mrp2, and reduced bile acid biosynthesis through downregulation of Cyp7a1 and Cyp8b1 can be achieved by treatment with synthetic and naturally occurring (i.e., bile acids) FXR ligands; however, synthetic FXR ligands do not display bile acid toxicity. It should be noted that FXR-mediated stimulation of canalicular bile flow via induction of Bsep could aggravate liver injury as observed in UDCA-fed mice with obstructive cholestasis.[19,233] UDCA induced Bsep expression and bile flow in these animals leading to increased biliary pressure with rupture of cholangioles causing bile infarcts and an increased mortality.[233] Unfortunately, many clinically relevant chronic cholestatic liver diseases such as primary sclerosing cholangitis (PSC) have a significant obstructive component implying a cautious use of FXR agonists in advanced disease stages. FXR also regulates the human phospholipid export pump MDR3, which may protect bile ducts by increasing phospholipid concentration in bile.[234] Besides modulation of bile acid transport and metabolism, FXR ligands have recently been demonstrated to have antifibrotic properties. Activation of the FXR/SHP cascade negatively regulates hepatic stellate cells leading to resolution of liver fibrosis.[235] In addition, FXR might also exert its antifibrotic properties via activation of PPAR$\gamma$, which reduces hepatic stellate cell activation.[236] FXR was also proposed as a novel therapeutic target for treating or preventing cholesterol gallstone disease, since pharmacological activation of FXR increases cholesterol solubility by enhancing biliary bile acid and phospholipid concentrations.[237] Although some compounds have been tested in animal models of cholestasis, these FXR agonists are not yet available for clinical use.

Long before knowing their exact mode of action, ligands for PXR (e.g., rifampicin) and CAR (e.g., phenobarbital and Yin Chin, a traditional Chinese herbal decoction) have been used for treatment of jaundice and pruritus in cholestatic liver diseases.[238−244] However, in most cases effects on liver function tests in human cholestasis were only moderate, and

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hepatotoxic side effects have been observed.[241−243,245−249] In rodents however, activation of PXR has been demonstrated to counteract LCA-induced liver toxicity. This was attributed to increased phase I and II metabolisms of LCA (i.e., hydroxylation via Cyp3a11 and sulfation via Sult2a1 and 3′-phosphoadenosine 5′-phosphosulfate synthase 2 (PAPSS2), an enzyme that generates the donor cofactor (PAPS) for the reaction)[68,69,150] and to downregulation of bile acid biosynthesis via repression of Cyp7a1. Moreover, PXR also induced the export pump MRP2/Mrp2[151,190] and administration of rifampicin led to MRP2 overexpression in otherwise healthy gallstone patients.[176] Mrp2 induction together with increased glucuronidation of bilirubin via PXR-induced UGT1A1/Ugt1a1[176,250] enhances bilirubin detoxification.[251]

Recently, 6,7-dimethylesculetin, the main active compound present in a herbal decoction (Yin Chin) used in traditional Chinese medicine to treat and prevent neonatal jaundice, has been identified as a CAR activator.[244] This substance and other CAR agonists coordinately regulate the bilirubin clearance pathway including uptake (Oatp1a4/Sclo1a4), glucuronidation (Ugt1a1), and excretion (Mrp2).[73,190] In addition to its effects on bilirubin metabolism, CAR stimulates expression of CYP3A4/Cyp3a11, SULT/Sult2a1, and PAPSS2 similarly to PXR, suggesting a coordinated regulation of this important bile acid detoxification pathway by both of these nuclear receptors.[74,151,152,191,206] Besides stimulation of bile acid detoxification, CAR also stimulates expression of the alternative export pumps Mrp3 and Mrp4,[119,152,191,192,204,206] which both are able to transport bile acids and their sulfate conjugates.[12−14,21] The functional relevance of these pathways is reflected by reduced liver injury in LCA-fed mice after CAR activation.[151,206]

While many studies showed that LCA-induced liver damage can be ameliorated by activation of PXR and CAR, not much is known about whether these strategies can also be applied in other models of cholestasis. Administration of PXR and CAR ligands to bile duct ligated mice markedly reduced serum bile acid levels and increased renal bile acid elimination.[191] In serum and urine of these animals, relative levels of polyhydroxylated bile acids were increased, which is in line with induction of phase I hydroxylation. Despite an improvement of cholestasis in this study, markers of liver injury were increased, possible caused by accumulating PXR and CAR agonists in biliary obstruction.[191] Although allowing a proof of principle, future studies are needed in other models to determine the safety of these agents. It has to be kept in mind that stimulating these receptors may interfere with various other metabolic pathways. Use of rifampicin in patients with cholestasis can be associated with liver toxicity in rare cases eventually causing liver failure,[246] and chronic CAR activation may promote hepatocarcinogenesis.[252,253]

Although not yet tested in models of cholestasis, application of VDR agonists might also have beneficial effects in cholestasis. The role of VDR ligands for cholestatic disorders remains to be determined, since, in normal liver, VDR expression is lower in hepatocytes than in nonparenchymal and biliary epithelial cells.[254]

Another therapeutic strategy in the treatment of cholestasis may represent the use of agonists for the peroxisome proliferator activated receptor alpha (PPARα). Cholesterol-lowering drugs such as fibrates and inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A reductase ("statins") are PPARα ligands,[255,256] and administration of these substances to patients with primary biliary cirrhosis resulted in a beneficial

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effect on liver function tests.[257−262] This could at least in part be explained by stimulation of the biliary phospholipid excretion pump Mdr2, since phospholipids protect the bile duct epithelium from detergent bile salts by formation of mixed micelles.[263−267] In addition, fibrates, but not statins, repress CYP7A1.[268−271] For the latter agents, pleiotropic antiinflammatory effects may contribute to improvements of surrogate parameters of cholestasis.[272] PPAR$\gamma$ agonists might also be of use in cholestasis, since these agonists inhibit hepatic stellate cell activation and counteract liver fibrosis in cholestasis.[236,273,274]

## 4. Summary and Outlook

Bile acid synthesis, metabolism, and transport are tightly regulated via a complex network involving NRs and hepatocyte-enriched transcription factors to maintain bile acid homeostasis. Through binding to specific nuclear receptors, bile acids can repress hepatic uptake and synthesis and induce phase I and II detoxification and subsequently export. However, these adaptive response mechanisms are not always sufficient to prevent bile acid toxicity in cholestasis. Beneficial effects of various synthetic NR ligands have been demonstrated in animal models of cholestasis augmenting the intrinsic (i.e., bile acid activated) adaptive pathways. Some NR ligands have also been used in human cholestatic liver disease. However, the therapeutic effects were not as convincing as in rodent models. Our increasing understanding of the molecular regulation of transport and detoxification systems should lead to the development of more specific and powerful therapies targeting NRs for the treatment of cholestatic liver disease.

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## Abbreviations Used

ASBT/Asbt (*SLC10A2/Slc10a2*), apical sodium-dependent bile acid transporter; BSEP/Bsep (*ABCB11/Abcb11*), bile salt

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export pump; CA, cholic acid; CAR (NR1I3), constitutive androstane receptor; CBDL, common bile duct ligation; CDCA, chenodeoxycholic acid; CYP, cytochrome P450 enzyme; CYP27A1/Cyp27a1, sterol 27-hydroxylase; CYP7A1/Cyp7a1, cholesterol 7α-hydroxylase; CYP8B1/Cyp8b1, sterol 12α-hydroxylase; DCA, deoxycholic acid; FTF (NR5A2), fetoprotein transcription factor; FXR (NR1H4), farnesoid X receptor; GR (NR3C1), glucocorticoid receptor; HNF1α (*TCF1*), hepatocyte nuclear factor 1 alpha; HNF4α (NR2A1), hepatocyte nuclear factor 4 alpha; LCA, lithocholic acid; LRH1 (NR5A2), liver receptor homologue; LXRα (NR1H3), liver X receptor; MDR1/Mdr1 (*ABCB1/Abcb1*), multidrug resistance gene 1; Mdr2 (*Abcb4*), multidrug resistance gene 2; MDR3 (ABCB4), human homologue to rodent Mdr2; MRP/Mrp (*ABCC/Abcc*), multidrug resistance associated protein; NR, nuclear receptor; NTCP/Ntcp (*SLC10A1/Slc10a1*), $Na^+$/taurocholate cotransporter; OATP/Oatp (*SLCO/Slco*), organic anion transporting peptide; OSTα/OST$\beta$/Ostα/Ost$\beta$, organic solute transporter alpha/beta; PFIC, progressive familial intrahepatic cholestasis; PPARα (NR1C1), peroxisome proliferator activated receptor alpha; PXR (NR1I2), pregnane X receptor; RARα (NR1B1), retinoic acid receptor alpha; RXRα (NR2B1), retinoid X receptor alpha; SHP (NR0B2), short heterodimer partner; SULT2A1, dehydroepiandrosterone sulfotransferase; UDCA, ursodeoxycholic acid; UGT; UDP-glucuronosyl transferase; VDR (NR1I1), vitamin D receptor.

Please note that human genes and their products are capitalized, whereas rodent genes and their products are written in lower case. Transporter genes are set in italics whereas gene products are set in roman font.

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