Bile Salt Transporters: Molecular Characterization, Function, and Regulation

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I. Normal Physiology of Bile Salt Transport

A. Basic Principles of Bile Secretion and Bile Salt Transport

Bile is a vital secretion, essential for intestinal digestion and absorption of lipids. Moreover, bile is an important route of elimination for environmental toxins, carcinogens, drugs, and their metabolites (xenobiotics). Bile is also the major route of excretion for endogenous compounds and metabolic products (endobiotics) such as cholesterol, bilirubin, and hormones (44, 262). Bile is primarily secreted by hepatocytes into minute channels arranged as a meshwork of tubules or canaliculi between adjacent hepatocytes. “Canalicular bile” accounts for ~75% of daily bile production in humans and is modified by secretory and absorptive processes as it passes along...
the bile ductules and ducts. The quantity of “ductular/ductal bile” varies with the species and responses to enteric hormonal stimuli and varies from as little as 5% in rats to as much as 25–40% of secretion in humans. Bile is further concentrated up to 10-fold in the gallbladder before reaching the intestine except for species like the rat where the gallbladder is absent (44, 262).

Bile secretion is an osmotic process driven predominantly by active excretion of organic solutes into the bile canaliculus, followed by passive inflow of water, electrolytes, and nonelectrolytes (e.g., glucose) from hepatocytes and across semipermeable tight junctions (42, 332). The main organic solutes of bile are bile salts, phospholipids, and cholesterol, which form mixed micelles in bile. The vectorial excretion of bile salts from blood into bile represents the major driving force for bile flow (“bile salt-dependent” bile flow). The human bile salt pool size is ~50–60 μmol/kg body wt and averages 3-4 g. Most of the bile salt pool is stored in the gallbladder during the fasting state (139, 210, 245). Canalicular excretion of reduced glutathione (GSH) and bicarbonate (HCO_3^-) constitute the major components of the “bile salt-independent” fraction of bile flow. However, HCO_3^- secretion occurs mainly at the level of bile duct epithelial cells (cholangiocytes), in response to stimulation by hormones and neuropeptides such as secretin, vasoactive intestinal peptide, bombesin, etc. (22, 39, 167).

Many organic biliary constituents including bile salts, cholesterol, and phospholipids are reabsorbed with high efficiency after reaching the small intestine and recirculate via the portal blood back to the liver. Thus the human bile salt pool circulates 6–10 times per day, resulting in a daily bile salt excretion of 20–40 g. Despite a high degree of intestinal bile salt conservation, 0.5 g of bile salts are lost through fecal excretion and must be replaced by de novo bile salt synthesis, which thus contributes to only 3–5% of the bile salts that are excreted into bile (139, 210, 245).

Bile salts may also undergo “cholehepatic shunting” from the bile duct lumen, via cholangiocytes and the periductular capillary plexus. Bile salt reabsorption by cholangiocytes may contribute in part to the conservation of bile salts and the generation of a hypercholeretic bile flow (123, 395). Although this pathway probably plays a minor role under normal physiological conditions, cholehepatic shunting of bile salts may become an important escape route for bile salts under cholestatic conditions when the bile duct epithelium proliferates. Moreover, bile salt uptake into cholangiocytes and gallbladder epithelial cells may also play an important role for cell signaling in the regulation of secretary and proliferative events within the biliary tree (7, 10, 67).

Bile salts are efficiently removed from portal blood by the liver via high-affinity, low Michaelis constant (K_m) transporting polypeptides in the basolateral sinusoidal membrane. Bile salts that escape the first-pass clearance by the liver are filtered at the glomerulus and excreted into urine, where they are reabsorbed by transporters in the brush border of the proximal convoluted tubule (386).

Most hepatic transport systems are not fully expressed until shortly before/after birth (19). Thus the fetus must rely entirely on elimination of “biliary” constituents via the placenta and maternal liver. This “placenta-maternal liver tandem” is responsible for protecting the fetal organism from potentially harmful compounds.

Recent cloning studies have characterized the molecular properties of most of the hepatobiliary transport systems required for uptake and excretion of bile salts and other biliary constituents in liver and extrahepatic tissues (Table 1, Fig. 1). These studies have done much to advance our understanding of the molecular basis of bile formation (205, 210, 245, 258, 259, 342, 358, 359). However, bile formation normally depends not only on the proper function of these transport systems, but also on an intact cytoskeleton required for the movement of vesicles and bile canicular contractions, and on junctional contacts that seal off the bile canaliculi and maintain cell polarity. Signal transduction mechanisms also regulate and coordinate these various processes. Although this review is focused on the molecular mechanisms involved in bile salt transport in the liver and the enterohepatic circulation, the interested reader may wish to consult other reviews that address these cellular mechanisms (15, 44, 98, 250, 251, 286).

B. Hepatocellular Bile Salt Transport

The hepatocyte is a polarized epithelial cell with basolateral (sinusoidal) and apical (canalicular) plasma membrane domains. Bile salts are concentrated in bile by active transport systems that are arranged in a polarized fashion (Table 1, Fig. 1). Hepatic uptake of biliary constituents (and their precursors) is initiated at the basolateral membrane, which is in direct contact with portal blood plasma via the fenestrae of the sinusoidal endothelial cells and the space of Disse. After uptake into hepatocytes, bile salts and other cholephiles reach the canalicular pole by diffusion either in the aqueous cytoplasm or within intracellular lipid membranes, depending on their hydrophobicity. The canalicular membrane represents the excretory pole of the hepatocyte and forms the border of the bile canaliculus. Canalicular excretion of biliary constituents represents the rate-limiting step of bile secretion since biliary constituents are excreted against high concentration gradients into bile. The basolateral and canalicular membranes differ in their biochemical composition and functional characteristics and are separated by tight junctions that seal off the bile canaliculi and hence form the only anatomical barrier maintaining the concentration gradients between blood and bile (38, 44).
TABLE 1. Nomenclature, location, and function of the major hepatobiliary membrane transporters in liver and extrahepatic tissues

<table>
<thead>
<tr>
<th>Name</th>
<th>Abbreviation (Gene Nomenclature)</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium-taurocholate cotransporting polypeptide</td>
<td>NTCP (SLC10A1)</td>
<td>Primary carrier for sodium-dependent conjugated bile salt uptake from portal blood</td>
</tr>
<tr>
<td>Organic anion transporting proteins</td>
<td>OATPs (SLC21A)</td>
<td>Multispecific organic anion transporting proteins for sodium-independent uptake of bile salts and a broad range of other organic anions and cations</td>
</tr>
<tr>
<td>Multidrug resistance-associated protein 3*</td>
<td>MRP3 (ABCC3)</td>
<td>Multispecific organic solute transporter, weakly expressed in normal liver but highly upregulated on basolateral membrane of hepatocytes in cholestasis; capable of extruding bile salt conjugates from liver</td>
</tr>
<tr>
<td>Multidrug resistance-1 P-glycoprotein*</td>
<td>MDR1 (ABCB1)</td>
<td>ATP-dependent excretion of various organic cations, xenobiotics, and cytotoxins into bile</td>
</tr>
<tr>
<td>Multidrug resistance-3 P-glycoprotein (phospholipid transporter)*</td>
<td>MDR3 (ABCB4)</td>
<td>ATP-dependent translocation of phosphatidylcholine from inner to outer leaflet of membrane bilayer; the phospholipid export pump</td>
</tr>
<tr>
<td>Multidrug resistance-associated protein-2 (Canalicular conjugate export pump)*</td>
<td>MRP2 (ABCC2)</td>
<td>Mediates ATP-dependent multispecific organic-anion transport (e.g., bilirubin diglucuronide, sulfates, glutathione conjugates) into bile; major determinant of bile salt-independent bile flow by GSH transport</td>
</tr>
<tr>
<td>Canalicular bile salt-export pump/(sister of P-glycoprotein)</td>
<td>BSEP (SPGP) (ABCB11)</td>
<td>ATP-dependent transport of monovalent bile salts into bile; stimulates bile salt dependent bile flow</td>
</tr>
<tr>
<td>Familial intrahepatic cholestasis-1</td>
<td>FIC1 (ATP8B1)</td>
<td>Potential aminophospholipid translocating ATPase (function not known); gene defect in Byler’s disease</td>
</tr>
<tr>
<td>Chloride-bicarbonate anion exchanger isoform-2</td>
<td>AE2 (SLC4A2)</td>
<td>Acid loader: Excretes bicarbonate into bile and stimulates bile salt-independent bile flow</td>
</tr>
<tr>
<td>Ileal (apical) sodium-dependent bile salt transporter</td>
<td>ISBT (ABST) (SLC10A2)</td>
<td>Located on the luminal membrane; functions to remove bile salts from bile; may facilitate bile salt removal during cholestasis; identical gene product to ileal transporter</td>
</tr>
<tr>
<td>Cystic fibrosis transmembrane regulator</td>
<td>CFTR (ABCC7)</td>
<td>Chloride channel on the luminal membrane; facilitates chloride entry into bile for exchange with HCO₃⁻ anions; mutations in CFTR can result in cholestasis</td>
</tr>
<tr>
<td>Chloride-bicarbonate anion exchanger isoform 2</td>
<td>AE2 (SLC4A2)</td>
<td>Located on luminal membrane; facilitates bicarbonate secretion into bile and contributes to bile flow independent of bile salts</td>
</tr>
<tr>
<td>Multidrug resistance-associated protein 3*</td>
<td>MRP3 (ABCC3)</td>
<td>Expressed on basolateral membrane of cholangiocyte; may be major transporter for returning bile salts to portal circulation from bile during obstructive cholestasis</td>
</tr>
<tr>
<td>Organic anion transporting polypeptide 3</td>
<td>Oatp3 (Slc21a7)</td>
<td>Sodium-independent uptake of bile salts from intestinal lumen</td>
</tr>
<tr>
<td>Ileal sodium dependent bile salt transporter</td>
<td>ISBT (ABST) (SLC10A2)</td>
<td>Primary carrier for sodium-dependent bile salt uptake from intestine by ileum; critical determinant of enterohepatic circulation of bile salts</td>
</tr>
<tr>
<td>Multidrug resistance-associated protein 3*</td>
<td>MRP3 (ABCC3)</td>
<td>Expressed on basolateral membrane of ileum and colon; may be major transporter for returning bile salts to portal circulation</td>
</tr>
<tr>
<td>Ileal sodium dependent bile salt transporter</td>
<td>ISBT (ABST) (SLC10A2)</td>
<td>Located on the luminal membrane of the proximal tubule; functions to take up bile salts from the glomerular filtrate; downregulated in cholestasis to facilitate bile salt excretion in urine; identical gene product to the ileal transporter</td>
</tr>
<tr>
<td>Multidrug-resistance-associated protein 2*</td>
<td>MRP2 (ABCC2)</td>
<td>Located on the luminal membrane of the proximal tubule, this transporter is an ATP-dependent multispecific organic anion transporter for divalent organic anion conjugates (sulfates, glucuronide, and glutathione conjugates) into urine; this gene product is identical to the canalicular conjugate export pump and is postulated to function to facilitate renal excretion of bile salt conjugates in cholestasis</td>
</tr>
</tbody>
</table>

GSH, reduced glutathione. * These transporters are members of the ATP-binding cassette family.
1. Basolateral bile salt uptake

Basolateral bile salt transport systems are essential for bile formation since ~95% of bile salts excreted into bile by the liver are reabsorbed on each pass through the intestine and undergo an “enterohepatic circulation” (139). This process is highly efficient with first-pass extraction rates of conjugated bile salts in the range of 75–90% depending on bile salt structure and is independent of systemic bile salt concentrations (241). Unconjugated bile salts are weak acids that are uncharged at the physiological pH in plasma and thus can traverse cell membranes by passive diffusion. However, taurine or glycine conjugated bile acids require an active transport mechanism for cellular uptake (241). Hepatic uptake of bile salts occurs against a 5- to 10-fold concentration gradient between the portal blood plasma and the hepatocyte cytosol and is mediated by both sodium-dependent and -independent mechanisms (262). Most substances cleared by the liver (including bile salts) are highly protein bound to serum albumin. Because only the unbound fraction of biliary constituents enter the liver, the ligand must dissociate upon contact with the sinusoidal membrane of the hepatocyte. Therefore, efficient extraction from albumin is an important step (293). Because simple dissociation from serum albumin is too slow (100, 382), this dissociation must be facilitated at the cell membrane, although proof for an hepatocellular albumin receptor is lacking (176, 271, 383). Under physiological conditions, bile salts are removed from sinusoidal blood predominantly by zone 1 (periportal) hepatocytes (119, 159). Hepatocytes in zone 3 downstream are only recruited at higher bile salt loads (215), such as may occur postprandially or under cholestatic conditions. Thus the normal liver has considerable functional reserve capacity for bile salt uptake. There are several different transport systems involved in hepatic bile salt uptake, and these include a high-affinity Na+/H- dependent bile salt transporter Ntcp/NTCP (Slc10a1/SLC10A1) and a family of multispecific organic anion transporters (Oatps/OATPs; Slc21a/SLC21A) that can mediate Na+/H- independent bile salt uptake.

1A) Sodium-dependent uptake via Ntcp/NTCP. The Na+-dependent pathway accounts for >80% of conjugated taurocholate uptake but <50% of unconjugated cholate uptake (210, 245). Because most bile salts are conjugated, the Na+-dependent transport system Ntcp/
NTCP is the most relevant Na\(^{+}\)-dependent bile salt uptake system. Bile salt uptake via Ntcp/NTCP is unidirectional with a sodium-to-taurocholate stoichiometry of 2:1, i.e., cotransport of two Na\(^{+}\) with one taurocholate molecule, and electrogenic, i.e., it is driven by both the transmembrane Na\(^{-}\) gradient which in turn is maintained by a Na\(^{-}\)-K\(^{+}\)-ATPase and the intracellular electrical potential derived from the outward diffusions of K\(^{+}\) (210, 245).

The basolateral Na\(^{+}\)-dependent bile salt transporter, the Na\(^{-}\)-taurocholate cotransporting polypeptide (Ntcp/NTCP), has been cloned from rat, human, mouse, and rabbit liver (57, 125, 127, 198). The rat liver Ntcp (gene symbol Slc10a1) consists of 362 amino acids with an apparent molecular mass of 51 kDa, 2 NH\(_{2}\)-terminal glycosylation sites, and 7 putative transmembrane domains (14, 127, 337), the latter being unique among sodium cotransporters (389). The Ntcp gene is located on rat chromosome 6q24 and spans 16.5 kb, with 4 exons separated by four introns (210, 245). Ntcp is localized exclusively at the basolateral membrane of differentiated mammalian hepatocytes and is hepatocyte specific (14, 337). Isolation of hepatocytes results in rapid reduction of Ntcp expression and loss of hepatocellular bile salt uptake in vitro (295). Ntcp is distributed homogeneously throughout the liver lobule/acinus (337). During rat development, Ntcp can first be detected between days 18 and 21 of gestation (43). Functionally, Ntcp transports all physiological bile salts (e.g., taurocholate, glycine- and taurine-conjugated dihydroxy and trihydroxy bile salts (210, 242, 245). Antisense experiments in Xenopus leavis oocytes injected with total rat liver mRNA have revealed a 95% reduction of Na\(^{-}\)-dependent taurocholate transport (126), suggesting that Ntcp is the major if not the only Na\(^{-}\)-dependent bile salt uptake system in rat liver. Although bile salts are the major physiological substrate for Ntcp, other compounds such as estrogen conjugates (estrone-3-sulfate), bromosulfophthalain, dehydroepiandrosterone sulfate, thyroid hormones, and the drug ONO-1301 can also be transported (210, 242, 245, 349). In addition, Ntcp also mediates uptake of drugs that are covalently bound to taurocholate such as chlorambucil-taurocholate (206).

In mouse liver, two alternatively spliced Ntcp isoforms (Ntcp1, Slc10a1; Ntcp2, Slc10a2) have been cloned. Ntcp is a 362-amino acid protein, while Ntcp2 is a truncated 317-amino acid protein produced by alternative splicing (57). Both Ntcps mediate Na\(^{-}\)-dependent taurocholate uptake when expressed in oocytes. However, mRNA levels of Ntcp2 are ~50-fold lower than Ntcp1, and its functional relevance under normal and pathological conditions remains to be determined (57).

Human NTCP (SLC10A1) consists of 349 amino acids and shares 77% amino acid identity with rat liver Ntcp. The functional properties of NTCP are very similar to rat Ntcp, although it has a higher affinity for taurocholate than the rat and mouse orthologs (125). The NTCP gene has been mapped to chromosome 14q24.1–24.2 (210, 245).

Microsomal epoxide hydrolase (mEH) has also been proposed to mediate Na\(^{-}\)-dependent bile salt uptake (365, 366, 399), including a microsomal isoform and an isoform localized to the basolateral membrane of hepatocytes. Expression of the cell-surface isoform of mEH in Madin-Darby canine kidney (MDCK) cells transfers/mediates Na\(^{-}\)-dependent DIDS-inhibitable taurocholate transport (366). Although mEH could mediate Na\(^{-}\)-dependent cholate uptake in hepatocytes, it has to be considered that >50% of cholate uptake is Na\(^{+}\) independent, and Ntcp also contributes to Na\(^{-}\)-dependent cholate uptake (210). Moreover, mEH (−/−) mice have no abnormalities in bile salt homeostasis, suggesting that mEH is not an important determinant for physiological bile salt transport (253). The microsomal isoform of mEH could be involved in vesicular bile salt transport from the basolateral to the canalicular membrane, but the physiological significance of this pathway is not clear (11).

Taken together, the current findings suggest that the features of Na\(^{-}\)-dependent bile salt uptake into hepatocytes can be largely explained by the molecular properties of Ntcp/NTCP.

B) Sodium-independent uptake via Oatps/OATPs. In contrast to Na\(^{-}\)-dependent bile salt uptake, Na\(^{+}\)-independent uptake of bile salts is determined by several different gene products. Oatps/OATPs are multispecific transporter systems with a wide substrate preference for mostly amphipathic organic compounds, including conjugated and unconjugated bile salts, bromosulfophthalain (BSP), bilirubin, DIDS, cardiac glycosides and other neutral steroids, linear and cyclic peptides, mycotoxins, selected organic cations, and numerous drugs such as pravastatin (for review, see Refs. 210, 242, 245). Na\(^{+}\)-independent bile salt uptake is quantitatively less significant than sodium-dependent uptake and appears to be largely mediated by facilitated exchange with intracellular anions (e.g., GSH, HCO\(_{3}^{-}\)) (210).

In rat liver, basolateral sodium-independent bile salt uptake is mediated by three members of the organic anion transporting protein (Oatp/OATP) family. Other members of the Oatp/OATP family do not appear to be bile salt carrier systems. Oatp1 (Slc21a1) from rat liver was the first cloned member of the Oatp family and is a 670-amino acid glycoprotein with an apparent molecular mass of 80 kDa and 12 putative transmembrane-spanning domains (152). Oatp1 is localized to the basolateral membrane of hepatocytes, the apical membrane of kidney proximal tubular cells, and choroid plexus epithelial cells (16, 28). Developmentally, Oatp1 expression precedes expression of Ntcp, and its mRNA can be detected already at day 16 of gestation in rat liver (210, 245).
of gestation in the developing rat liver (91). Oatp1 is a highly versatile and multispecific transport system that transports a wide variety of amphipathic substrates with differing affinities, including bile salts (although with lower affinities than Ntcp), nonbile salt organic anions, organic cations, neutral steroids, and small peptides. More specifically, Oatp1 transports bile salts (taurocholate, cholate, glycocholate, taurochenodeoxycholate, taurosulfocholate, taurodeoxycholate, taurohyodeoxycholate), organic anionic dyes such as BSP, bilirubin monoglucuronide, thyroid hormones including triiodothyronine (T3) and thyroxine (T4), steroid hormones (aldosterone, dexamethasone, cortisol) and steroid conjugates [17β-estradiol glucuronide, estrone-3-sulfate, dehydroepiandrosteronsulfate (DHEAS)], leukotriene C4 (LTC4) and other glutathione conjugates (dinitrophenylglutathione), the anionic magnetic resonance imaging contrast agent gadoterate (Gd-EOB-DTPA, or gadolinium-ethoxybenzyl-diethylenetriamine-pentacetic acid), neutral steroids such as ouabain, the dipeptidic angiotensin-converting enzyme inhibitors enalapril and temocaprilat, the peptidomimetic thrombin inhibitor CRC 220, the endothelin receptor antagonist BQ-123, the opioid receptor antagonists D-penicillamine-enkephalin and Leu-enkephalin, biotin, fexofenadine, and the bulky type II organic cations D-penicillamine-enkephalin and Leu-enkephalin, biotin, fexofenadine, and the bulky type II organic cations APD-ajmalinium and recuronium. Importantly, in contrast to Oatp1, bilirubin monoglucuronide, BSP, LTC4, and Gd-EOB-DTPA (gadoxetate) are not transported via Oatp2 (203, 210, 242, 245). Moreover, Oatp2 transports ouabain with higher affinity and is unique in mediating high-affinity transport of the cardiac glycoside digoxin (263). Hence, Oatp1 appears to prefer amphipathic organic anions, whereas Oatp2 has an extended substrate preference for neutral compounds (203, 210, 245). Similar to Oatp1, the driving force for Oatp2-mediated uptake appears to be exchange with GSH and GSH conjugates (228).

The third Oatp family member involved in hepatic bile salt uptake in rat liver is Oatp4 (Slc21a10). Oatp4 is the full-length isoform of the rat liver-specific transporter 1 (rLst-1) (58). Although three rLst-1 splice variants have been detected (70), full-length Oatp4 represents quantitatively and functionally the most relevant protein in rat liver (58). Compared with rLst-1, Oatp4 is more highly expressed in liver and contains an additional 35 amino acids, resulting in a predicted transmembrane topology characteristic of all Oatps (58). Oatp4 shares 43% amino acid identity with Oatp1 and 44% identity with Oatp2 (210, 245). While rLst-1 transports only taurocholate, Oatp4 also transports BSP, estrone-3-sulfate, estradiol 17β-glucuronide, DHEAS, prostaglandin E2, LTC4, the thyroid hormones T3 and T4, and gadoxetate (58). Similar to Oatp1
and Oatp2, Oatp4 is a multispecific transporter with high affinities for BSP, DHEAS, LTC4, and anionic peptides. In addition, Oatp4 appears to be particularly involved in the hepatic clearance of anionic peptides including microcystin (a toxin derived from algae) and cholecystokinin (a gastrointestinal peptide hormone) (210, 245).

Oatp3 (Slc21a7) is not expressed in liver in contrast to initial reports (1) but may be important for intestinal bile salt uptake (370) (see sect. ID). Oatp3 has a similar broad substrate specificity but much lower affinities than Oatp4 (59). Thus, while Oatp4 works in concert with Oatp1 and Oatp2 in the basolateral membrane of rat hepatocytes, Oatp3 is a multispecific transport system in the small intestine (59).

An evolutionary ancient liver specific Oatp has been characterized in a primitive vertebrate, the small skate (Raja erinacea) with substrate similarities to rat Oatp4 and human OATP-C (55). This Oatp shares only ~40-50% amino acid identity with other liver-specific OATPs/Oatps and is most closely related to human OATP-F in brain. These findings emphasize the early evolutionary development of the Slc21a/SLC21A transporter family.

Taken together, the transport characteristics of Oatp1, -2, and -4 can account for the bulk of Na+-independent bile salt uptake in liver (210, 245).

In humans, three liver-specific OATPs (OATP-A, OATP-C, and OATP-8) transport bile salts (208). For bile salt uptake, OATP-C is the most relevant isof orm (210, 245).

The first human OATP identified in human liver was OATP-A (SLC21A3) (207). It consists of 670 amino acids and exhibits only a 67% amino acid homology to rat Oatp1 (which is not an ortholog as suggested by low amino acid identity and different substrate specificity), 73% with Oatp2, 42% with Oatp4, and 44% with human OATP-C. Although Oatp-A was originally cloned from human liver (207), its hepatic expression level is low, and its contribution to overall hepatic bile salt uptake is probably minor (245). It is predominantly expressed in human cerebral endothelial cells where it may play a role in the blood-brain barrier (104). OATP-A transports bile salts such as taurocholate, cholate, tauroursodeoxycholate, in addition to BSP, estrone-3-sulfate, DHEAS, the magnetic resonance imaging contrast agent Gd-B 20790, the opioid receptor antagonists D-penicillamine-enkephalin, and deltorphin II, fentanyl, and the bulky type II organic cations APD-ajmalium, recuronium, N-methylquinine, and N-methylquinidine. Transport rates were highest for the organic cation N-propylajmalium and the peptidomimetic drug CRC 220. In addition, OATP-A mediates unique high-affinity transport of the bulky lipophilic organic cations methylquinine and methylquinidine (210, 245).

OATP-C (SLC21A6) also known as LST-1 and OATP2 consists of 691 amino acids and is selectively expressed at the basolateral membrane of hepatocytes (2, 142, 189). OATP-C exhibits the highest amino acid identity (64%) with rat Oatp4 (210). As a result of gene duplication in humans, both OATP-C and OATP-8 represent the human orthologs of rodent Oatp4. OATP-C transports taurocholate (with slightly lower affinity than NTCP), bilirubin monoglucuronide, conjugated steroids, eicosanoids, thyroid hormones, and peptides (210). The substrate specificity of OATP-C is very comparable to rat Oatp4 and, more specifically, includes taurocholate, bilirubin monoglucuronide, BSP, estrone-3-sulfate, estradiol 17β-glucuronide, DHEAS, prostaglandin E2, thromboxane B2, LTC4 and LTE4, the thyroid hormones T3 and T4, and pravastatin (210). Although OATP-C exhibits large overlapping substrate specificities with other OATPs of human liver (208), a unique property is its capacity to transport unconjugated bilirubin (77, 78). In contrast to OATP-A, OATP-C shows a substrate preference for organic anions and does not include amphipathic organic cations. Of note, OATP-C transports taurocholate with slightly lower affinity than NTCP, although current data suggest that it represents an important Na+-independent bile salt uptake system in human liver (208, 210). Polymorphisms in OATP-C associated with variable degrees in plasma membrane expression may represent an important factor influencing drug disposition (354). In addition, a recently identified OATP-C mutation severely interferes with normal basolateral OATP-C expression and function (J. König and D. Kepp ler, personal communication).

OATP8 (SLC21A8) is 80% identical with OATP-C and is also expressed at the basolateral membrane of human hepatocytes (190). Thus OATP-C and -8 are expressed in a liver-specific fashion. The data on bile salt transport by OATP8 are controversial since bile salts were not transported when OATP8 was expressed in mammalian cells (190), but they were when OATP8 was expressed in oocytes (208, 210). Interestingly, OATP8 (and rat Oatp4) has been identified as hepatic uptake systems for cholecystokinin (148).

OATP-B (SLC 21A9) is also expressed at the basolateral membrane of human hepatocytes but does not transport bile salts (208, 351). Although OATP-B is predominantly expressed in liver, it is not liver specific and is found in many other tissues (208) including placenta (341) (see sect. IF).

Hence, although sodium-independent bile salt transport is an intrinsic feature of several Oatps/OATPs, each of these polyspecific transport systems exhibits additional substrate specificities and preferences that may even be specific for certain substrates (e.g., digoxin for Oatp2 and OATP8). For many of these substrates, Oatp/OATP-mediated uptake appears to be the main if not the only uptake route, based on the similarities of $K_m$ values between oocyte expression systems and total liver.

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2. Basolateral bile salt efflux

Basolateral efflux systems belonging to the multidrug resistance (MRP/Mrp) subfamily normally are expressed at very low levels but may be induced under cholestatic conditions. In humans, this family currently comprises six members (MRP1–6), at least four of which (MRP1, -2, -3, -6) have been demonstrated in liver (37, 180). The hepatic expression of MRP4 and MRP5 proteins is low, and their physiological function and (sub)cellular localization are not yet known (140). Three members of this family have also been identified in rat (Mrp2, -3, -6), five in mouse (Mrp1, -2, -4, -5, -6), and one in rabbit (Mrp2) (180). The founding member of the MRP family, MRP1 (Mrp1 in rodents), was initially cloned from a multidrug resistant small lung cancer cell line (73) and is minimally (if at all) expressed in normal liver (73), where it has been reported to be localized to the basolateral membrane of hepatocytes (240). However, potential antibody cross-reactivity with other Mrps should be considered when interpreting these early reports. Mrp3/MPR3 and Mrp6/MRP6 have also been localized to the basolateral membrane of hepatocytes, while Mrp2/MPR2 is localized to the canalicular membrane. At the hepatocellular level, rat Mrp3 is normally only expressed in the terminal cells in the lobule surrounding the pericanalicular vein (89, 192, 331). Mrp3/MPR3 has also been localized to the basolateral membrane of cholangiocytes (89, 194, 299, 308, 331) and intestinal epithelia (135, 186, 300, 308), where it may be involved in the cholehepatic and enterohepatic circulation of bile salts. Under normal conditions, expression of Mrp3/MPR3 is also very low in hepatocytes in contrast to cholangiocytes. Human MRP3 is mainly expressed in the periportal region of the lobule in addition to cholangiocytes (37, 308). Similar to Mrp2/MPR2, Mrp1/MPR1 and Mrp3/MPR3 are ATP-dependent export pumps whose spectrum (although with different affinities) include glucuronide and glutathione conjugates of endogenous and exogenous compounds (180, 191). Mrp1/MPR1 (179, 180) and Mrp3/MPR3 (6, 137, 138) have been shown to transport divalent bile salts such as sulfated taurocholate and taurocholodeoxycholate with high affinity. In addition, Mrp3 can also transport monovalent bile salts such as tauro- and glycocholate (6, 137, 138), while human Mrp3 transport only glycocholate with low affinity, but not taurocholate to a significant degree (5, 398). Mrp1 and Mrp3/MPR3 are normally expressed at very low levels in hepatocytes, but are dramatically upregulated in cholestasis in rats (89, 136, 268, 285, 331, 352, 367). Because Mrp1/MPR1 and Mrp3/MPR3 are able to transport the divalent anionic bile salts such as sulfated and glucuronidated bile salts that are eliminated into urine in cholestasis, the induction of Mrp1 and Mrp3/MPR3 during cholestasis may explain the shift toward renal excretion of bile salts as a major mechanism for bile salt elimination in patients with chronic, long-standing cholestasis (290, 339). Similarly, Mrp4 RNA has been recently shown to be upregulated in bile salt-fed mice, suggesting that this transporter could also be involved in basolateral bile salt efflux (314). However, further studies will have to demonstrate whether Mrp4 is localized to the basolateral membrane of hepatocytes. Mrp6 is localized predominantly to the lateral membrane of hepatocytes in rat liver where it mediates transport of the anionic cyclopentapeptide and endothelin receptor antagonist BQ-123; of note, other cyclic peptides such as endothelin and vasopressin are transported by Mrp2 but not by Mrp6 (233). Mrp6 is constitutively and highly expressed in hepatocytes and kidney (193). Recent evidence suggests that Mrp6 may be involved in transport of glutathione conjugates and leukotrienes (LTC₄) (146). Mutations of the MRP6 gene are associated with pseudoxanthoma elasticum, although the functional link underlying this observation remains unclear (27, 146, 294).

Members of the Oatp/OATP family also remain candidates for bile salt efflux at the basolateral membrane, since studies in Xenopus laevis oocytes have shown that Oatp1 and -2 are able to operate as bidirectional exchangers (228), although this remains to be demonstrated for hepatocytes.

3. Intracellular bile salt transport

The mechanisms involved in the transcellular movement of bile salts across hepatocytes from the basolateral to the canalicular membrane are poorly understood. With physiological bile salt loads, a considerable fraction is bound to intracellular binding proteins (e.g., glutathione-S-transferases, 3-hydroxyesteroid dehydrogenase, fatty acid-binding proteins in rat liver; a 36-kDa bile acid binding protein in human liver) and diffuses to the canalicular membrane in a protein bound form (for review, see Ref. 4). In addition, free, unbound intracellular bile salts may reach the canalicular membrane by rapid diffusion. At high bile salt loads, increasing partition of hydrophobic bile salts occurs into intracellular organelles including endoplasmatic reticulum, Golgi apparatus, and other membrane-bound compartments (4). Early reports of a direct vesicle-dependent fraction of transcellular bile salt transport can now be better explained by vesicular targeting of their respective transport systems (183).

4. Canalicular bile salt excretion

Canalicular bile secretion represents the rate-limiting step in bile formation. The canalicular membrane contains both ATP-dependent and ATP-independent transport systems (Table 1, Fig. 1). ATP-dependent transport systems in the liver are members of the ATP-binding cassette (ABC) superfamily, and they transport biliary constituents against steep concentration gradients across
the canalicular membrane that are often in the range of 100- to 1,000-fold and are driven by ATP hydrolysis. A typical ABC transporter consists of 12 or more membrane-spanning domains that determine the substrate specificity and two intracellular nucleotide-binding loops that are highly conserved and contain the Walker A and B motifs for binding and hydrolysis of ATP (140). Most canalicular ABC proteins belong to the multidrug resistance P-glycoprotein (MDR) family also known as ABC-B family or the multidrug-resistance protein (MRP) family (ABC-C) (140, 178). Two other families, ABC-A and ABC-G, may also be relevant, since at least three ABC-A members (ABCA1, -2, -3) and three ABC-G half transporters (ABCG2, -5, and -8) are highly expressed in liver (94, 229).

The canalicular membrane contains a bile salt export pump (Bsep/BSEP) for monovalent bile salts, a conjugate export pump (Mrp2/MRP2) for divalent bile salts, and various other amphipathic conjugates, including GSH (a major determinant of bile salt-independent canalicular bile flow), a multidrug export pump (Mdr1/MDR1) for bulky amphipathic organic cations (e.g., various drugs), a phospholipid flippase (Mdr2 in rodents/MDR3 in humans) for phosphatidylcholine, a P-type ATPase (Fic1/FIC1) mutated in hereditary cholestasis but whose function is still unknown, and a Cl-/HCO3- exchanger (AE2) for HCO3- excretion. The latter represents the only functionally relevant ATP-independent transport system at the canalicular membrane (238, 243). All other transporters, except FIC1 (which is a P-type ATPase), belong to the ABC superfamily. AE2 and MR2 are the major driving forces for bile salt-independent bile flow, while BSEP drives bile salt-dependent flow (205, 258, 259, 342, 359).

A) Canalicular excretion of monovalent bile salts via Bsep/BSEP. The canalicular bile salt export pump Bsep/BSEP (Abcc11/ABCB11) originally was known as the sister of P-glycoprotein (Spgp/SPGP). Following the partial cloning of Spgp in pig liver (68), the full-length cDNA was cloned from rat liver (69, 112). Functional expression of rat Spgp in SF9 insect cells demonstrated that Spgp functions as an ATP-dependent bile salt transporter with transport characteristics comparable with canalicular rat liver plasma membrane vesicles (112). These functional data, together with hepatocyte-specific expression of Spgp on the surface of canalicular microvilli, indicate that Spgp represents the mammalian canalicular bile salt export pump. The rat Bsep is a 1,321-amino acid protein with 12 putative membrane-spanning domains, four potential N-linked glycosylation sites, and a molecular mass of ~160 kDa. Bsep was localized by immunofluorescence microscopy and immunogold labeling studies to canalicular microvilli and to a canalicular subpopulation of membrane vesicles (112). When ontogenic expression of Bsep was compared with that of Mrp2, it became evident that Bsep is almost undetectable before birth, whereas Mrp2 was already observed in livers of 16-day-old fetuses (400), indicating that biliary excretion of monovalent bile salts does not take place until birth. Bsep transports various bile salts at rates in the same order of magnitude as ATP-dependent transport in canalicular rat liver plasma membrane vesicles. In addition to taurocholate, Bsep also mediates ATP-dependent transport of glycocholate, taurochenodeoxycholate, and tauroursodeoxycholate (210, 245). Rat Bsep mediates low-level resistance to taxol, but not to other drugs (e.g., vinblastine, digoxin) that form part of the multidrug resistance phenotype (69). Transfection studies with mouse Bsep also revealed only slightly enhanced vinblastine efflux, indicating that Bsep is probably not of major relevance for detoxification of xenobiotics (218).

The mouse Bsep gene was localized to a region on mouse chromosome 2, which has also been linked to genetic gallstone susceptibility, although the detailed mechanisms remain unclear (117, 213). In contrast to humans with a hereditary BSEP defect (PFIC-2), targeted inactivation of this gene in mice results in nonprogressive but persistent intrahepatic cholestasis, due to the de novo formation and biliary excretion of muricholic acid and a novel tetra-hydroxylated bile salt in mice (376). Of note, bile flow is minimally reduced and bile salt excretion is only reduced to 30%, suggesting that additional bile salt transporter(s) may exist in mice. Although the molecular identity of this alternative transport system remains to be determined, Mrp2 is a likely transporter for tetrahydroxy bile salts (245). Alternatively, it remains possible that sulfated and glucuronidated dianionic bile salts, which also are Mrp substrates, account for most of the secreted bile salts in these animals (180). Mouse Bsep contains several phosphorylation sites that appear to be involved in regulating bile salt transport capacity by Bsep (264).

Bsep is highly conserved during vertebrate evolution as suggested by the recent cloning of a Bsep ortholog from the liver of the small skate, Raja erinacea, a 200 million-year-old marine vertebrate (23, 54). Notably, the sites of published BSEP mutations in humans are also conserved from the skate Bsep ortholog, and mutations of several of these sites inhibit skate Bsep transport function in SF9 cells (54).

Identification of the gene responsible for a subtype of progressive familial intrahepatic cholestasis (PFIC-2) has led to the discovery of the human BSEP gene. The human BSEP gene is mutated in patients with PFIC-2, characterized by absence of BSEP from the canalicular membrane and extremely low biliary bile salt concentrations that are <1% of normal (see sect. mA) (155, 344). This suggests that BSEP is the major canalicular bile salt transport system. After several years of effort, the human BSEP has now been functionally expressed by two groups and appears similar to studies in human canalicular membrane vesicles with respect to bile salt affinity and substrate specificity (53, 265).
B) Canalicular excretion of divalent bile salts via Mrp2/MRP2. The bilirubin conjugate export pump (Mrp2/ MRP2) (Abcc2/ABCC2), functionally also known as the canalicular multispecific organic anion transporter (cMOAT), was originally cloned from rat liver (49, 280), followed by human (353) and mouse liver (101). Mrp2/ MRP2 mediates the canalicular excretion of a broad range of organic anions; most of these are divalent amphipathic conjugates with glutathione, glucuronate, and sulfate formed by phase II conjugation in the hepatocyte (e.g., bilirubin diglucuronide) (76, 191, 274). Canalicular excretion of GSH is also mediated through Mrp2/ MRP2 (279). Divalent bile salts with two negative charges such as sulfated tauro- or glycolithocholate are transported by Mrp2/MPR2, whereas monovalent bile salts are not substrates for Mrp2/MPR2 (179, 180). However, mutation of a single amino acid confers transport capacity for monovalent bile salts to Mrp2 (149). Other Mrp/MPR isoforms such as Mrp1/MPR1 and Mrp3/MPR3 are located at the basolateral membrane where they may serve as a compensatory overflow system under cholestatic conditions when canalicular (Mrp2/MPR2) excretory function is impaired. The mouse Mrp2 gene was localized to a region on mouse chromosome 19D2 (214, 363), which has also been linked to genetic gallstone susceptibility (213). The human Mrp2 gene has been mapped to chromosome 10q24 (363). Mutations of the MRp2 gene result in the Dubin-Johnson syndrome characterized by impaired canalicular excretion of a broad range of endogenous and exogenous amphipathic compounds (see sect. IV.A); the rat model for this syndrome is the transport deficient (TR−) and Groningen Yellow (GY) rat (Wistar strain) or Eisai hyperbilirubinic (EHBR) rat (Sprague-Dawley strain), which were pivotal models for the functional characterization of cMOAT and subsequent cloning of Mrp2 (281).

C) Other canalicular transport systems involved in bile secretion and their relation to bile salt excretion. Once bile salts have been excreted into bile, they stimulate the release of phosphatidylcholine and cholesterol from the outer leaflet of the canalicular membrane (272), which then in turn form mixed micelles in bile. By doing so, bile salt toxicity to the bile duct epithelium is avoided, which would otherwise occur due to an unopposed detergent bile salt action. The phospholipid flippase Mrd2/MDR3 (Abcb4/ABCB4) ensures the continuous supply of phosphatidylcholine to the outer leaflet of the canalicular membrane (327, 362).

In addition to biliary excretion of phospholipids, bile is also a major pathway for elimination of cholesterol, which is mostly derived from plasma high-density lipoprotein (HDL). Hepatocellular uptake of HDL cholesterol is mediated via a scavenger receptor (SR-BI) (3, 158, 195). An ATP-dependent cholesterol transporter (ABCA1) mediates reverse cholesterol transport from macrophages into HDL particles, removes absorbed cholesterol from enterocytes, and is mutated in individuals with Tangier disease and familial HDL deficiency with an increased risk for atherosclerosis and premature coronary artery disease (34, 48, 301). ABCA1 is highly expressed in liver but probably does not play a major role in biliary cholesterol excretion, since Abca1 knockout mice also do not demonstrate defects in bile salt-induced cholesterol excretion (A. Groen, personal communication). Mutations of two highly homologous genes (ABCG5 and ABCG8) that encode the plant sterol sitosterol transporters, sterolin-1 and -2, respectively, have been identified in patients with sitosterolemia, a disease characterized by impaired biliary excretion of dietary sterols. ABCG5 and ABCG8 are also highly expressed in liver (26, 144, 222, 229) and may play a key role in hepatic cholesterol excretion (222, 273) as suggested by a fivefold increased biliary cholesterol excretion in mice with transgenic over expression of human ABCG5 and ABCG8 (397).

Fic1/FIC1 (ATP8B1) a P-type ATPase mutated in “familial intrahepatic cholestasis” belongs to a family of putative aminophospholipid transporters. Fic1/FIC1 has been localized to the canalicular membrane and the bile duct epithelium (361) but is also highly expressed in extrahepatic tissues including the intestine and pancreas (50, 96). The detailed function of Fic/FIC1 is unclear, but mutations of this transporter result in variants of familial intrahepatic cholestasis, suggesting that it must play an important direct or indirect role in canalicular bile salt excretion (50). Of note, these patients have a prominent reduction in the biliary excretion of hydrophobic bile salts such as cholate and chenodeoxycholate relative to cholate conjugates, indicating that Fic1/FIC1 could directly excrete highly hydrophobic bile salts (335). Apart from this, Fic1/FIC1 could also play an indirect role in bile secretion by maintaining the canalicular membrane asymmetry between the inner and the outer layer by maintaining phosphatidylethanolamine and serine within the inner bilayer of the plasma membrane or regulating the docking of vesicles fusing with the canalicular membrane (276). Strong expression of Fic1/FIC1 in extrahepatic tissues such as pancreas, small intestine, and kidney suggests a more general role in the regulation of secretory processes (50) and may explain some of the extrahepatic features associated with these syndromes such as pancreatitis, diarrhea, and nephrolithiasis. Fic1 (−/−) mice have normal biliary bile salt excretion but accumulate bile salts when fed orally due to abnormal regulation of intestinal bile salt absorption. The detailed mechanisms remain to be resolved (283).

C. Cholangiocellular Bile Salt Transport

Although contributing only 3–5% to the total liver cell mass, bile duct epithelial cells (cholangiocytes) play an
important role in normal bile secretion (39, 355). Large and medium-sized but not small (<30 μm in diameter) intrahepatic bile ducts contain several transport systems for secretory and absorptive functions (168) (Table 1, Fig. 1). Biliary constituents including bile salts, glucose, and drugs may be transported from bile into cholangiocytes. However, only a minority of bile salts are in solution in bile as free monomers at the level of the bile ducts, and thus only small amounts of bile salts might be available for absorption by cholangiocytes (245).

D. Intestinal Bile Salt Transport

Bile salts, cholesterol, and phospholipids undergo extensive enterohepatic cycling/enterohepatic circulation, thereby returning these biliary constituents to the liver for reexcretion into bile (139, 156, 245) (Table 1, Fig. 1). The most efficient bile salt conservation mechanism is the uptake of conjugated bile salts in the terminal ileum via a Na\(^{+}\)-dependent mechanism (74, 342). In addition, a Na\(^{+}\)-independent anion exchanger has been identified in proximal rat jejunum (12). Passive diffusion of unconjugated bile salts also occurs in small and large intestine (246).

1. Apical uptake of bile salts into enterocytes

Na\(^{+}\)-dependent bile salt uptake occurs via the ileal bile salt transporter (Isbt/ISBT or lbat/IBAT) also known as the apical sodium-dependent bile salt transporter (Asbt/ASBT). This transport system has also been identified in the apical membrane of cholangiocytes and proximal renal tubular cells. Isbt was originally cloned from hamster intestine (387), followed by human (388), rat (320), rabbit (198), and mouse (302) ileum. Size (48 kDa), membrane topology, and transport characteristics are similar to Ntcp/NTCP (35% amino acid identity) (79, 129, 342). Isbt is expressed biphasically during rat development, with the first expression on day 22 of gestation, followed by a transient decrease and a sharp increase at postnatal days 17 and 18 (320, 322). Both primary and secondary conjugated and unconjugated bile salts are substrates for Isbt/ISBT, the highest affinity being reported for conjugated dihydroxy bile salts (267). However, in contrast to Ntcp/NTCP, which transports some nonbile salt substrate in addition to bile salts, the substrate specificity of Isbt/ISBT appears to be strictly limited to bile salts. ISBT is the major intestinal bile salt uptake system in humans as emphasized by ISBT mutations that result in bile salt malabsorption (267, 388).

The Na\(^{+}\)-independent bile salt transporter Oatp3 (Slc21a7) is 80–82% identical to Oatp1 and Oatp2 and has transport characteristics similar to Oatp2, with a range of amphipathic anions as its substrates, including bile salts (59, 370). Oatp3 was originally cloned from retina and was found to be expressed in the brush-border membrane of jejunal enterocytes (1, 370). Oatp3 mRNA transcripts were detected throughout the entire small intestine (as well as brain, lung, and kidney), but Oatp3 protein is predominantly located to the apical surface of jejunal epithelial cells (1, 370), consistent with a role of Oatp3 as the Na\(^{+}\)-independent transport system which has been functionally localized to the jejunum. Bile salt uptake into jejunal brush-border membrane vesicles is stimulated by an in-to-out HCO\(_3\)\(^{-}\) gradient, similar to Oatp1 (303). However, it remains to be determined whether this mechanism...
also applies to Oatp3. The relative importance of Oatp3 for intestinal bile salt uptake compared with Isbt also remains to be clarified (342). Interestingly, OATP-A has been proposed as the human Oatp3 ortholog (370), and human OATP-A has been detected in human intestine (245).

In addition to uptake systems in the apical membrane, the apical brush-border membrane also contains excretory systems, including members of the Mdr and Mrp family (e.g., Mdr1, Mrp2) (Fig. 1). As far as bile salt transport is concerned, Mrp2 could play a role as an alternative excretory route for sulfated/glucuronidated bile salts (in analogy to the role of Mrp2 in kidney, see sect. I E).

2. Intracellular bile salt transport in enterocytes

As with other cells involved in bile salt transport, the information about the molecular mechanisms of intracellular bile salt transport in enterocytes is limited. Photoaffinity labeling studies have identified a 14-kDa cytosolic intestinal bile acid-binding protein (I-Babp) that is cytoplasmatically attached to Isbt (115, 196). I-Babp probably represents the most important protein for transcellular bile salt movement through enterocytes (197). The functional ileal bile salt uptake complex is multimeric and comprises four Isbt dimers and four I-Babps (196). Similar ontogenic expression patterns of Isbt and I-Babp as well as their response to bile salts and dexamethasone suggest that both transport systems might be controlled by similar if not identical regulatory mechanisms (145, 320).

3. Basolateral efflux of bile salts from enterocytes

At a functional level, an anion exchange mechanism has been demonstrated at the basolateral membrane of intestinal cells (381). Recently t-Asbt (which can function as an anion exchanger) has been reported to be expressed twofold higher at the mRNA level than the full-length Asbt in rat ileum (217). Another potential candidate for bile salt efflux from enterocytes is Mrp3/MRP3, which has also been identified in both rat and human small intestine (135, 186, 300, 308). Mrp3/MPR3 is expressed in the basolateral membrane in all intestinal segments but is lowest in duodenum and markedly increased in the terminal ileum and colon (330). This is in contrast to Mrp2/MPR2, which is expressed primarily in the apical membrane of the proximal intestine (65, 300, 348). The high expression of Mrp3/MPR3 in terminal ileum provides a mechanism to return bile salts to the portal circulation. Mrp3/MPR3 may also play an important role in drug absorption in the intestine.

E. Renal Bile Salt Transport

Bile salts that escape first-pass clearance by the liver are filtered at the glomerulus from plasma into urine, where they are reabsorbed in the proximal convoluted tubule (386) (Table 1, Fig. 1). Thus, under normal conditions, urinary bile salt losses are minimized. Under cholestatic conditions however, renal excretion of bile salts may become a major alternative elimination route for elimination of (mainly divalent sulfated and glucuronidated) bile salts (290), which may be attributed to increased passive glomerular filtration of (elevated) serum bile salts and active tubular excretion of (mainly divalent) bile salts, together with reduced tubular bile salt conservation.

1. Apical transport systems in proximal renal tubules

The apical plasma membrane of the proximal renal tubular cells contains transport systems for bile salt reabsorption as well as excretion into urine (342). Bile salts are reabsorbed by the apical Na\(^+\)-dependent bile salt transporter Isbt in the proximal convoluted tubule (71). In addition to Isbt, Mrp2 (306) and Oatp1 (28) have also been localized to the apical brush-border membrane. Mrp2 may be involved in tubular excretion of organic anions (e.g., para-aminobenzoic acid) under normal conditions and increased urinary excretion of sulfated/glucuronidated bile salts under cholestatic conditions (306). In contrast to its basolateral localization in hepatocytes, Oatp1 is localized to the apical membrane of the S3 segment of proximal renal tubules (28). The relative contribution of Oatp1 to tubular bile salt uptake remains unclear. Moreover, Oatp3 (Slc21a7) has also been reported to be expressed in rat kidney (1), although this remains controversial (342). By analogy to the rat, Isbt (74), OATP-A (207), and MRP2 (305) have also been localized in human proximal kidney tubular cells.

2. Basolateral efflux systems in proximal renal tubules

Little is known about the basolateral counterparts of the apical transport systems described above. Recent evidence suggests a potential role for Mrp1 (285). However, Mrp3 has recently been localized to the basolateral membrane of the proximal renal tubule in rats (330). Therefore, it will also be of particular interest to see whether Mrp3 also plays an important role for basolateral efflux, e.g., of bile salts.

F. Placental Bile Salt Transport

Because the fetal liver is immature and ontogenetic expression of hepatobiliary transporters is not detectable until shortly before birth (19), bile salts undergo minimal biliary excretion by the fetal liver, although bile salts are synthesized by the fetal liver in utero (260). Instead, they are eliminated by the maternal liver after vectorial translocation from the fetal to the maternal circulation via the
placenta ("placenta-maternal liver tandem"). The blood-placental barrier of term placenta begins at the fetal side with the endothelial cells of fetal capillaries, cytotrophoblast (absent in term placenta), and syncytiotrophoblasts that face the maternal blood with their apical surface. Distinct transport systems have been identified at a functional level at the basolateral (fetal-facing) and apical (maternal-facing) membrane of the trophoblast. However, the molecular identity of these transport systems has not yet been resolved, and data are still quite fragmentary (342).

1. Basolateral (fetal-facing) trophoblast membrane

Bile salt uptake occurs via Na⁺-independent, bidirectional, and HCO₃⁻ trans-stimulatable mechanisms, consistent with a role for OATPs/Oatps. Notably, partial OATP-A (SLC21A3) transcripts have been identified in term placenta (342). Recently, OATP-B (SLC21A9) was localized to the basal syncytiotrophoblast and cytotrophoblast membranes, where it may be involved in the placental uptake of fetal-derived sulfated steroids (341). Preliminary data indicate the presence of Oatps 1, 2, and 4 mRNA transcripts in rat placenta (J. J. Marin, personal communication). Moreover, recent studies have localized MRP3 in fetal blood endothelia of term placenta and syncytiotrophoblast layer, suggesting a potential role for MRP3 in the extrusion of fetal bile salts through endothelial and syncytiotrophoblast barriers (343). Similarly Mrp1, -2, and -3 transcripts have recently been detected in rat placenta, although their subcellular localization remains to be determined (Marin, personal communication).

2. Apical (maternal-facing) trophoblast membrane

Both ATP-dependent and ATP-independent bile salt transport systems have been identified, the latter appearing to be the predominant transport mechanism (46, 342). However, BSEP remains a candidate, since partial Bsep/BSEP transcripts have been identified in term placenta (342).

II. REGULATION OF BILE SALT TRANSPORTERS IN NORMAL PHYSIOLOGY

The functional expression of membrane transport proteins can be regulated at several levels, including gene transcription, events that control translation of RNA, and posttranscriptional activity (see Table 2 and Figs. 2 and 3). Although the mechanisms that control gene transcription of membrane transporters are still incompletely un-

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Table 2. Nuclear hormone receptors and other transcription factors that regulate bile salt metabolism enzymes and transporters

<table>
<thead>
<tr>
<th>Nuclear Hormone Receptors and Other Transcription Factors</th>
<th>Activating Ligand for the Nuclear Receptor</th>
<th>Regulation of Bile Salt Metabolism Enzymes and Transporter Genes</th>
<th>Expected Physiological Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>LXR (liver X receptor)</td>
<td>Oxysterols</td>
<td>CYP7A1 (cholesterol 7-α hydroxylase)</td>
<td>Upregulates bile salt synthesis from cholesterol via CYP7A1</td>
</tr>
<tr>
<td>SHP-1 (short heterodimeric protein-1)</td>
<td>None</td>
<td>CYP7A1 (cholesterol 7-α hydroxylase), Ntcp</td>
<td>Downregulates bile salt uptake via Ntcp and bile salt synthesis via CYP7A1</td>
</tr>
<tr>
<td>RAR (retinoic acid receptor)</td>
<td>All trans-retinoic acid</td>
<td>Ntcp, Mrp2</td>
<td>Upregulates Ntcp and Mrp2</td>
</tr>
<tr>
<td>FXR (farnesoid X activated receptor)</td>
<td>Hydrophobic bile salts</td>
<td>SHP-1, Bsep/BSEP, I-BABP, OATP8</td>
<td>Upregulates SHP-1; Bsep/BSEP I-BABP and OATP8</td>
</tr>
<tr>
<td>PXR (pregnane X receptor in rodents), SXR (steroid X receptor in humans)</td>
<td>Xenobiocids; ursodeoxycholate, lithocholate; pregnenolone 16α-carbonitrile (PCN) in rodents; rifampin (in humans)</td>
<td>Oatp2 in rodents; CYP3A4, Mtp2</td>
<td>Upregulates Oatp2 and Mrp2; upregulates CYP3A and stimulates detoxifying bile salt hydroxylation</td>
</tr>
<tr>
<td>CAR (constitutive androstene receptor)</td>
<td>Xenobiocids</td>
<td>CYP3A, Mtp2</td>
<td>Stimulates hepatic drug metabolism, and drug conjugate export pump. Mtp2</td>
</tr>
<tr>
<td>NFκB (nuclear factor κB)</td>
<td>Cytokines</td>
<td>MDR1, Ntcp, Mdr2</td>
<td>Stimulates upregulation of Mdr1B</td>
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<tr>
<td>HNF3β (hepatic nuclear factor-β)</td>
<td></td>
<td>Ntcp, Oatp1, Bsep, Mdr2</td>
<td>Downregulates these transporters when overexpressed in mice</td>
</tr>
<tr>
<td>HNF4α</td>
<td></td>
<td>Ntcp, Oatp1, Oatp2, Oatp-4, Isbt, human OATP-C, OATP-8</td>
<td>Maintains Ntcp and Oatp1 expression; upregulates Bsep and downregulates Mdr2 in knock-out mouse</td>
</tr>
<tr>
<td>HNF1α</td>
<td></td>
<td>Ntcp, Oatp1, Oatp2, Oatp-4, Isbt, human OATP-C, OATP-8</td>
<td>Loss of expression of Ntcp, Oatp1, Oatp2, and Isbt in knock-out mouse</td>
</tr>
<tr>
<td>AP-1 (activating protein-1)</td>
<td>? Bile salts</td>
<td>Isbt, human OATP-C, OATP-8</td>
<td>Upregulates Isbt in vitro</td>
</tr>
<tr>
<td>PTF (fetal transcription factor)</td>
<td>? Bile salts</td>
<td>MRP3 CYP8B1</td>
<td>Upregulates MRP3 in vitro; downregulates CYP8B1 in vitro</td>
</tr>
<tr>
<td>PPARα (peroxisome proliferator activator-α)</td>
<td>Fibrates, fatty acids, eicosanoids, leukotrienes, NSAIDS</td>
<td>ISBT</td>
<td>Upregulates ISBT in vitro</td>
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derstood, a number of factors may be involved including constitutive and inducible DNA binding proteins that bind to upstream regulatory elements (RE) in the gene promoters. These transcription factors often act together to either stimulate or inhibit gene expression (17). Recent interest has focused on a group of nuclear hormone receptors (NHR) that are activated by various ligands such as steroids, retinoids, and hormones (61, 62). The nuclear receptor superfamily is divided into four major subgroups based on their dimerization and DNA binding properties (for reviews, see Refs. 66, 169, 236, 237). Class I contains the classical steroid hormone receptors such as for estradiol, progesterone, testosterone, cortisol, and aldosterone. Class II receptors comprise the receptors for vitamin D₃ (which recently has been shown to be also activated by the hydrophobic bile salt lithocholate, Ref. 234), T₃, and all-trans-retinoic acid. The latter (all-trans-retinoic acid receptor RAR) is also involved in the regulation of hepatobiliary transporter genes as outlined below. Other class II receptors involved in the regulation of bile salt synthesis and transport include the farnesoid X receptor (FXR) that binds oxysterols; the pregnane X receptor (PXR) whose high-affinity ligands are fatty acids, eicosanoids, fbrates, and NSAIDS; liver X receptor (LXR) that binds oxysterols; and the estrogen receptor (ER) whose human ortholog is the steroid and xenobiotic receptor (SXR) (392). PXR and SXR receptors are the targets for catatotic steroids and certain xenobiotics. Class II receptors heterodimerize with the retinoid X receptor (RXR) enabling high-affinity DNA binding to direct repeats (DR) separated by spacer nucleotides of variable number (DR₁–₅), which then leads to activation of gene transcription. Because heterodimer formation largely depends on the availability of RXR, limiting cellular/nuclear RXR concentrations may result in a trans-repressive effect.

In addition to these ligand-activated nuclear receptors, other factors such as the hepatocyte nuclear factor (HNF) family of liver-enriched transcription factors including HNF-1 (318, 319), HNF-3 and CCAAT/enhancer binding protein (C/EBP), as well as sterol responsive element binding protein (SREBP) and nuclear factor kappa B (NF-kB) also appear to play an important role in the regulation of hepatobiliary transporter expression (60, 257, 360) (Table 2).

A. Transcriptional Regulation of Bile Salt Transporters

1. Role of nuclear hormone receptors in regulation of bile salt transporters

Table 2 and Figure 2 list several of the bile salt transporters whose expression appears to be regulated by one or more NHRs. Bile acids are ligands for the nuclear hormone receptor FXR, which together with its heterodimeric partner, the RXR, acts as a transcription factor for several bile salt transporters, including the hepatic bile salt export pump Bsep and the ileal bile acid binding protein I-Babp (235, 277, 373). In addition, the expression of short heterodimeric protein (SHP)-1, which acts as a transcriptional repressor is itself regulated by FXR and can downregulate the expression of several genes including Ntcp and cholesterol-7a-hydroxylase CYP7A1, the rate limiting enzyme in bile salt synthesis. Recent studies suggest that the bile salt lithocholate and its 6-hydroxylated metabolite are also ligands for the PXR, resulting in the

![Diagram of transcriptional regulation of bile salt transporters](http://physrev.physiology.org/)

**FIG. 2.** Role of ligand-activated transcription factors in the transcriptional regulation of bile salt synthesis and transport. Conversion of cholesterol to bile salts by CYP7A1 and SB1 is stimulated by oxysterol-activated liver X receptor (LXR). Bile salts inhibit their own synthesis by farnesoid X receptor (FXR)-dependent activation of SHP-1, which in turn suppresses CYP7A1 and SB1 transcription. Bile salts also suppress retinoic acid receptor (RAR)-dependent genes such as Ntcp via the same pathway. Activation of FXR by bile salts stimulates transcription of Bsep, Mrp2, I-Babp, and OATP8. Activation of pregnane X receptor (PXR) by bile salts induces transcription of Oatp2 and Mrp2. Finally, liver receptor homolog-1 (LRH-1) may mediate induction of MRPL by bile salts in human enterocytes. RXR, retinoid X receptor; FTF, fetoprotein transcription factor.
transcription of CYP3A, an enzyme involved in hydroxylation and detoxification of bile salts (334, 393). Two α1-fetoprotein transcription factor (FTF) also known as liver receptor homolog-1 (LRH-1)-like elements have also been described in the promoter region of Mrp3 that function as bile salt response elements (147). Thus a picture is emerging where bile salts may regulate the expression of several important bile salt-transporting and -metabolizing systems by binding to various nuclear receptors (Fig. 2).

A) RXR. RXR is the obligate heterodimeric partner for the class II nuclear receptors and is activated by binding of 9-cis-retinoic acid. Thus alterations in the expression of RXR would be capable of influencing the expression of a large number of bile salt synthesis and transporter genes. RXRα is highly expressed in liver, kidney, muscle, lung, and spleen, whereas two other RXR isoforms, RXR-β and -γ, are not as highly expressed (371).

Although RXR-α knock-out mice do not survive beyond midgestation, the RXR-α gene has been deleted in adult animals specifically in the liver using cre-mediated recombination under the influence of an albumin promoter (371). These studies demonstrate that multiple metabolic pathways in the liver that are regulated by RXR heterodimerization are markedly altered when RXR-α is absent. These include FXR, which functions with RXR to enhance SHP expression, thereby suppressing the conversion of cholesterol to bile salts. The absence of RXR led to a significant increase in CYP7A1 mRNA (371). RXR/FXR appears to have a dominant negative influence on CYP7A1 expression, since RXR/LXR normally promotes the expression of CYP7A1 to stimulate conversion of cholesterol to bile salts. Other class II nuclear receptors that influence hepatic metabolic pathways are also impaired in the absence of RXR-α including RXR/CAR-β and RXR/PXR (371), leading to marked reductions in mRNA levels for liver fatty acid binding proteins and CYP4A1 and CYP2B10 and CYP3A1.

Thus the expression of RXR is one mechanism through which a coordinated effect on multiple gene products that influence bile salts, fatty acid, cholesterol, steroid, and xenobiotic metabolism, might be coordinated.

B) RAR. RAR-α together with its heterodimer partner RXR activate the hepatobiliary transporters Ntcp and Mrp2 by binding to retinoid response elements in the promoter regions of these genes. The liver is the major storage site for vitamin A. Retinoids stimulate expression and activity of RXR/RAR-dependent genes such as Ntcp and Mrp2 (81, 345).

C) FXR/bile acid receptors. FXR functions as a heterodimer with RXR and binds with high affinity to inverted repeat response elements (IR-1) where consensus receptor binding hexamers are separated by a single nucleotide. Initial studies demonstrated that induction of the human ileal bile acid binding protein (I-BABP) by bile salts was dependent on FXR/RXR binding to the highly conserved IR-1 (G/A GGTA A TAACCT) (118, 165, 166, 235).

FXR is expressed in tissues where bile salts are transported including the liver, intestine, and kidney and is activated by bile salts with the rank order of potency: chenodeoxycholate (CDCA) > deoxycholate (DCA) = lithocholate (LCA) > cholate (CA) (277). Evidence that FXR is the nuclear bile acid receptor (BAR) came from studies where expression plasmids containing murine and human FXR were transfected into monkey kidney CV-1 cells or human hepatoma HepG2 cells and exposed to bile salt metabolites (235). In these experiments CDCA was the most potent activator, with a half-maximal effective concentration (EC50) of 50 and 10 μM for murine and human FXR, respectively. Amino acid residues aspartagines (354) and isoleucine (372) confer sensitivity of human FXR to CDCA (75). DCA and LCA also stimulated FXR expression but to a lesser extent, whereas other sterols including cholesterol, oxysterols, steroid hormones, and other bile acid metabolites had no effect (235, 277). Particularly noteworthy was the absence of an activating effect by ursodeoxycholate (UDCA), a commonly used bile acid for the treatment of cholestasis (235), and β-muricholic acid, a prominent bile acid that accumulates in the cholestatic rat model (316). Cotransfection assays indicated that FXR/RXR heterodimers were required for bile acids to maximally transactivate the luciferase reporter. In the same studies, FXR was found to repress transcription of the gene encoding for cholesterol 7α-hydroxylase (CYP7A1), the rate-limiting enzyme in bile acid synthesis from cholesterol while stimulating the expression of the human intestinal bile acid binding protein (I-BABP) (235), a 17-kDa protein with high affinity for bile acids, that modulates bile salt uptake in the terminal ileum. Studies in Caco-2 cell lines, derived from human enterocytes, show that bile salts increase I-BABP expression, a finding consistent with the effects of bile diversion and cholyystamine administration in mice where I-Babp expression is diminished (118). Deletion and mutation analyses have established that the FXR/RXRa heterodimer activates I-BABP gene expression by binding to bile acid response elements in its promoter as demonstrated in human, mouse, and rabbit (118). Subsequent studies have demonstrated that FXR activates the expression of the transcriptional repressor SHP-1, which functions in a dominant manner to inhibit CYP7A1 in the mouse (see below). Thus bile salts feedback to regulate their own synthesis by binding to FXR and subsequent activation of SHP (Fig. 2). Subsequent studies confirm that both human, rat, and mouse BSEP/Bsep promoters are transcriptionally activated by FXR (13, 110, 287) and that bile salts increase BSEP expression in primary human hepatocytes or HepG2 cells with the same rank order
of potency that activates FXR (314). Conversely, LCA decreases BSEP expression by antagonizing FXR activation (396). Recent studies have demonstrated transactivation of Mrp2 (173) and the human OATP8 promoter by FXR (162). In contrast to hepatic bile salt transporters and I-BABP, the ileal sodium-dependent bile salt transporter Isbt was not regulated by FXR, although the studies confirmed a role for FXR in regulation of I-Babp.

In summary, the findings that FXR is abundantly expressed in tissues that express bile salt transporters, that CDCA, DCA, and LCA and their conjugates bind to FXR at physiological concentrations that regulate gene transcription and are highly effective activators of FXR when expressed in cellular expression systems, provide strong evidence that bile salts are the natural ligand for this NR.

Confirmatory evidence for the importance of FXR in the regulation of expression of several bile salt transporters came from studies with targeted disruption of the nuclear receptor FXR (326). When a 1% cholic acid diet was fed to wild-type mice, no ill effects were observed. Ntcp and CYP7A1 were both downregulated while Bsep was upregulated, thereby limiting the toxic accumulations of bile salts in the liver. In addition, SHP was also upregulated, facilitating downregulation of Ntcp and reducing expression of CYP7A1. However, when the FXR null mice were fed cholic acid, no changes in the expression of Ntcp, CYP7A1, Bsep, or SHP were observed and the mice developed severe hepatic necrosis, wasting, and 30% died by 7 days (326). Altogether these findings emphasize the importance of FXR in the regulation of hepatic bile salt transport and support prior studies that suggested that the downregulation of Ntcp and upregulation of Bsep in models of cholestasis might function to protect the liver from accumulation of hepatotoxic levels of bile salts (220) (Fig. 2).

D) **SHP-1.** Bile salts are known to repress the expression of CYP7A1, thereby providing feedback regulation for conversion of cholesterol to bile salts. Conversely, cholesterol stimulates forward regulation of CYP7A1, the rate-limiting enzyme for bile salt synthesis from cholesterol (231). The nuclear receptor LXR-α is known to be activated by the oxysterol cholesterol derivative 24,25(S)-epoxycholesterol and binds together with its heterodimeric partner RXR to an LXR response element in the CYP7A1 promoter, thereby inducing CYP7A1 expression. However, the CYP7A1 promoter does not contain response elements for FXR. In contrast, the nuclear receptor SHP-1, which lacks a DNA binding domain, is capable of repressing CYP7A1 expression by binding and repressing the transcriptional activity of liver receptor homolog (LRH)-1, another monomeric (116, 230) orphan nuclear receptor that acts as a competence factor and is required for constitutive CYP7A1 expression (Fig. 2). SHP-1 from mice, rats, and humans contains RXR/FXR binding sites in the promoter regions of all three genes (116). When HepG2 cells were transfected with FXR expression vectors controlled by a rat or human SHP-1 promoter, a potent synthetic nonsteroidal ligand (GW4064) increased expression of a luciferase reporter gene significantly, indicating that SHP-1 expression is directly regulated by FXR/RXR in these species (116). Bile acid feeding markedly upregulates the expression of SHP-1 mRNA while reducing the expression of CYP7A1 in the mouse (230). Thus bile salts are able to regulate their own synthesis from cholesterol by stimulating the expression of SHP-1, which in turn inhibits the expression of CYP7A1 by blocking the function of LRH-1. Bile salt synthesis may also be regulated by sterol 12α-hydroxylase (CYP8B1), an enzyme whose gene also contains an LRH-1 response element which may be targeted by SHP-mediated suppression (128). Thus the synthesis of bile salts is exquisitely regulated by the complex interaction of at least five nuclear receptors including RXR, LXR, FXR, SHP-1, and LRH-1 (Fig. 2). However, partial maintenance of negative feedback regulation of bile salt synthesis in SHP null mice suggests the existence of SHP-independent pathways (181, 374), indicating that redundant mechanisms regulate this critical step in bile salt homeostasis (311). Because bile salt feeding downregulates Ntcp expression in the mouse (326), it is likely that this downregulation results from SHP-1 inhibiting the expression of this bile salt transporter. Indeed, recent evidence suggests that inhibition of Ntcp promoter activity by cholic acid in vitro is mediated through bile salt-induced expression of the FXR target gene SHP-1 and subsequent direct inhibition of RXR, resulting in inhibition of retinoid transactivation of Ntcp by bile salts (85). These findings provide an explanation for downregulation of Ntcp expression in cholestasis (see sect. vii). To add to the complexity, SHP-1 can also inhibit transactivation of HNF-4 and RXR (223). Finally, ligands that activate or inhibit SHP-1 expression would be candidates for pharmacological manipulation of bile salt synthesis and of potential benefit in cholestatic liver disease.

Studies in primary rat hepatocytes indicate that taurolcholate as well as tumor necrosis factor (TNF)-α are strong activators of c-Jun NH₂-terminal kinase (JNK) and that SHP-1 is a direct target of activated c-Jun (122). Mutations in a putative activated protein-1 (AP-1) element in SHP-1 suppresses c-Jun-mediated activation of the SHP-1 promoter, indicating that bile acid activation of the JNK signaling cascade is important in regulating CYPA1 levels in rat hepatocytes (122). Hydrophobic bile salts are also potent inducers of cytokine expression (TNF-α and interleukin-1) in macrophages (252), indicating that bile salt-induced cytokines themselves could mediate part of the bile salt effects. However, these effects appear to be restricted to unconjugated bile salts. Importantly, both bile salts and cytokines are known activators of the JNK...
pathway resulting in the formation of AP-1 (122, 134). Of note, an AP-1 element has also been identified in the rat Isbt promoter, which could mediate bile salt effects on Isbt expression (333) (Fig. 2).

E) FTF (also known as LRH-1 or CYP7A1 promoter binding factor). A putative bile salt response element has been identified in the promoter region of human MRP3 isolated from Caco-2 cells. In this study, CDCA stimulated MRP3 mRNA threefold in a dose- and time-dependent manner. Bile salt response elements were identified in the region −229/−138 and included two FTF-like elements. Since MRP3 is located on the basolateral membrane of enterocytes, these findings suggest that bile salts may regulate their enterohepatic circulation from the intestine (147). Moreover, this mechanism could possibly also explain upregulation of Mrp3/MRP3 in hepatocytes and cholangiocytes under cholestatic conditions with bile salt retention. Preliminary studies suggest that an LRH-1 response element may regulate Isbt expression (63) and could mediate bile salt effects. Conversely, another study suggests that bile acid-induced FTF may suppress CYP8B1 expression, possibly by interfering with constitutive transactivation via HNF4α, which maintains basal CYP8B1 transcription (394).

F) SXR/PXR. The human SXR and its rodent ortholog PXR function as xenobiotic receptors that regulate the expression of CYP3A genes involved in detoxification pathways for many drugs (223, 390, 391). These cytochrome P-450 enzymes catalyze hydroxylation reactions that are usually the first step in drug detoxification pathways. Targeted disruption of the mouse PXR gene abolishes the ability of xenobiotics to induce CYP3A. Recent studies suggest that bile salts may also serve as functional ligands for SXR and PXR, thereby upregulating CYP3A enzymes (334, 393) and possibly transporters for hepatic uptake of drugs (e.g., Oatp2) (121) (Fig. 2). In contrast to FXR, the rank order of ligand potency is different: 3-keto-lCA > LCA > DCA = CA (334, 393). LCA is a potent cholestatic bile salt that produces severe hepatic necrosis when fed to mice. Treatment of mice with pregnolone-16-α-carbonitrile (PCN), a potent inducer of CYP3A, enhances the formation of hydroxylated metabolites of lithocholic acid and protects mice from the toxic effects of this bile salt (334, 393). This protective effect of PCN was abolished in PXR (+/−) mice (334, 393). Feeding LCA to PXR (+/−) mice also failed to change the expression of FXR and SHP, two other nuclear receptors previously implicated in bile acid repression of CYP7A1 expression (116, 230). Together these studies suggest that SXR and its rodent counterpart, PXR, may serve as functional bile salt receptors and facilitate upregulation of CYP3A, thereby enhancing metabolism of potentially toxic bile salts. While studies of SXR and PXR expression will be necessary in patients and animal models with cholestatic liver injury, the antibiotic rifampicin, another potent ligand for SXR and inducer of CYP3A, has been shown to be effective in the treatment of the symptoms of pruritus in cholestatic liver diseases (21, 56). It has been speculated that rifampicin may stimulate 6α-hydroxylation of bile acids, leading to glucuronidation by UDP-glucuronosyl transferases and excretion of bile salts by previously described alternative pathways in the urine (18, 47, 289, 385). This may also explain why cholestasis also improves in some patients treated with rifampicin (56). Studies in FXR/BAR nullizygous mice, where the expression of the bile salt export pump is diminished and hepatic levels of bile salts increase, report strong upregulation of CYP3A11 and CYP2B10. Mrp3 and particularly Mrp4 mRNA levels were also upregulated and enhanced by bile salt feeding in FXR knock-out mice (314). These studies support the notion that there are compensatory changes in hepatic transporters that mediate alternative pathways for bile salt excretion that are mediated by bile salts via nuclear receptors. Interestingly, the hydrophilic bile salts UDCA and its taurine conjugate TUDCA, which are weak activators of FXR, are potent stimulators of PXR and CYP3A4 in human hepatocytes, suggesting a possible mechanism for beneficial effects of UDCA in chronic cholestatic liver diseases (314).

SXR also activates MDR1 gene expression, mediating the effects of drugs (e.g., paclitaxel, Taxol) on the expression of P-glycoprotein (350). Other studies suggest that PXR is also involved in regulation of Oatp2 (334), explaining how microsomal-inducing chemicals upregulate Oatp2 expression (121, 128). Further evidence that PXR might regulate Mrp2 transcription is provided by studies on the effects of herbicides in mice in vivo (384) and xenobiotics (e.g., vincristine, tamoxifen, or the “classic” PXR-ligand rifampicin) in isolated rat hepatocytes in vitro (174). On the basis of these observations, it is likely that SXR/PXR mediate the regulation of Mrp2 and Mrp3 by drugs and various toxicins (e.g., cisplatin, rifamycin) (312). These findings also suggest that there is a highly coordinated regulation of hepatic endo-/xenobiotic (phase 1) metabolism (via Cyps) and excretion of their (phase 11) conjugated metabolites (via Mrp2). Thus a picture is beginning to emerge where SXR/PXR coordinate the regulation of uptake, metabolism, and efflux of drugs and potential toxins including bile salts when they accumulate in the liver.

G) Constitutive androstane receptor. Like FXR and PXR, constitutive androstane receptor (CAR) binds DNA as a heterodimer with RXRα but is highly and constitutively expressed in the liver. CAR is inhibited by androstan metabolites (for review, see Ref. 377). In contrast to other class II NHRs, CAR is also located in the cytoplasm and translocates to the nucleus after exposure to a diverse group of compounds including phenobarbital, which is the prototype (380). Recent studies indicate that CAR and PXR bind to common response elements in the
promoters of CYP2B and CYP3A with DR-3, D-4, or everted repeats with a 6-bp spacer. Thus these two signaling pathways appear to interact in the regulation of xenobiotic drug metabolism (346). FXR also binds with high affinity with RXR-α to response elements in the rat Mrp2 promoter that are shared with CAR and PXR (172). Thus bile salts as well as CAR and PXR ligands can regulate Mrp2 expression, since both naturally occurring (CDCA) as well as synthetic (GW4064) FXR ligands induce the expression of MRP2/Mrp2 mRNA in human and rat hepatocytes (172). Moreover, CAR activators have been shown to induce Mrps1–3 in rat liver but not in extrahepatic tissues such as kidney and intestine (65).

2. Role of other transcription factors in regulation of bile salt transporters

A) AP-1. Two AP-1 consensus sites have been identified in the proximal promoter of the rat ileal apical sodium-dependent bile salt transporter Isbt. Both c-jun and c-fos bind to these elements and enhance promoter activity in transfected cell lines (92).

B) HNF-1α. HNF-1α is a “liver-enriched” transcription factor that regulates transcription of several hepatocyte-specific genes including the bile salt transporter Ntcp and plasma proteins such as albumin, α1-antitrypsin, fibrinogen, etc. (for review, see Ref. 360). Two isoforms have been identified: HNF-1 (or HNF-1α) and vHNF (or HNF-1β). HNF-1α is predominant in hepatocytes but is also present in epithelial cells of other organs including renal proximal tubules, stomach, small intestine, colon, and pancreas, whereas HNF-1β has an even wider distribution. Both factors can form homo- and heterodimers that bind to a 15-nucleotide-long consensus sequence with a palindrome structure (A/GT/TAAT). HNF-1α homodimers are the major transcription factors in hepatocytes. HNF-1α is also regulated by HNF-4, another liver-enriched transcription factor (200). HNF-1α negatively regulates its own expression and that of other HNF-4-dependent genes by negative feedback inhibition of the main activation domain of HNF-4 (200). HNF-1α plays an important role in the constitutive expression of Ntcp (81, 170, 356). Expression of Ntcp, Oatp1, and Oatp2 are significantly reduced in the livers of HNF-1α knock-out mice (318), and Isbt is not expressed in the terminal ileum, leading to fecal bile salt loss. HNF-1α also has a direct role in activating transcription of the Isbt gene (318). HNF-1α also regulates the expression of the nuclear bile acid receptor FXR/BAR (318), which in turn plays a critical role in the transcriptional regulation of Bsep, SHP, and I-Babp.

Human HNF-1α is required for the expression of OATP-C (SLC21A6) as well as OATP8 (SLC21A8) and mouse Oatp4 (Slc21a6) (161). Thus HNF-1α seems to play a major role in bile salt transporter expression and homeostasis.

C) HNF-3β. Transgenic mice that overexpress HNF-3β have reduced hepatic levels of expression of Ntcp and Mdr2 (291). This finding could be explained by studies demonstrating that HNF-3β transactivates the liver-enriched homeobox gene (Hex), which in turn positively regulates the Ntcp promoter (84).

D) HNF-4. Recent studies in conditional gene knock-out mice for HNF-1α and HNF-4α report reduced levels of basolateral bile salt and organic anion uptake systems (Ntcp, Oatps), indicating a central role to these transcription factors in maintaining basolateral transporter expression (99, 133, 175). Part of these effects could indirectly be explained by HNF-4 effects on HNF1 expression (200).

E) Signal transducers and activators of transcription. Signal transducers and activators of transcription (STATs) consist of a family of signal transduction proteins activated by binding of extracellular polypeptides (growth factors and cytokines) to transmembrane receptors (for review, see Ref. 141). STAT5 activates the rat Ntcp promoter by binding to two closely spaced consensus sites that may explain upregulation of Ntcp expression by growth hormone and prolactin (103).

F) C/EBPs. C/EBPs are a family of leucine zipper transcription factors involved in the regulation of various aspects of cellular differentiation and proliferation, metabolic function (e.g., gluconeogenesis, carbohydrate metabolism), and response to inflammatory insults in hepatic, adipose, and hematopoietic tissues (288; for review, see Ref. 224). The human Ntcp and rat Ntcp promoters contain several putative C/EBP-α and -β binding sites (81, 170, 317). A C/EBP element is required for maximal retinoid transactivation of the rat Ntcp promoter by RXR-α:RAR-α (81). Further putative C/EBP binding sites have also been identified in the rat Mrdr1b and human MDR3 and MRP2 promoters (257, 340), suggesting that this transcription factor could play an important role in the regulation of hepatobiliary transporter expression.

G) PPAR-α. Recent studies have suggested a physiological role for PPAR-α in mediating human ISBT gene regulation (160). This is the first evidence that PPAR-α is involved in bile salt homeostasis by regulating intestinal bile salt uptake.

B. Posttranscriptional Regulation of Bile Salt Transporters

There is considerable evidence that several bile salt transporters in hepatocytes are regulated posttranscriptionally and that their cell surface expression can be stimulated by recruitment from intracellular pools (183).
1. Hepatocellular basolateral transporters

Studies in isolated rat hepatocytes indicate that cAMP stimulates sodium bile salt cotransport via a protein kinase A-mediated event that can be potentiated by increases in intracellular calcium and downregulated by activation of protein kinase C (120). This stimulatory effect of cAMP is associated with increased content of Ntcp in the plasma membrane and a decrease of Ntcp in endosomal compartments and does not involve protein synthesis (254). Subsequent studies showed that Ntcp is a serine/threonine phosphoprotein and that cAMP results in Ntcp dephosphorylation (255), possibly leading to retention of Ntcp in the plasma membrane and subsequent stimulation of Ntcp-mediated bile salt uptake. Further studies indicate that okadaic acid, an inhibitor of protein phosphatase 2A, prevents cAMP-stimulated translocation of Ntcp in part by decreasing cAMP-induced increases in cytosolic Ca\(^{2+}\). However, okadaic acid increased Ntcp phosphorylation without inhibiting basal taurocholate uptake. Therefore, increased phosphorylation due to inhibition of protein phosphatase 2A does not affect Ntcp activity. These studies suggest that cAMP-mediated signal transduction is maintained by protein phosphatases, but it remains unclear whether cAMP-mediated phosphorylation is due to inhibition of a kinase or activation of other protein phosphatases (255). Inhibitors of phosphatidylinositol (PI) 3-kinase and actin filament formation also block the ability of cAMP to translocate Ntcp to the plasma membrane and increase taurocholate uptake in rat hepatocytes (90, 378, 379). Oatp1 activity also can be mediated by alterations of its phosphorylation (114).

Phosphorylation of specific tyrosine residues on the cytoplasmic tail of rat Ntcp appears to be involved in targeting of this basolateral transporter to this specific plasma domain (347). Truncation of the 56-amino acid cytoplasmic tail inhibits delivery of a green fluorescent protein (GFP)-Ntcp construct to the plasma membrane in COS-7 and MDCK cells. Two tyrosine residues, Tyr-321 and Tyr-307, have been identified in site-directed mutagenesis experiments, with Tyr-321 representing the major basolateral sorting determinant (347).

2. Hepatocellular canalicular transporters

Current evidence suggests that several members of the superfamily of ABC transporters that are functionally expressed at the apical canalicular domain reside within submembranous endosomal pools that can be mobilized in response to several stimuli (Fig. 3) (45, 184, 185, 296). Studies in the isolated perfused rat liver indicate that infusions of cAMP increase the biliary excretion of bile salts by stimulating the intracellular vesicle transport system (131) and that simultaneous infusions of taurocholate further augment secretion of phospholipids and bile salts (132). Administration of dibutyryl cAMP to rat hepatocyte couples results in expansion of the apical domain as well as increased and enhanced canalicular excretion of fluorescent Mrp2 substrates. These findings were associated with enhancement of apical membrane expression of Mrp2 as assessed by immunofluorescence staining (296). Dibutyryl cAMP treatment also stimulates excretion of bile salt fluorescent substrates in hepatocyte couples (45). The effects of cAMP are acute and thus not dependent on protein synthesis. Inhibitors of microtubule function also block these effects, supporting a role for microtubules in this regulatory process.

Studies in the intact rat, using intravenous administration of dibutyryl cAMP as well as taurocholate, also demonstrate a rapid and selective increase in Mdr1 and Mdr2 as well as Mrp2 and Bsep in canalicular membrane vesicles isolated from these livers. This finding correlates functionally with increased ATP-dependent transport of substrates for these apical membrane transporters (109). This process is inhibited by colchicine as well as wortmannin, an inhibitor of PI 3-kinase, supporting a role for microtubules and PI 3-kinase in this process. Cycloheximide treatment has no effect on these phenomena, emphasizing that these are posttranscriptional events (248, 249). Additional studies in isolated canalicular membrane vesicles from rat liver suggest that 3′-phosphoinositide products of PI 3-kinase also have direct posttranslational effects on Mrp2 and Bsep but not Mdr1 transport activity (184, 248).

Taurocholate and cAMP appear to stimulate recruitment of ABC transporters from separate pools since simultaneous administration of these two agents augments the canalicular expression levels of these transporters above that observed with only a single agonist. Furthermore, these studies suggest that the majority of the bile salt export pump protein resides within an intracellular, presumably endosomal compartment, from which they are mobilized to the canalicular domain in response to metabolic need (185).

The bile salt tauroursodeoxycholic acid (TUDCA), which is widely used to improve bile secretory function in patients with intrahepatic cholestasis, also can stimulate the insertion of transport proteins into the canalicular apical membrane under certain physiological and cholestatic conditions. Studies in isolated rat hepatocytes and perfused livers indicate that TUDCA but not UDCA is capable of stimulating canalicular exocytosis by producing a sustained elevation in intracellular calcium in the cytosol (30, 31). This process involves depletion of inositol 1,4,5-trisphosphate (IP\(_3\))-sensitive microsomal Ca\(^{2+}\) stores by an IP\(_3\)-independent mechanism that leads to mobilization of extracellular sources of calcium through Ni\(^{2+}\)-sensitive Ca\(^{2+}\) channels in the plasma membrane (31). This sustained increase in intracellular calcium is associated with translocation of the Ca\(^{2+}\)-sensitive isoform, α-PKC, to the plasma membrane, a process that...
facilitates exocytosis (33). Studies in isolated perfused rat livers demonstrate that TUDCA can also reverse the cholestatic effects of taurolithocholate (31). TLCA translocates the calcium-insensitive isoform protein kinase C-ε (PKCε), phosphatidylinositol 3-kinase (PI3-K), and mitogen-activated protein kinases (MAPK). Hyperosmolarity and pathological stimuli such as endotoxin (lipopolysaccharide, LPS)/cytokines, biliary obstruction (CBDL), phalloidin, and oxidative stress result in retrieval of membrane transporters with a reduction of the number of functional transporters in the canalicular membrane. In addition to retrieval, impaired targeting with reduced insertion of transport proteins into the canalicular membrane may also contribute to cholestasis. The retrieved membrane transporters may initially undergo reinsertion from a subapical vesicular compartment, but later are degraded by the lysosomal or ubiquitin-proteasome pathway. Posttranslational modifications of transport proteins [e.g., phosphorylation (P), phosphoinositide (PI)-3-kinase products (PI-P1,3)] may rapidly modify transport activity. Cis-inhibition [from cytoplasmic side, e.g., cyclosporin A (CSA) and other drugs] and trans-inhibition [from canalicular side, e.g., estradiol 17β-glucuronide (E217G)] of efflux pumps may be particularly relevant for drug-induced cholestasis.

Other studies in rats indicate that TUDCA also stimulates the bile excretory mechanism by enhancing the canalicular membrane transport of bile salts (99, 130). This process is thought to be mediated by an increase in bile salt excretory capacity, which is regulated by microtubule-dependent apical targeting of vesicles that contain the bile salt transporters and is dependent on signaling pathways that involve mitogen-activated protein (MAP) kinases (211, 309). In perfused rat liver, TUDCA activates signaling pathways that involve PI 3-kinase, Ras, a small monomeric GTP binding protein (211, 248), and extracellular signal-regulated kinase (Erk)-type MAP kinases. These stimulatory effects of TUDCA on taurocholate excretion can be blocked by inhibitors of PI 3-kinase. Recent studies suggest that TUDCA-induced stimulation of bile salt excretion is accompanied by p38 MAP kinase-dependent insertion of the bile salt export pump, Bsep, from subcanalicular regions into the apical canalicular domain of hepatocytes (212).

In mice, UDCA feeding results in upregulation of overall expression of canalicular Bsep and Mrp2 without any effect on Mdr2 or basolateral Ntcp (99). Both bile salt and glutathione excretion were also enhanced, indicating that these findings are functionally relevant. Changes in overall protein expression may go hand in hand with stimulation of their insertion into the canalicular membrane. Thus a complex signaling pathway, which is not yet fully elucidated, appears to be involved in the regulation
of intracellular trafficking of the bile salt transporter proteins.

The signals for retrieval of canalicular transport systems have been less well studied. Hyperosmolarity and pathological stimuli such as endotoxin (lipopolysaccharide, LPS)/cytokines, biliary obstruction (CBDL), phalloidin, and oxidative stress result in retrieval of membrane transporters with a reduction of the number of functional transporters in the canalicular membrane (202, 278, 298, 310). In addition to retrieval, impaired targeting with reduced insertion of transport proteins into the canalicular membrane may also contribute to cholestasis. The retrieved membrane transporters may initially undergo reinsertion from a subapical vesicular compartment, but the latter are degraded by the lysosomal or ubiquitin-proteasome pathway (for review, see Refs. 184, 185) (Fig. 3). Anchoring proteins (e.g., radixin), which cross-link transporters with actin filaments, are essential for proper localization of transporters as suggested by defective bilirubin excretion and loss of Mrp2 from the canalicular membrane in radixin-deficient mice (182).

III. BILE SALT TRANSPORT DEFECTS IN CHOLESTATIC LIVER INJURY

As the molecular mechanisms of bile formation and bile salt transport have become clearer, they have provided additional insight into the mechanisms of cholestasis. However, this pathophysiological process is complex and may result not only from defects in the regulation and expression of the membrane transporters that determine the bile secretory process but also numerous intracellular events that impair signal transduction pathways, alter the function of cytoskeletal proteins, affect the expression/localization of tight and gap junctional proteins, and modify the targeting of intracellular vesicles that maintain cell polarity. The focus of this section is on the effects of hereditary and acquired forms of cholestatic liver injury on the expression of membrane transporters that transport bile salts (see Refs. 41, 154, 220, 258, 358 for additional information on the molecular pathophysiology of cholestasis).

A. Genetic Defects in Bile Salt Transport Proteins (FIC1, BSEP, MRP2) and MDR3

Mutations in genes that code for several canalicular membrane transporters result in inherited progressive cholestatic liver disorders in infancy known as progressive familial intrahepatic cholestasis (PFIC) (153, 154) (Fig. 4). Although rare homozygous recessive disorders, these diseases provide critical information concerning the important role that these transporters play in the normal formation of bile and the pathogenesis of cholestasis. One of these transporters is the canalicular bile salt export pump (BSEP), another is a phospholipid export pump (MDR3), whose function is critical to the formation of bile salt mixed micelles in bile, and the third (FIC1) is without known function. Mutations in these transporters (FIC1, BSEP, and MDR3) account for the phenotypic expression of PFIC-1, -2 and -3.
1. Fic1/FIC1

Homozygous mutations in FIC1 result in PFIC-1 described in an Amish kindred and known as Byler’s disease (72, 96, 335, 361) and in Inuit populations in Greenland (187). Mutations in the same genomic regions are also associated with a rare benign form of intrahepatic cholestasis in adults known as benign recurrent intrahepatic cholestasis, or BRIC, and found primarily in Scandinavian families (50, 364). Mutations in this gene result in a profound reduction of bile salts in bile, which is most pronounced for hydrophobic toxic bile salts such as lithocholate and chenodeoxycholate. PFIC-1 is a progressive ultimately fatal pediatric cholestatic disorder characterized by normal γ-glutamyltransferase levels in the serum, the absence of bile duct proliferation on liver histology, as well as frequent extrahepatic manifestations such as diarrhea, malabsorption, and pancreatitis. Electron microscopic studies reveal highly characteristic lesions of the canalicular membrane as well as accumulation of intracellular membrane components within the canalicular lumen known as “Byler’s bile.” Symptoms of diarrhea persist after liver transplantation, indicating a more general role of FIC1 in the (intestinal) defense against toxic bile salts. In line with such a hypothesis, preliminary/recent findings indicate that the FIC1 knock-out mouse accumulates bile salts in serum while maintaining normal bile salt excretion, suggesting that the primary defect is in regulating intestinal bile salt absorption (283).

It remains to be determined how mutations in the same regions of the gene can result on the one hand in a progressive fatal cholestatic disorder of infancy while on the other account for a benign recurrent cholestatic process in adults. Equally unclear is the finding that some members of BRIC families have mutations that result in cholestatic disease while others with similar mutations do not express the cholestatic phenotype. These apparent paradoxes suggest that other genetic and/or environmental factors may be required as a “second hit” for the cholestatic phenotype to be expressed.

2. Bsep/BSEP

Mutations in the canalicular membrane bile salt export pump (BSEP), formerly known as the sister of P-glycoprotein, account for the syndrome of PFIC-2 (344). This important discovery provides compelling evidence for the role of this member of the ABC gene family in determining bile salt-dependent bile flow. More than 30 mutations have now been described in families with this familial disorder (52). Patients with PFIC-2 resemble Byler’s disease phenotypically, but these families are unrelated to the Amish Byler family. PFIC-2 has been described in families from Saudi Arabia and Europe. A recent report indicates that BSEP mutations may also present as a compound heterozygote in an adolescent with BRIC (209), suggesting that different mutations may account for differences in severity of the disease. Again, it is likely that environmental factors also influence the phenotypic expression of these mutants.

A recent study has examined the effect of seven of the PFIC-2 associated missense mutations when introduced into rat Bsep and expressed in MDCK and Sf9 cells (375). G238V, E297G, G982R, R1153C, and R1268Q mutations prevent the protein from trafficking to the apical membrane, whereas the G238V mutant seems to be rapidly degraded by proteasomes. Most but not all of these mutations (C336S and D482G) also abolish bile salt transport activity when assessed in Sf9 cells (54, 314, 375, 376).

3. Mdr2/MDR3

Patients with PFIC-3 have mutations in MDR3 that encode for the phospholipid export pump at the canalicular membrane. This protein functions as a phospholipid flipase and translocates phosphatidylethanolamine from the inner to the outer surface of the canalicular membrane bilayer. Bile salts are also not substrates for Mdr2/MDR3, yet when this phospholipid export pump is deficient, bile salts do not form micelles and can result in damage of cholangiocytes and progressive cholestatic liver injury (PFIC-3) (80, 87).

The pathophysiological consequences of this genetic defect were first established in the Mdr2 knockout mouse (327). These animals have a complete deficiency of phospholipid in bile and eventually develop a portal inflammatory response and bile ductular proliferation. Fibrosis ensues, and with time these animals develop a sclerosing cholangitis and biliary-type cirrhosis and hepatocellular carcinomas (239, 327). Expression of the human MDR3 in Mdr2 (−/−) animals restores the normal phenotype and the ability of the mice to excrete phospholipid (329). Transplantation of Mdr3 transgenic hepatocytes into Mdr2 (−/−) mice also corrects this abnormality (86). Lipoprotein-X is an abnormal lipoprotein that accumulates in the circulation in cholestatic liver injury, probably as a result of the regurgitation of bile into plasma. However, this lipoprotein is absent in the bile duct-obstructed Mdr2 (−/−) mouse. These studies indicate that phospholipids must first be excreted into bile for this abnormal serum lipoprotein to be formed (275).

Several recent studies have reported MDR3 mutations in children and teenagers with progressive cholestasis and intrahepatic gallstones (151). Recent studies have emphasized the wide phenotypic spectrum of MDR3 deficiency ranging from neonatal cholestasis and cholestasis of pregnancy to cirrhosis in adults (151). Children with missense mutations have a less severe disease and more often benefit from UDCA (151). These mutations led to truncations and absence of the MDR3 protein at the canalicular membrane in some and to single-base pair
changes in others. Only individuals with the latter defect (missense mutations) responded clinically to treatment with the bile salt UDCA, probably because the protein could still be expressed. Mdr2 heterozygote (+/−) mice excrete half the normal level of phospholipids into bile but are phenotypically normal. Mothers of homozygous MDR3-deficient children with PFIC-3 are also obligate heterozygotes. This partial defect in phospholipid excretion predisposes them to the development of cholestasis during the third trimester of pregnancy when estrogen levels rise and further impair bile secretion (150). MDR3 deficiency may account for one-third of patients with high GGT-PFIC (PFIC-3) in European patients (151) but only 2% of Taiwanese infants with this syndrome (64), suggesting a role for additional gene defects. Hereditary MDR3 defects are also involved in intrahepatic and gallbladder cholesterol gallstone formation and the pathogenesis of cholestasis of pregnancy (67, 151). MDR3 deficiency may be linked to cholesterol gallstone formation through defective biliary excretion of phospholipids resulting in cholesterol supersaturation of bile. Reduced MDR3 levels have also been found in oriental patients with intrahepatic bile duct stones, suggesting a role for heterozygotes with MDR3 defects in the pathogenesis of this syndrome (324).

These uncommon disorders emphasize the importance of the normal function of the phospholipid export pump and the relevance of mutations in this transporter to the pathogenesis of certain cholestatic diseases. Polymorphisms in the expression of MDR3 might predispose individuals to cholestatic liver injury, particularly if they become exposed to drugs or environmental toxins that are potential cholestatic agents. A few studies in patients with primary biliary cirrhosis and alcoholic cholestatic liver disease report normal MDR3 mRNA levels (93, 401, 403). The only acquired disease with abnormal (reduced) biliary phospholipid excretion identified in adults so far is TPN-induced cholestasis, although no information on MDR3 expression data is available so far (88). Sclerosing cholangitis-like changes in Mdr2 (+/−) mice (99a, 239) suggest that MDR3 defects could be involved in adults with PSC. Further studies are needed to determine the role that MDR3 mutations and polymorphisms play in idiosyncratic cholestatic disorders in the adult.

4. Mrp2/MRP2

The mutant TR−/−/GY/EHBR rats have mutations in the Mrp2 gene that result in a stop codon and premature termination of protein translation (171, 282, 369). In humans, mutations in MRP2 produce the Dubin-Johnson syndrome, a cause of hereditary conjugated hyperbilirubinemia. Mutations in this gene in the rat model and humans result in defects in the excretion of a variety of amphipathic organic anions, including divalent bile acids, conjugated bilirubin, coproporphyrin isomer series 1, leukotrienes, as well as many other compounds such as BSP, indocyanin green, oral cholecystographic agents, antibiotics such as ampicillin and ceftriaxone, and heavy metals. When several of the MRP2 mutant genes described in Dubin-Johnson syndrome are expressed in HepG2 cells, the mutated protein is unable to be target to the apical membrane but is retained within the endoplasmic reticulum and subsequently degraded in proteosomes (177).

GSH, oxidized GSSG, and GSH conjugates are endogenous substrates for Mrp2. GSH is a low-affinity substrate for Mrp2 and a major determinant of bile salt-independent bile flow that is significantly reduced in the TR−/−/GY/EHBR rat model (157). It is likely that polymorphisms in Mrp2/MPR2 and agents that impair the Mrp2/MRP2 transporter expression and function will diminish GSH excretion and lead to cholestatic injury. In addition, such mutations could also indirectly result in cholestatic through impaired excretion of potentially cholestatic metabolites of drugs and hormones. Mrp3/MPR3 expression is also increased after common bile duct ligation in the rat and in hepatocytes in patients with the Dubin-Johnson syndrome and the TR−/−/GY/EHBR rat where canalicular expression of Mrp2 is genetically absent (136, 192). These responses may be viewed as a protective adaptation in situations where MRP2/Mrp2 function is impaired (89).

B. Acquired Defects in Bile Salt Transport Proteins in Cholestasis

Studies in several experimental models of cholestasis including common bile duct ligation (CBDL), estrogen/ethinyl estradiol (EE), and endotoxin (LPS) administration as well as more limited studies in acquired cholestatic diseases in humans provide information on the effects of this form of liver injury on the expression of bile salt transport proteins in the liver and other tissues (for review, see Ref. 220). In general, there is a common pattern of responses that serves to partially protect the hepatocyte from the retention of toxic bile salts, but also contributes to/aggravates systemic bile salt retention (Table 3, Fig. 5). The transport of bile salts from portal blood into hepatocytes is diminished by downregulation of several bile salt transporters on the basolateral membrane including Ntcp, Oatp1, and r-Lst1 (incomplete splice isoform of Oatp4) but not Oatp2 and Oatp4. At the same time, bile salt export mechanisms in hepatocytes continue to be expressed (Bsep) and in some instances may be upregulated (Mrp3) (89, 331). Mrp3 should be able to transport bile salts back into the systemic circulation, since Mrp3 has a high affinity for lithocholate sulfate and other bile salt conjugates that accumulate in the cholestatic liver (138).

Cholangiocyte bile salt transporters are also affected during cholestasis (Table 3, Fig. 5). Bile ducts proliferate
in most cholestatic conditions, resulting in a marked increase in the number of cholangiocytes. The ileal sodium-dependent bile salt transporter (Isbt), located on the luminal membrane of cholangiocytes, may function to remove bile salts from bile if the cholestatic hepatocyte continues to excrete bile salts. Mrp3 is located on the basolateral membrane of the cholangiocyte and continues to be expressed on proliferating cholangiocytes (331). As a result of this proliferative activity, the total amount of this transporter is upregulated in the cholestatic liver.

Changes in the expression of several renal and intestinal bile salt transporters (Ibst and Mrp2) also take place during cholestasis, thereby facilitating the urinary and intestinal excretion of bile salts and bilirubin (219, 352). Altogether, these changes in expression of liver, kidney, and intestinal bile salt transporters can be interpreted as an attempt to mitigate tissue damage from hepatic and systemic retention of bile salts (Table 3, Fig. 5).

Adaptive regulation of the expression and function of these membrane transporters occurs by several different mechanisms, including 1) alterations in gene transcription mediated by elements that interact with regulatory elements in gene promoters as described in more detail in section II (Table 3); 2) posttranscriptional changes in mRNA processing or message stability; or 3) posttranscriptional changes that result either in defects in protein

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<th>Transporter</th>
<th>Change in Expression During Cholestasis</th>
<th>Proposed Mechanisms*</th>
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<tr>
<td>Ntcp/NTCP</td>
<td>Downregulation</td>
<td>Ntcp gene expression</td>
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<td>Oatps/OATPs</td>
<td>Downregulation of rodent Oatp1 and human OATP2</td>
<td>IL-1β-mediated downregulation of RXR/RAR NHR</td>
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<tr>
<td>Mrp3/MRP3</td>
<td>Highly upregulated on basolateral membrane of hepatocytes in cholestasis</td>
<td>Transcriptional regulation in hepatocytes not yet determined</td>
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<tr>
<td>Isbt</td>
<td>Expression maintained after common bile duct obstruction</td>
<td>Not known but may be related to bile salt binding to FXR resulting in upregulation of the ileal-bile acid binding protein that associates with Ibst</td>
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<td>CFTR</td>
<td>Not known</td>
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<td>AE2</td>
<td>Continues to be expressed in bile duct proliferation after bile duct obstruction, reduced in PBC</td>
<td>Not known</td>
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<td>Mrp3/MRP3</td>
<td>Continues to be expressed after common bile duct ligation in rodents and PBC in humans</td>
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<tr>
<td>Oatp3</td>
<td>Not known</td>
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<tr>
<td>Isbt</td>
<td>Downregulated after common bile duct obstruction</td>
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<td>Mrp3/MRP3</td>
<td>Continues to be expressed after common bile duct obstruction</td>
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<tr>
<td>Mrp2</td>
<td>Upregulated by posttranscriptional mechanisms after common bile duct ligation</td>
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Data are from animal models and cholestasis in patients. PBC, primary biliary cirrhosis; RAR, retinoic acid receptor; FXR, farnesoid X receptor; SHP, short heterodimeric protein; IL, interleukin; RXR, retinoid X receptor; NHR, nuclear hormone receptor; HNF, hepatocyte nuclear factor.

* Most of these proposed mechanisms of regulation in cholestasis remain to be confirmed by in vivo studies in cholestatic animal models and in humans and are inferences based on in vitro studies and knowledge of their physiological regulators.
trafficking or the regulation of the proteins activity though phosphorylation/dephosphorylation reactions or direct inhibition by substrates/compounds (Fig. 3). Because the half-life of these membrane transporters is several days, acute changes in the activity of transporter function are likely to occur by a posttranscriptional mechanism, whereas transcriptional regulatory responses would be expected in chronic adaptive responses.

1. Effects of cholestasis on basolateral membrane bile salt transporters in hepatocytes (Ntcp/NTCP, Oatps/OATPs, Mrp1/MRP1, Mrp3/MRP3)

A) Ntcp/NTCP. This transporter is uniformly downregulated in all experimental models of cholestasis and in cholestatic liver disease in humans (220). Nuclear run-on studies in the CBDL and LPS-treated rat indicate that this downregulation occurs at least in part at the level of gene transcription (106, 356). After inhibition of gene transcription, Ntcp protein disappears rapidly from the sinusoidal membrane throughout the hepatic lobule. In children with biliary atresia, Ntcp mRNA is diminished but increases if biliary drainage is restored by a successful portoenterostomy (Kasai procedure) (321). Levels of hepatic Ntcp mRNA correlate inversely with bile salt levels in bile duct-obstructed rats and humans, respectively (108), and with levels of total serum bilirubin in patients with biliary atresia (321). These changes in Ntcp expression are not reproduced by choledochocaval fistulas or by partial bile duct ligation, suggesting that bile retention rather than the magnitude of bile salt flux is important in regulation of Ntcp gene transcription (108). However, cholate feeding to mice reduces expression of Ntcp, suggesting that endogenous bile salts retained during cholestasis could also be involved in the regulation of Ntcp gene expression (99, 326). The promoter of Ntcp in the rat contains several regulatory elements including HNF-1, as well as an RXR/RAR retinoic acid element. Bile salt-induced SHP-1 inhibits retinoid transactivation (via RXR:RAR) of the rat Ntcp promoter in vitro (85) (Table 3). Along these lines, bile duct ligation in mice induces SHP-1 expression in parallel with accumulation of serum bile salt levels and precedes downregulation of Ntcp by several hours (402). Cholestatic doses of LPS result in loss of HNF-1 and RXR:RAR activities in rat liver and decrease Ntcp expression in bile ducts to the systemic circulation followed by their renal excretion. Enterohepatic recirculation of BS+ is impaired as a result of reduced hepatic excretion and decreased expression and function of ileal Isbt.
parallel with reductions in bile flow (356) (Table 3). Reductions in RXR mRNA levels are also part of the acute phase response. In hamster liver, LPS and proinflammatory cytokines, including interleukin-1β and TNF-α, induce a rapid dose-dependent decline in three distinct RXR mRNAs and proteins RXR-α, -β, and -γ within several hours (24). Stress pathway activation induces phosphorylation of RXR via both mitogen-activated protein kinase (223, 226). Finally, LPS also reduced kinase-4 and its downstream mediator JNK, resulting in phosphorylation of RXR via both mitogen-activated protein kinase (24). Stress pathway activation induces phosphorylation of Ntcp and Mrp2 observed in models of cholestatic liver injury. Finally, preliminary studies indicate that EE administration downregulates expression of genes containing RXR/RAR response elements. Vitamin A stores are diminished in cholestatic liver diseases (269) and could influence the reduced expression of Ntcp and Mrp2 observed in models of cholestatic liver injury. Finally, preliminary studies indicate that CBDL in the rat results in rapid loss of RXR, RAR-α, and SHP nuclear protein and that these acute effects may be mediated in part by cytokines, particularly interleukin-1β (36, 82).

B) Oatp1, -2, -4/OATP-A and -C. Studies in rat models of CBDL, EE, and LPS administration indicate that all of these cholestatic models result in downregulation of Oatp1 (220). mRNA levels remain unchanged after EE treatment, suggesting that EE administration downregulates Oatp1 mainly by posttranscriptional mechanisms (325). The expression of Oatp2, which is higher in the brain than the liver, and Oatp4 is not reduced after CBDL in the rat (107). This finding is in contrast to the expression of the liver-specific transporter (rlst1), the incomplete splicing isoform of Oatp4, which is markedly diminished after CBDL in the rat (164). In contrast to these findings, clinical studies in patients with primary sclerosing cholangitis indicate that OATP-A mRNA expression is increased, suggesting that OATP-A may be able to function in reverse in cholestasis and help to extrude organic anions from the cholestatic hepatocyte (204). However, OATP-C is reduced in patients with alcohol and inflammation-induced cholestatic liver injury (40, 403) and primary sclerosing cholangitis (PSC) (270) and primary biliary cirrhosis (PBC) (401). Further studies will be necessary to clarify the functional implications of these changes in expression of Oatp/OATPs in cholestasis considering the considerable overlap in substrates. Preservation of some Oatps/OATPs under cholestatic conditions (e.g., rat Oatp2 and -4 in CBDL rats; human OATP-A in PSC) could mediate monovalent bile salt efflux by reversal of the transport direction, although this remains highly speculative. The relative contributions of maintained Oatps/OATPs and upregulated Mrps/MRP s to basolateral efflux of monovalent and divalent bile salt conjugates under cholestatic conditions remain to be determined (210).

C) Mrp1/MRP1, Mrp3/MRP3. Mrp1 is substantially upregulated during cholestasis produced by LPS (367), while Mrp3 is induced in common bile duct obstruction but not in EE-induced cholestasis. Upregulation of MR P3 is also seen in patients with obstructive cholestasis (323) and PBC (401). Studies in the rat indicate that Mrp3 expression is normally expressed only on pericanalicular venous hepatocytes but is progressively upregulated after CBDL so that by 14 days, Mrp3 is expressed throughout the hepatic lobule (331). This increase in Mrp3 parallels the progressive downregulation of the canalicular homolog Mrp2. Mrp3/MRP3 expression is also increased in hepatocytes in patients with the Dubin-Johnson syndrome and the TR−/GY/EHBR rat where canalicular expression of Mrp2/MPR2 is genetically absent (136, 192, 282, 369). In addition, Mrp3 is also upregulated in FXR (−/−) mice with reduced canalicular Bsep levels (314). These observations suggest that Mrp3 may facilitate efflux of substrates into sinusoidal blood that normally would be extruded into bile by Mrp2/MPR2 (314). Because sulfated bile salt conjugates are synthesized in the cholestatic liver and are high-affinity substrates for Mrp3 as demonstrated in vitro in cellular expression systems (138), we speculate that Mrp3/3MRP3 may function as an important efflux pump in cholestasis, thereby protecting hepatocytes from the accumulations of toxic levels of bile salts (138). In summary, upregulation of basolateral MR P3 during cholestasis may not only explain the appearance of conjugated bilirubin in plasma, but may also provide an alternative efflux route for bile salts from hepatocytes into the sinusoidal blood which together with downregulation of basolateral uptake systems (NTCP and OATP2) prevents further accumulation of potentially toxic biliary constituents, particularly bile salts within cholestatic hepatocytes (219). Recent data suggest that the inverse changes of reduced NTCP and induced MR P3 expression spatially and temporally coincide and are most pronounced in the periphery of liver lobules and cirrhotic nodules in PBC livers (401).

2. Effects of cholestasis on bile salt transporters on the canalicular membrane of the hepatocyte (Bsep/BSEP, Mrp2/MPR2, Mdr2/MDR3, Fic1/FIC1)

A) Bsep/BSEP. In contrast to downregulation of Ntcp and Mrp2 in animal models of cholestasis (LPS, EE, CBDL), Bsep expression is only modestly impaired and bile salt excretion continues, albeit at reduced levels, even in the face of complete bile duct obstruction (221). In contrast, many cholestatic agents, including cyclosporin A, glibenclamide, rifamycin, rifampin (336), triggilitzone (102), and bosentan (97) cis-inhibit ATP-dependent taurocholate transport in vitro in isolated rat liver
canalicular membrane vesicles. Some compounds (estradiol 17β-glucuronide) trans-inhibit Bsep in rat liver only after excretion into bile as evidenced by the absence of cholestatic effect in TR−/GY/EHBR mutants which lack Mrp2 (143). This estrogen metabolite also fails to inhibit Bsep when Bsep is expressed in an Sf9 insect cell line, unless Mrp2 is also coexpressed (336). Both murine and human Bsep/BSEP contain FXR response elements in their promoter, suggesting that increases in liver levels of bile salts that are specific ligands for FXR may help to maintain Bsep expression during cholestatic liver injury. Bsep protein expression is also maintained in normal amounts and location in clinical studies in patients with biliary atresia (188) and PBC (401). Together these observations suggest that expression levels of members of the Mdr/MDR P-glycoprotein family (Bsep, Mrd1, and Mdr3; see below) are relatively well preserved during cholestatic liver injury, compared with other canalicular proteins such as Mrp2 or the basolateral bile salt transporters Ntcp and Oatp1.

B) Mrp2/MDR2. Expression of Mrp2 at the mRNA and protein level is markedly downregulated in all experimental animal models of cholestasis including CBDL, EE, and LPS (220). Impairment of Mrp2 expression and transport function in endotoxin models of cholestasis provides an explanation for why sepsis commonly results in elevation of serum levels of conjugated bilirubin (357). Reduced Mrp2 mRNA expression may be explained through impaired retinoid transactivation via RXR-RAR, which may be inhibited via bile acid-induced SHP-1 or cytokine-mediated downregulation of RXR transcription and RXR phosphorylation. Reductions in both liver RXR-α and RAR RNA, nuclear protein levels, and DNA binding to the Mrp2 promoter cis-levels in liver contrast with their maintenance of expression in the kidney and may account for the organ-specific adaptive regulation seen following CBDL in the rat (36, 82, 83). Nevertheless, studies of MRp2 expression in patients with cholestatic alcoholic hepatitis suggest that MRp2 mRNA is not significantly reduced in this inflammatory disorder (403). In line with these observations in vivo, LPS also fails to downregulate MRp2 expression in human liver slices despite being biologically active as indicated by induction of nitric oxide and proinflammatory cytokines (95). Similar observations have been made in late-stage PBC (401). These findings emphasize that observations made in cholestatic animal models may not necessarily always apply to cholestatic disorders in humans. However, recent findings that FXR response elements are contained within the MRp2 promoter suggest that bile salts should be capable of upregulating this transporter in humans (172).

C) Mdr1a,b/MDR1. While bile salts are not nominal substrates for this P-glycoprotein, and no mutations have been described to date that result in cholestatic liver disease, certain drugs and substrates for Mdr1/MDR1 may also inhibit the bile salt export pump if they accumulate in the liver. Interestingly, unlike most basolateral membrane transporters that are downregulated in response to cholestatic liver injury, the molecular expression of P-glycoprotein-170 (Mdr1/MDR1) is upregulated in both animal models of cholestasis and in cholestatic disorders in humans (220). Cholestasis induced by CBDL, LPS, or α-naphthlisothianate (ANIT) all increase the expression of Mdr1 mRNA (313, 357, 367). The level of expression correlates with the severity of the cholestasis, as manifested by the height of serum levels of plasma bilirubin and alkaline phosphatase (313). Levels of MDR1 in biopsies from patients with bile duct obstruction, inflammatory cholestasis (403), and PBC reveal similar findings (266, 401). Exogenous stimuli including heat shock, ultraviolet light, chemotherapeutic agents, carbon tetrachloride, and carcinogens also increase the transcription of the Mdr1 gene promoter (261). Recent studies suggest that Mdr1 can be upregulated through the action of cytokines such as TNF-α, which initiates a signal transduction cascade that results in translocation of NFκB to the nucleus and stimulation of Mdr1 gene transcription (297).

Unlike other canalicular export pumps, genetic defects in hepatic MDR-1 P-glycoprotein (170) have not been identified in humans. Bile flow also remains normal in knockout mouse models of Mdr1a and Mdr1b, and organic cation excretion is only modestly impaired. However, organic cation transport is significantly impaired in double knock-out [Mdr1a,b (−/−)] mice despite normal bile production (328).

Many drugs (nearly 80% by some estimates) are organic cations and are substrates for Mdr1/MDR1. Thus it is possible that polymorphisms in the expression of Mdr1/MDR1 might diminish the capacity of the liver to excrete these substrates and lead to increased retention of these compounds in the hepatocyte (35). Cationic drugs normally excreted via Mdr1/MDR1 such as cyclosporin A, rifampin, and rifamycin competitively inhibit the activity of the bile salt export pump both in isolated rat canalicular membrane vesicles (336) and when expressed in Sf9 insect cells (336). Whether polymorphisms in MDR1 lead to hepatic retention of these cationic drugs and inhibition of Bsep in human liver remains to be established.

D) Mdr2/MDR3. Most studies indicate that the expression of Mdr2/MDR3 is not altered in acquired forms of cholestasis in humans and rats (41, 220). However, polymorphisms in the expression of MDR3 may predispose to acquired cholestatic disorders as discussed in the genetics section.

E) Fic1/FIC1. This transporter is unchanged in experimental (CBDL) and acquired human cholestasis (96, 335, 403).
3. Adaptive responses of bile salt transporters in cholangiocytes

It is not clear if Isbt in the luminal membrane of cholangiocytes has a significant function under normal physiological conditions, but its expression is upregulated when bile ducts proliferate in the bile duct obstructed rat (219). Under these circumstances Isbt may function to remove bile salts from the obstructed biliary lumen in response to continued excretion of bile salts at the canalicular membrane of hepatocytes (Fig. 5). Recent studies indicate that isolated large cholangiocytes from rat liver are capable of sodium-dependent taurocholate uptake and that during bile duct obstruction, Isbt expression is also induced on the luminal membrane of small cholangiocytes (9, 225). Isbt protein levels are also increased in total liver homogenates after 2 wk of bile duct ligation when cholangiocyte proliferation is pronounced (219). These findings suggest that bile duct proliferation may be an adaptive response to the retention of toxic bile salts and feedback to facilitate bile salt clearance from the obstructed biliary lumen (25, 217, 219, 225).

Mrp3/MRP3 is also normally expressed at the basolateral membrane of rat and human cholangiocytes and remains at this location as cholangiocytes proliferate during cholestasis induced by bile duct obstruction (137, 194, 331). Given the high affinity of Mrp3 for cholestatic bile salts, it is likely that Mrp3/MRP3 plays the major role in the return of bile salts from cholangiocytes to the systemic circulation when they are reabsorbed from the biliary lumen (67, 113). MDR1 is also strongly expressed in proliferating ductules from patients with PBC where it might have a protective role (401). Although mutations have not been identified in human MDR1 and bile salts are not substrates for this transport protein, altered patterns of expression of this transporter might reduce the capacity of the cholangiocyte to defend itself from toxic compounds. In such circumstances cholangiopathies might develop.

4. Adaptive responses of bile salt transporters in kidney

Bile salt secretion in urine is markedly increased in both cholestatic animal models and in clinical cholestatic disorders (71, 219, 338, 388). Recent studies in bile duct-ligated rats indicate that Isbt mRNA and protein are downregulated, resulting in reduced levels of expression on the luminal membrane of the proximal tubule of the rat kidney, a change that is associated with a diminished capacity of rat renal brush-border membranes in the kidney to reabsorb bile salts from the glomerular filtrate (219). Mrp2 is also located at the apical membrane of the proximal tubule (305, 306). However, in contrast to Isbt, Mrp2 protein expression is upregulated on the apical membrane of the rat renal proximal tubule (219) and is associated with an increased ability to excrete the Mrp2 substrate para-aminohippurate (352) (Fig. 5). Conjugated bilirubin, tauroliothocholate sulfate, and bile all increase Mrp2 expression in renal proximal tubular epithelial cells (352). This adaptive response would facilitate the renal excretion of divalent organic anions such as bile salt sulfates and glucuronides that accumulate during cholestasis. Mutations in the Isbt gene in humans result in primary bile salt malabsorption at the terminal ileum (267), but the effect of these mutations on cholangiocyte or renal handling of bile salts is not known. Nevertheless, reciprocal differences in expression of Isbt and Mrp2 between cholangiocytes and kidney suggest that there may be tissue-specific factors that regulate the expression of these two bile salt transporters. Preliminary studies indicate that cholestasis induced by CBDL in the rat results in the rapid loss of the NHRs, RXR-α, FXR, and SHP expression in the liver but not the kidney where renal levels of FXR and SHP RNA and protein were increased (82). Thus differences in transporter expression in liver and kidney may be related to different patterns of NHR responses to cholestasis in the two tissues.

5. Adaptive responses of bile salt transporters in intestine

Studies on the effects of bile diversion and bile duct obstruction on the pattern of expression of this transporter have been conflicting, with some showing down-regulation (304) and others not (20). Isbt is not regulated by the NHR FXR. However, bile salts do effect the expression of the ileal bile salt binding protein, I-Babp, a 17-kDa protein that contains an FXR response element in its promoter (118). FXR nullizygous mice do not upregulate I-Babp when fed excessive amounts of cholic acid compared with the wild-type control animals (326). Because I-Babp binds to the cytoplasmic domain of Isbt (199), it is likely that bile salts can regulate the functional expression of the bile salt transporter through effects on the expression of I-Babp (65, 300, 348). A recent study suggests that MRP3 expression in enterocytes is induced by bile acids via LRH-3/FTF (147).

6. Placenta

Maternal cholestasis (e.g., CBDL in pregnant rats) impairs bile salt transfer from the fetus to the mother by impairing bile salt transport at both the basolateral (fetal-facing) and apical (maternal-facing) membrane (232, 315). The molecular basis remains to be clarified. Some of the functional changes, however, appear to be related to reduction of trophoblast tissue (232).

IV. LIVER REGENERATION

Two-thirds partial hepatectomy of rat liver induces liver regeneration and results in changes of bile salt trans-
porter expression (111, 368). At the basolateral membrane of hepatocytes, Ntcp mRNA and protein is markedly diminished. Oatp1 and -2 RNA levels decline while Oatp1 protein expression falls and then recovers to normal levels while Oatp2 protein remains depressed. Mdr1 RNA expression is increased by partial hepatectomy. At the canalicular membrane, Bsep RNA expression is variably expressed, but the protein remains unchanged. Mrp2 RNA and protein expression are either unchanged or diminished. In contrast, Mdr1 and Mdr2 RNA levels are increased. Functional correlates of these molecular responses to partial hepatectomy have not been determined.

V. SUMMARY

Advances in the field of bile salt transport have greatly accelerated since the advent of molecular cloning. Classic biochemical and physiological studies, long the traditional areas of study, have given way to an era of functional genomics whereby the function and regulation of the bile salt transporters are being understood at a molecular level. In this review we have presented what is currently known about the normal physiology of bile salt transport in the liver and other extrahepatic tissues, that is, the field in greater detail. As we wrote this review, we were impressed with the rapid rate of acceleration of publications in this field, making it impossible to remain entirely current. We also apologize to those whose work we overlooked or were unable to include because of space. A number of recent reviews are also cited in the text for the reader interested in following one or more aspects of this field in greater detail.

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