Pharmaceutical Research, Vol. 24, No. 10, October 2007 (© 2007)
DOI: 10.1007/s11095-007-9289-1

Expert Review

Bile Acid Transporters: Structure, Function, Regulation and Pathophysiological Implications

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Received February 7, 2007; accepted February 28, 2007; Published online April 3, 2007

Abstract. Specific transporters expressed in the liver and the intestine, play a critical role in driving the enterohepatic circulation of bile acids. By preserving a circulating pool of bile acids, an important factor influencing bile flow, these transporters are involved in maintaining bile acid and cholesterol homeostasis. Enterohepatic circulation of bile acids is fundamentally composed of two major processes: secretion from the liver and absorption from the intestine. In the hepatocytes, the vectorial transport of bile acids from blood to bile is ensured by Na^+ taurocholate co-transporting peptide (NTCP) and organic anion transport polypeptides (OATPs). After binding to a cytosolic bile acid binding protein, bile acids are secreted into the canaliculus via ATP-dependent bile salt excretory pump (BSEP) and multi drug resistant proteins (MRPs). Bile acids are then delivered to the intestinal lumen through bile ducts where they emulsify dietary lipids and cholesterol to facilitate their absorption. Intestinal epithelial cells reabsorb the majority of the secreted bile acids through the apical sodium dependent bile acid transporter (ASBT) and sodium independent organic anion transporting peptide (OATPs). Cytosolic ileal bile acid binding protein (IBABP) mediates the transcellular movement of bile acids to the basolateral membrane across which they exit the cells via organic solute transporters (OST). An essential role of bile acid transporters is evident from the pathology associated with their genetic disruption or dysregulation of their function. Malfunctioning of hepatic and intestinal bile acid transporters is implicated in the pathophysiology of cholestatic liver disease and the depletion of circulating pool of bile acids, respectively. Extensive efforts have been recently made to enhance our understanding of the structure, function and regulation of the bile acid transporters and exploring new potential therapeutics to treat bile acid or cholesterol related diseases. This review will highlight current knowledge about structure, function and molecular characterization of bile acid transporters and discuss the implications of their defects in various hepatic and intestinal disorders.

KEY WORDS: bile acids; cholestasis; enterohepatic circulation.

INTRODUCTION

Bile acids are distinctive molecules in being poorly metabolized after their synthesis in the liver to conserve their pool for various physiological roles in the body (1–3). After their synthesis, bile acids are secreted into the bile and are then avidly absorbed from the intestine (4). Bile acids in the portal blood are taken up by the hepatocytes and are re-secreted to continue cycling between the intestine and the liver establishing their enterohepatic circulation (4–6). Maintaining a balance between bile acid synthesis, secretion and intestinal re-absorption is vital since every aspect of their homeostasis is intricately linked to various important physiological processes (3,7,8). Biosynthesis of bile acids represents a catabolic process by which the hydrophobic insoluble cholesterol is converted into the soluble molecules of bile acids that are easily secreted (9–12). This de novo synthesis, which is negatively regulated by its end products bile acids, accounts for about 50% of cholesterol elimination from the body signifying the physiological role of bile acids in cholesterol homeostasis (9,10). In the intestinal lumen, bile acids are essential for the absorption of lipids, cholesterol and lipid-soluble vitamins owing to their amphipathic nature and ability to form mixed micelles via which fat is delivered through the aqueous luminal milieu to the brush border membrane of intestinal epithelial cells for absorption (2,4). Bile acids also affect intestinal and colonic epithelial function and integrity by triggering various signaling pathways impinging on various processes such as absorption of electrolytes and induction of apoptosis (13–17). The recovery of these highly significant compounds is efficiently achieved by their intestinal retrieval via active transport processes across the intestinal epithelium and transferring them back to the liver by the portal blood (1,4). The conservation of bile acids by their enterohepatic circulation leads to the accumulation of large mass of these

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0724-8741/07/1000-1803/0 © 2007 Springer Science + Business Media, LLC

organic solutes that flux through the hepatocytes with a high transport rate providing the main driving force for bile flow (1,18). To accomplish their enterohepatic circulation, bile acids must cross plasma membrane barriers in the intestine and the liver.

Importantly, the majority of the circulating bile acids is conjugated to taurine or glycine amino acids and they, at physiological pH, exist in the form of membrane impermeable anions (4,19). Therefore, the transcellular movement of bile acids is accomplished by specialized transporters localized to the apical or the basolateral membrane of the polarized epithelial hepatocytes and eneterocytes (4–6). Indeed, the functional multiplicity of circulating bile acids and their involvement in cholesterol homeostasis has generated a great deal of interest to investigate the detailed mechanisms of their transport pathways in normal physiology and the contribution of their derangement to the pathophysiology of several biliary and cholesterol related disorders such as cholestatic liver diseases and atherosclerosis (4–6). Recent development in molecular biology techniques provided us with invaluable tools to determine the molecular identity of hepatic and intestinal bile acid transporters and to explore the molecular mechanisms of their regulation. Significant body of research regarding bile acid transporters and their regulation has profoundly enhanced our ability to treat several biliary related disorders and provided potential molecular targets for development of therapeutic modalities (4,20–24). The current review provides a comprehensive summary of the recent knowledge regarding hepatic and intestinal bile acid transporters. An attempt has been made to update the reader with the most recent advances pertaining to structure, function and regulation of these transporters. We will also briefly highlight the knowledge about bile acid transporters expressed outside intestinal and hepatic tissues including those of the renal epithelial cells and the cholangiocytes. Further, the importance of bile acid transporters in normal physiological states or during the development of biliary and cholesterol related disorders is discussed.

HEPATOCELLULAR TRANSPORT OF BILE ACIDS

The transport of bile acids across the hepatocytes represents the secretory component of their circulation and is attributed to their movement from the portal blood to lumen of bile canaliculus. Bile acid flux through the hepatocytes occurs against a concentration gradient and requires an active transport processes to maintain high concentrations in the bile (1,6). This secretory process is governed by distinct active carrier-mediated transport systems expressed in a polarized fashion in the hepatocytes (Fig. 1) (4–6). Several transporters on the basolateral (sinusoidal) membrane of the hepatocytes initiate the transport of bile acids (25). Once inside the hepatocyte, bile acids are transferred transcellularly via various mechanisms (26) to the apical (canalicular) pole for their destined secretion by active apical carrier-mediated transport processes into the bile (4). These polarized hepatic transport processes of bile acids will be reviewed in this section and their regulation and the implication of their perturbation in disease states will be discussed.

Basolateral Transport of Bile Acids

The basolateral transport of bile acids represents the first step in clearance of bile acids from portal blood into the hepatocytes (25). The basolateral membrane of the hepatocytes is in direct contact with the space of Disse that receives its content including bile acids from the portal blood through large pores (fenestrae) of the sinusoidal endothelium (1,25). The majority of bile acids reaches the space of Disse bound to albumin and therefore, needs to be dissociated from their binding protein to be able to flux through the membrane (25). Bile acid dissociation from albumin is facilitated by conformational changes in the structure of albumin upon its contact with the basolateral membrane of the hepatocytes (27). Bile acid transport across the basolateral or sinusoidal membrane is highly efficient with about 75–90% of conjugated bile acids extracted from the first pass of the portal blood through hepatic lobules (6,25). Interestingly, the liver possesses high capacity for extracting bile acids since their clearance occurs largely by hepatocytes occupying liver acinar zone 1 that are in close proximity to the portal vein (periportal) whereas zone 3 (perivenous) hepatocytes are involved only in cases of high loads of bile acids such as cholestasis (6,28–30). Bile acids extraction from the portal blood mainly involves two major processes: Na^+-dependent and Na^+-independent uptake of bile acids into the hepatocytes (4,6). Also, several other transporters located on the basolateral membrane are responsible for bile acids efflux from the hepatocytes (Fig. 1) (4,6).

Na^+-dependent Bile Acid Transport

Earlier studies have characterized the functional features of hepatic Na^+-dependent bile acid uptake in various experimental models of purified basolateral plasma membrane vesicles, isolated hepatocytes and perfused rat liver (25). The salient characteristics of this transport process include a dependency on Na^+ (31,32), electrogenic nature (33,34), and a high affinity for conjugated bile acids with apparent K_m ranging between 15–56 μM for taurocholic acid (TC) (25). In hepatocytes, this uptake process is driven by an inwardly Na^+ gradient that is maintained by the activity of Na^+/K^+ ATPase (5,6). Although several studies strongly indicate that hepatic Na^+-Dependent Taurocholic Cotransporting Polypeptide (NTCP) is the main transporter mediating this process, several reports also suggest the involvement of microsomal-epoxide hydrolase as well (5,6).

(a) Na^+-dependent Taurocholic Cotransporting Polypeptide (NTCP): Na^+-dependent uptake activity in the hepatocytes has been shown to be predominantly mediated by the function of Na^+-dependent taurocholic cotransporting polypeptide (NTCP) (4–6). NTCP is expressed in the liver and its polypeptide is localized exclusively to the basolateral membrane of the hepatocytes (35–37). Recently, Ntcp expression in rat was detected in the luminal membrane of rat pancreatic acinar cells where it may be involved in clearing bile acids that leak to pancreatic terminal acini (36,38). The NTCP-mediated bile acid uptake in pancreatic cells is associated with cell injury (38). NTCP exhibits

functional features concurring with those previously described for hepatic Na^+-dependent uptake of bile acids (35). Also, there is a parallel decrease in both Na^+-dependent bile acid uptake and the expression of NTCP in de-differentiated rat primary hepatocytes and in human hepatoma HepG2 cell line strongly indicating the pivotal role of NTCP in this transport process (39,40). In this regard, Hagenbuch *et al.* (41) have shown that Xenopus oocytes injected with total mRNA extracted from rat hepatocytes exhibited Na^+-dependent bile acid uptake process that was inhibited by targeting of Ntcp mRNA with antisense oligonucleotides. These elegant experiments provided compelling evidence that NTCP, at least in rat liver, represents the major route for Na^+-dependent uptake of bile acids.

Structure-Function. NTCP (or SLC10A1) belongs to SLC10A gene family of transporters that also include another Na^+-dependent bile acid transporter (SLC10A2) (35,36). NTCP was initially cloned in rat liver and subsequently in mouse, rabbit and human (35,36,42–44). NTCP gene maps to chromosomes 6q24 and 14q24 in rat and humans, respectively, (35,36). In mouse, NTCP gene is located on

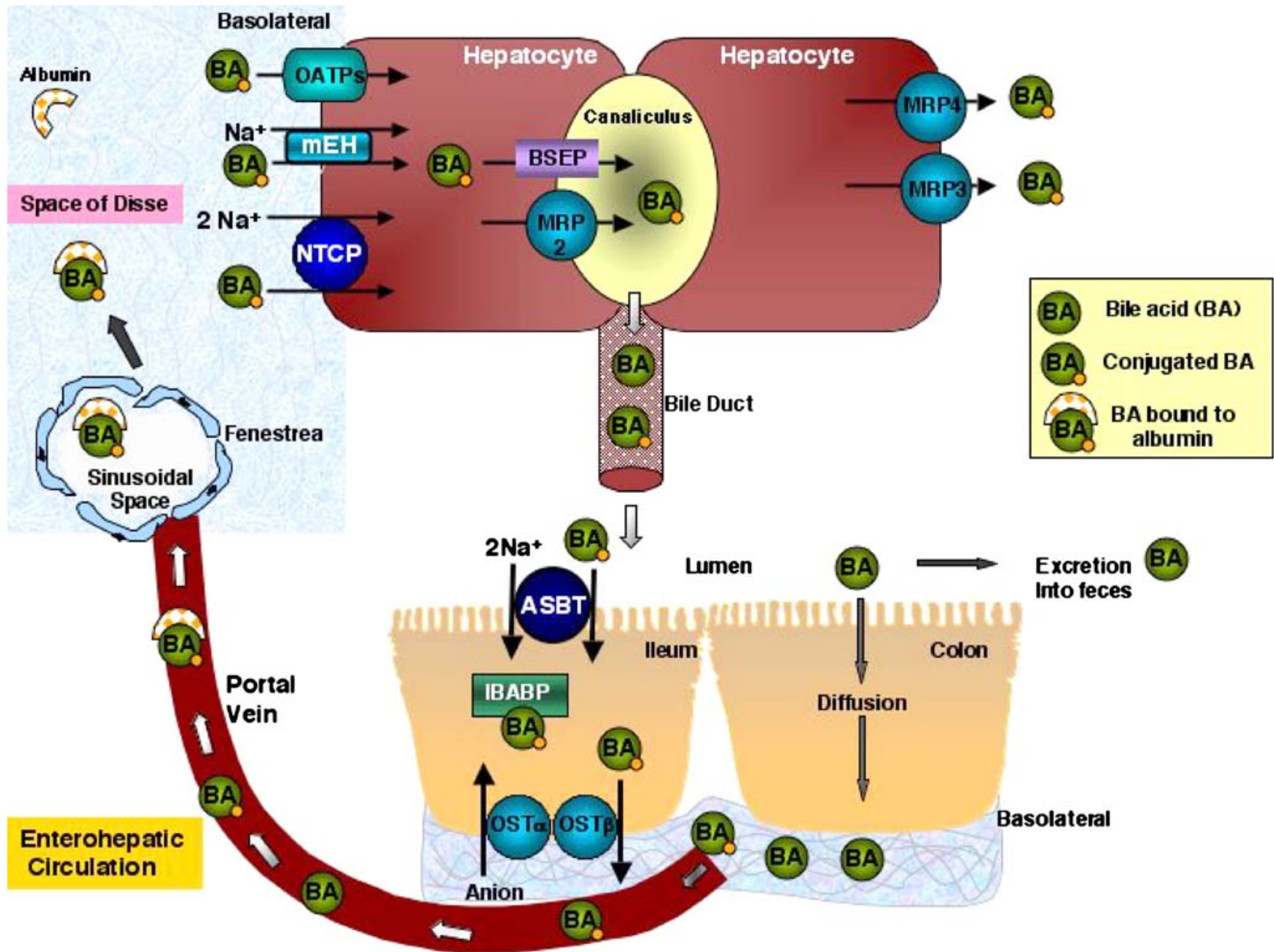

Fig. 1. Enterohepatic circulation of bile acids. Bile acids reach the space of Disse through the large fenestrea of the sinusoids largely as albumin-bound complexes. After their dissociation from albumin, bile acids are transported across the hepatocytes to the canaliculus. Bile acids transport across the basolateral membrane of the hepatocytes is mainly mediated by the Na^+-dependent Taurocholic Cotransporting Polypeptide (*NTCP*). Microsomal epoxide hydrolase (*mEH*) and Organic Anion Transporting Polypeptides (*OATPs*) also transport bile acids in a Na^+-dependent and Na^+-independent manner, respectively. Bile acids efflux across the basolateral membrane of hepatocytes occurs via the Multidrug Resistance Proteins MRP3 and MRP4. The secretion of bile acids across the canalicular membrane occurs via two members of the ATP-binding cassette transporters: Bile acids Export Pump (*BSEP*) that transports monovalent bile acids, and Multidrug Resistance Protein MRP2 that transports divalent sulfated or glucuronidated bile acids. Bile acids are delivered to the intestinal lumen through bile duct where they aid in emulsifying dietary lipids. Some of the secreted bile acids are de-conjugated in the intestinal lumen by the action of the bacterial flora. The majority of both conjugated and unconjugated bile acids are efficiently absorbed from the intestinal lumen and only about 5 % get excreted. While unconjugated bile acids may passively diffuse across the small intestinal and colonic epithelia, bile acids are actively absorbed in the distal ileum via Na^+-dependent Apical Sodium Dependent Bile acid Transporter (*ASBT*). The intracellular transport of bile acids across the enterocytes is facilitated by the Ileal Bile Acid Binding Protein (*IBABP*) while they efflux through Organic Solute Transporter α and β (*OSTα/OSTβ*). Bile acids re-enter the portal blood completing their enterohepatic circulation.

chromosome 12q D1 and was found to transcribe two variants differing in their C-terminal (43,45). Human NTCP consists of 349 amino acids with an apparent mass of 56 kDa (42). Computer analysis predicted a topology of seven transmembrane domains for NTCP with an intracellular C-terminal and an extracellular N-terminal (42). Previous experimental evidence suggested a topology of nine membrane-spanning domains in which two re-entrant membrane sequences are associated with rather than spanning the plasma membrane (46). Interestingly, recent evidence indicated that the two segments of NTCP polypeptide that associate with the membrane are part of a P-loop structure that is essential for its transport function (47).

NTCP has been shown to mediate the transport of both conjugated and unconjugated bile acids but with a higher affinity to the former in a Na^+-dependent manner with a stoichiometry of 2:1 (48). Additionally, NTCP transports sulfated bile acids such as chenodeoxycholate-3-sulfate and taurolithocholate-3-sulfate (23,49). Also, steroid sulfates such as oesterone-3-sulfate as well as drug conjugated chlorambucil taurocholate have been shown to be substrates for NTCP (23,50). Although the binding site for bile acids has not been identified, information about the structure-function of NTCP is emerging (36). The fact that thiol reagents are inhibitors of NTCP transport activity suggested that certain cysteine (C) residues of the protein are important for binding and transporting the substrate (36). Indeed, mutation in C^{266} abrogated the thiophilic inhibition of NTCP without affecting its basal activity (51,52). However, mutations in C^{96}, C^{98} and C^{250} cysteine residues abolished the transport activity of NTCP (52,53). These observations clearly indicated that C^{266} residue is located near the substrate binding site to confer inactivation of the transporter upon binding to thiol reagents while cysteine 96, 98 and 250 residues are essential for the transport function of NTCP (36). With respect to the binding of Na^+, the negatively charged aspartate (D) 115 and glutamate (E) 257 that are located on the extracellular loops 1 and 3 have been shown to be critical for sodium binding and transport function of NTCP (52). No hepatic disorder has yet been described that is associated with NTCP mutations. However, Ho *et al.* have recently identified ethnicity-dependent polymorphisms in human *NTCP* gene associated with decreased transport function (54). These findings suggest that certain mutations in human *NTCP* gene may lead to alteration in its function and may play a role in the development of hypercholanemia (54).

Regulation and Pathophysiological Implications. Elucidation of molecular regulation of NTCP is important to enhance our understanding of the pathophysiology of diseases, such as liver cholestatic disorders, and to aid in the discovery of better therapeutic modalities. In fact, NTCP expression has been shown to be altered in certain physiological challenges such as pregnancy (55), and in pathological conditions such as advanced stage primary biliary cirrhosis (56) and cholestatic alcoholic hepatitis (57). Also, NTCP undergoes posttranscriptional modulation in which its function is rapidly stimulated in response to activation by various signaling molecules (58). Identifying molecular components of NTCP regulation will reveal the mechanisms by which NTCP is coordinately regulated with other transporters of the enterohepatic circulation and will identify potential targets for choleretic reagents that enhance bile formation.

Importantly, NTCP gene expression has been shown to be suppressed by high levels of bile acids as an adaptive response to reduce their entry into the hepatocytes (58). Bile acids elicit their influence on gene transcription via the activation of FXR nuclear receptor (58). FXR does not bind NTCP promoter but rather indirectly affect its activity (58,59). In the hepatocytes, activated FXR induces the expression of short heterodimer partner (SHP), which in turn blocks the stimulating effect of retinoic acid receptor and retinoid X receptor RAR/RXR heterodimer on NTCP promoter (Fig. 2) (60). Also, the suppression of the hepatic nuclear factor HNF1α was recently suggested to be involved in bile acid-induced inhibition of NTCP expression (61).

Endotoxin and proinflammatory interleukin IL1β also reduced NTCP expression by the down regulation of RAR/RXR complex (62,63). The effect of IL1β on RAR/RXR heterodimer was recently shown to be mediated by c-Jun N-terminal Kinase (JNK)-dependent pathway (64). Glucocorticoid receptor was recently shown to directly bind and activate NTCP promoter, similar to its effect on ileal Na^+-dependent bile acid transporter ASBT, in a ligand-dependent manner (65). This activation by glucocorticoid was blocked by FXR-induced expression of SHP but was augmented by PGC-1α coactivator (65). The transcription factor STAT5, member of *S*ignal *T*ransducers and *A*ctivators of *T*ranscription, mediates the upregulation of NTCP expression by prolactin (66). STAT5 activates NTCP promoter via binding to the interferon gamma-activated sequence-like elements (GLEs) (66). Interestingly, NTCP expression is down regulated during pregnancy despite the increase in the level of STAT5 (58). This may suggest that a STAT5-independent pathway is activated during pregnancy that

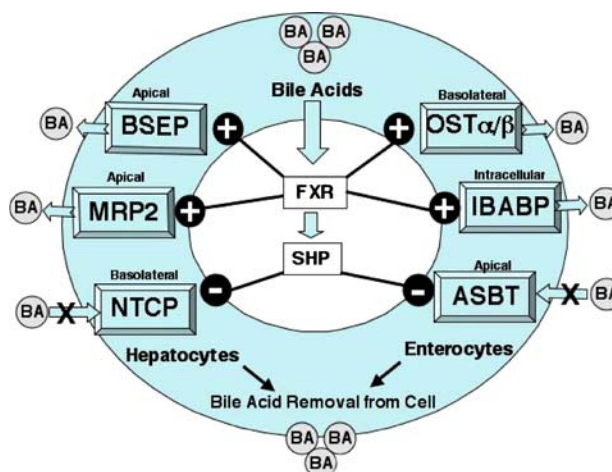

Fig. 2. Bile acid induced regulation of hepatic and intestinal bile acid transporters. Bile acids activate farnesoid X receptor (*FXR*), which in turn inhibits their uptake while stimulating their efflux in a concerted manner to prevent their intracellular accumulation. FXR induces the expression IBABP and OSTα and β in the enterocytes and the expression of BSEP and MRP2 in the hepatocytes. FXR activation stimulates the expression of small heterodimer partner (*SHP*) that suppresses the expression of ASBT and NTCP in the enterocytes and hepatocytes, respectively.

overrides the stimulatory effect of STAT5 on NTCP expression (58).

Previous studies have also shown a rapid increase in hepatic Na^+-dependent bile acid transport by cAMP via vesicular trafficking of NTCP from intracellular pool to the basolateral membrane (58). This mode of regulation is converged via a signaling pathway that is dependent on actin-cytoskeleton, intracellalur Ca^{++}, PI3K, PKCζ and protein phosphatase B2 (58). Recently, Anwer *et al.* presented strong experimental evidence suggesting that cAMP may increase NTCP translocation by the dephosphorylation of serine-226 residue of NTCP polypeptide (67). This dephosphorylation appears to retain NTCP polypeptide on the plasma membrane increasing its surface expression and stimulating its activity (67).

(b) Microsomal Epoxide Hydrolase (mEH): Several studies have also suggested a role for microsomal epoxide hydrolase in Na^+-dependent bile acid uptake (6). This enzyme is essential for the metabolism of many xenobiotics and is present in two distinct topological forms that are derived from the same polypeptide (68). Type II form has been shown to be targeted to the plasma membrane and is capable of mediating DIDS-sensitive Na^+-dependent bile acid uptake (69). The physiological significance of this protein and its contribution to the total basolateral Na^+-dependent bile acid transport is still controversial (6). Mice lacking the expression of mEH have no apparent abnormalities in bile acid homeostasis (70). However, Zhu *et al.* have recently identified a point mutation in a patient with hypercholanemia associated with 85% decrease in mEH protein with no alteration in the level of NTCP expression (71). Moreover, *in vitro* analysis revealed that the identified mutation in mEH gene significantly decreased its promoter activity (71). The authors argued that a compelling experimental evidence indicate that mEH is more efficient for transporting glyco-conjugates of bile acids compared to NTCP (71). Since the majority of circulating bile acids in humans are conjugated to glycine (1), its possible that mEH is responsible for hepatic uptake of higher percentage of bile acids compared to NTCP and that mutations in mEH lead to severe hypercholanemia (71). The generation of NTCP knockout mice in the future may provide more insights into the role of NTCP and mEH in Na^+-dependent bile acid uptake across the basolateral membrane of the hepatocytes.

Na^+-independent Bile Acid Transport

The other major pathway for bile acids uptake across the basolateral membrane of the hepatocytes occurs in a Na^+-independent manner (4,6). This transport process is responsible for most of the uptake of unconjugated bile acids in the portal blood (25). Although Na^+-independent transport of the hydrophobic unconjugated bile acids could be explained by passive diffusion (25), earlier studies supported the presence of a carrier-mediated transport process (72). Bile acid transport via this pathway occurs in exchange with an intracellular anion (6,25). Intriguingly, a wide spectrum of different amphipathic organic anions along with bile acids could be transported via this Na^+-independent transport process (6,25). The Na^+-independent bile acid and organic anions uptake in hepatocytes has been shown to be mediated by several members of the SLC21 (recently renamed as SLCO) *O*raginc *A*nion *T*ransporting *P*olypeptides (OATP) superfamily of transporters (4,6,25). These plasma membrane transporters are glycoproteins with a common structure consisting of predicted 12 transmembrane domains with both N- and C-terminal protruding in the cytosol and a consensus superfamily signature that is located between the third extracellular loop and transmembrane domain 6 (73,74). OATPs protein have been shown to mediate the exchange of extracellular organic anion or bile acid with intracellular HCO_3^- or glutathione (GSH) hence indicating that they are also involved in GSH efflux (74). In the following section, we will focus on the human OATPs that are involved in bile acid uptake in human hepatocytes with a little mention to their rodent orthologs. We will use the new nomenclature system for these transporters that has been recently formulated by Hagenbuch and Meier (74).

(a) Organic Anion Transporting Polypeptide OATP1A2: OATP1A2 (previously known as OATPA) is mainly expressed in the brain and liver with higher expression level in the brain (75). It was also shown to be expressed in liver and colon cancer cell lines (23,76,77). OATP1A2 has an apparent molecular mass of 85 kDa containing 670 amino acids and its gene (*SLCO1A2*) is mapped to chromosome 12q12 (23,74). The hepatic orthologs in rodents are Oatp1a1 and Otap1a4 (previously called Oatp1 and Oatp2, respectively), (74). OATP1A2 polypeptide in the liver is localized to the basolaetral membrane of the hepatocytes and is involved in the Na^+-independent bile acid uptake (6). Intriguingly, rat Oatp1 was shown to be homogenously expressed throughout the hepatic lobule whereas Oatp2 expression is concentrated in the perivenous and pericentral region of the lobule (78,79). Along with bile acids, OATP1A2 is capable of transporting a variety of amphipathic organic compounds including steroid conjugates; thyroid hormones; prostaglandins; peptides such as the thrombin inhibitor CRC-220; drugs like chlorambuciltaurocholate, fexofenadine and ouabain; organic anion bromosulfophthalein (BSP); and organic cations such as rocuronium, N-methyl -quinine and -quinidine (74). Of note, the unique features of OATP1A1 compared to other human OATPs are illustrated by the most diverse amphipathic substrate profile and by being the only OATPs transporter to facilitate the transport of organic cations as well (80).

(b) Organic Anion Transporting Polypeptide OATP1B1: Formerly known as OATP-C, this organic anion transporter represents a 691 amino acid polypeptide with a molecular mass of 84 kDa (81). OATP1B1 gene (*SLC1O1B1*) is localized on chromosome 12q12 and its expression is strictly limited to the liver where it is localized to the basolateral membrane of the hepatocytes (74). The rodent ortholog is Oatp1b2 (previously named Oatp4) (74). The strict liver expression pattern of OATP1B1 indicates its

essential role in hepatic clearance of organic compounds (74,81). In contrast to OATP1A2 function, OATP1B1 was suggested to be charge-selective and restricted to organic anions (6). Indeed, it facilitates the transport of wide spectrum of organic substrates and several drug compounds such as benzylpenicillin, methotrexate, pravastatin and rifampicin (6,74). Interestingly, a number of polymorphisms in the *SLC01B1* gene have been identified that were associated with a decrease in OATP1B1 function and protein maturation (82,83).

(c) Organic Anion Transporting Polypeptide OATP1B3: The gene encoding for OATP1B3, is also mapped to chromosome 12q12 and its expression is restricted to the basolateral membrane of the hepatocytes (74,84,85). OATP1B3 (previously referred to as OATP8) is also expressed in various human cancer tissues and cell lines (84). OATP1B3 represents a glycoprotein of molecular mass of 120 kDa that consists of 702 amino acids (85). Similar to the member of OATP superfamily, OATP1B3 possesses a wide spectrum of substrates (74). However, it appears that OTAP1B3 is unique in transporting the intestinal peptide cholecystoknin 8 (CCK-8) and the cardiac glycosides digoxin (80).

Regulation of OATPs and Pathophysiological Implications. No disease has been described as a result of impaired function of any of OATP transporters (73). However, due to their central role in the first pass clearance of numerous drugs and organic compounds, advanced knowledge of the mechanisms of OATP regulation is fundamental for drug development and therapy (73). Alterations in OATP function and expression may interfere with the bioavailability or toxicity of certain drugs and subsequently confound their therapeutic effects (73). Accumulated evidence indicate that OATP transporters are regulated at the transcriptional level. Previous studies have demonstrated that the expression of rat Oatp1a1, Oatp1a2 and Oatp1b2 undergoes ontogenic regulation and their protein expression was detected only during the first 4 weeks after birth (73). Also, the hepatic expression of Oatp transporters was shown to be reduced in knockout mouse models of hepatic nuclear factor HNF1α and HNF4α indicating a central role for these transcription factors in basal Oatp expression in the liver (86,87). Rat Oatp1a1 expression in kidney is upregulated by testosterone but inhibited by estrogen leading to a gender-based difference in its renal expression (88). Interestingly, sex hormones do not influence Oatp1a1 expression in the liver indicating a tissue-specific type of regulation (88–90). Furthermore, the nuclear receptor pregnane X receptor has been shown to stimulate the promoter activity of rat Oatp1a4 and ligands of PXR such as rifampicin, lithocholic acid and pregnenolone-16α-carbonitrile (PCN) induces Oatp1a1 expression (91).

Importantly, bile acids appear to reduce the expression of several OATP transporters. For example, cholate feeding to mice resulted in a decrease in the expression of Oatp1a1 and Oatp1b2 (92). Also, the expression of these rodent Oatps was shown to be reduced in several models of cholestatic liver diseases such endotoxin-induced cholestasis and bile duct ligation (73). In humans, OATP transporters are differentially regulated in cholestasis. While the expression of OATP1B1 is decreased in patients with primary sclerosing cholangitis (93), OATP1B3 expression is found to be induced by bile acids (94). This observation suggests that the upregulation of OATP1B3 by bile acids may maintain sufficient hepatic extraction of xenobiotics and peptides during cholstasis (74). Similarly, the expression of OATP1A2 remains unaltered or even increased in cases of primary sclerosing cholangitis (23).

Post-transcriptional regulation has been reported for rat Oatp1a1 (73). Previous studies have shown that activation of protein kinase C (PKC) results in down-regulation of rat Oatp1a1 function via increasing its phosphorylation without altering its expression on plasma membrane (95). Similarly, digoxin transport via rat Oatp1a4 was also shown to be decreased by PKC activators (95). These findings indicate that the phosphorylation state and not only changes in the expression of OATPs should be considered in evaluating their function during pathological cases (95).

Bile Acid Efflux

Under normal physiological conditions, bile acid uptake (influx) represents their predominant transport across the basolateral membrane of hepatocytes whereas their efflux is negligible (5). However, bile acid efflux is upregulated during cholestatic conditions to facilitate bile acids efflux from the hepatocytes (5). The process of bile acid efflux across the basolateral membrane of the hepatocytes is mediated by members of the multidrug resistance proteins (MRPs) subfamily (6). This subfamily of transporters consists of at least six members (MRP1-6) with four expressed in the liver (6). MRP3 and MRP4 (96,97) are localized to the basolateral membrane of the hepatocyte, whereas MRP2 is expressed on the canalicular membrane (6). These transporters function as ATP-dependent pumps with a range of substrates including glucuronide and glutathione conjugates of endogenous and exogenous compounds (6). MRP1 was initially cloned from a multidrug-resistant human lung cancer cell line (6). It is expressed at a very low level in the liver and is capable of exporting di-anionic bile acids such as sulphated taurolithocholic and taurochenodeoxycholate (6). The hepatic expression of human MRP3 is also very low, however, it is induced in patients with Dubin–Johnson syndrome and patients with primary biliary cirrhosis (98). Human MRP3 also mediates the export the monovalent glycocholic bile acid with low affinity (K_m=0.248 mM) but not taurocholic acid (99). The fact that MRP3 is upregulated during cholestasis and support bile acid efflux across the basolateral membrane of heptocytes suggests its involvement in bile acid shift towards renal excretion for elimination (6). However, recent studies of Zelcer *et al.* provided evidence that bile acid homeostasis was unaltered in mice lacking the expression of MRP3 (100). Furthermore, these studies demonstrated that level of bile acids and their uninary excretion remained unchanged after bile duct ligation in $Mrp^{-/-}$ mice (100). The authors concluded that Mrp3, at least in mice, does not contribute to bile acid efflux (100). Further studies are needed to determine the role of MRP3 in bile acid efflux in humans.

Intracellular Transport of Bile Acids

Despite our increased understanding of bile acid transport across plasma membrane of the hepatocytes, the mechanisms of their transcellular movement are still not

fully understood (6). Two mechanisms have been proposed for the transcellular transport of bile acids from the sinusoidal to the canalicular membrane of the hepatocyte: intracellular trafficking and vesicle-mediated transport (5,6). Under normal physiological conditions, the majority of bile acids is transported via intracellular trafficking pathway whereas the vesicle-mediated pathway is involved when bile acid load is imposed on the liver (5).

Evidence for the intracellular trafficking of bile acids is obtained from studies demonstrating rapid cytosolic diffusion of fluorescent derivatives of bile acid before their canalicular secretion (5,19). This movement is driven by basolateral to canalicular membrane concentration gradient and is proposed to be mediated by intracellular binding proteins (5,19). Several hepatic intracellular bile acid-binding proteins were identified in rat liver including the 3α-hydroxysteroid dehydrogenase (3α-HSD), glutathione S-transferase and liver fatty acid binding protein (L-FABP) (5,26). Interestingly, inhibition of 3α-HSD by indomethacin in isolated hepatocytes and rat liver perfusion resulted in a displacement of bile acids from the cytosol and their redistribution out of the cells into the media (19,101). These findings indicated that 3α-HSD is the major cytosolic bile acid-binding protein in rat liver and suggested that binding of bile acids to intracellular proteins is essential to retain them in the cytosol and prevent their efflux (19). The expression of both 3α-HSD and L-FABP was shown to be under the control of FXR transcription factor suggesting that bile acids induce their expression (102). Also, HNF1α transcription factor is critical for the expression of both 3α-HSD and L-FABP of (86). In humans, a 36 kDa bile acid-binding protein has been identified (5,19). Although the affinity of this protein for bile acids is higher than the affinity of its counterparts in rat, the exact role of this protein in bile acid transport remains to be investigated (5,19).

The involvement of vesicle-mediated intracellular hepatic transport of bile acids was concluded from several observations that indicated the partitioning of hydrophobic bile acids into membranous intracellular organelles such as endoplasmic reticulum and Golgi apparatus (5,19). The movement of vesicles containing bile acids facilitates their intracellular transport to the canalicular membrane (5,19). This hypothesis was further supported by the fact that inhibitor of microtubule assembly, colchicine, decreases canalicular biliary secretion after a large load of bile acids (19). These observations, however, should be cautiously interpreted since the inhibition of microtubule assembly influence the targeting of several membrane proteins and is not specific for bile acid-containing vesicles (6).

Canalicular Transport of Bile Acids

Canalicular bile acid transport is a critical component of their enterohepatic circulation and represents the rate-limiting step in hepatic excretion and bile formation (6). The excretion of bile acids into the bile across the canalicular membrane occurs against a steep concentration gradient that ranges between 100–1,000 fold (103). While bile acid influx across the basolateral membrane is largely driven by inwardly directed Na^+ gradient or outwardly directed GSH and HCO_3^- gradient, the canalicular excretion of bile acids occurs via transporters whose function depends on ATP hydrolysis (6,103). The major transporters involved in mediating hepatic bile acid excretion include Bile Salt Export Pump (BSEP) that is responsible for transport of monovalent bile acids and the Multidrug Resistance Protein MRP2, which is the main transporter of divalent bile acids (6,103–105). Interestingly, mutations in these canalicular transporters are known to result in hereditary liver diseases further affirming the pivotal role of canalicular bile acid transport and its importance in maintaining hepatic homeostasis (24).

Bile Salt Export Pump (BSEP)

BSEP is the predominant transporter of the canalicular membrane responsible for the excretion of monovalent bile acids into the bile (6,103). This is evident from the fact that mice lacking the expression of BSEP have impaired bile secretion and mild cholestasis (106). BSEP is exclusively expressed in the liver where its polypeptide is localized to the canalicular membrane of the hepatocytes (107).

Structure Function. BSEP, or ABCB11, is a member of the multidrug resistance P-glycoprotein that belongs to the ATP-binding cassette (ABC) superfamily of transporters (5). It was first cloned from pig liver and subsequently from human, rabbit, rat and mouse (104,108,109). Human BSEP gene maps to chromosomes 2q24 and encodes for a protein with a molecular mass of 150–170 kDa (104,110). Similar to other ABC transporters, BSEP polypeptide consists of 12 transmembrane spanning domains with two typical intracellular nucleotide-binding domains with Walker A and B motifs for binding and hydrolysis of ATP (104). The functional features of BSEP have been examined in various heterologous expression systems and were found to be similar to those previously described in human or rat canalicular plasma membrane vesicles (104). BSEP mediates the transport of conjugated monovalent bile acids with high affinity for taurochenodeoxycholate > taurocholate > tauroursodeoxycholate glycocholate (110,111). Low rate transport of some organic compounds via BSEP was also reported, however, the relevance of such a transport is uncertain (6).

Regulation and Pathological Implications. Given the central role of BSEP in bile acid secretion and bile flow, it is predicted that modulation of its function and expression is vital for the integrity of hepatic function and bile formation in response to different physiological and pathological challenges. The expression of BSEP occurs during the postnatal period and its expression is down regulated, similar to NTCP, after the de-differentiation of the heptocytes as occurs when in culture (39,112). Defects in BSEP function lead to impeded bile flow and cholestasis (24,103). Particularly important, mutations in BSEP were found in patients with type 2 progressive familial intrahepatic cholestasis (PFIC2) (24,103). Therefore, mechanisms of BSEP regulation were extensively investigated and accumulative evidence indicate several modes of modulation both at the transcriptional and post-transcriptional level.

BSEP expression was shown to undergo a positive feed forward regulation by its substrate, bile acids (92). Bile acid-activated farsenoid X receptor (FXR) forms a heterodimer

with retinoid X receptor (RXR) and activates BSEP promoter activity upon binding to specific bile acid response *cis*-element (Fig. 2) (11,104). This mechanism underlies the observation that Bsep mRNA and protein are induced in mice following a challenge with a large dose of bile acids (113). Also, FXR deficient mice exhibited low level of BSEP expression that was not induced by bile acid-enriched diet (102). Recent findings demonstrated that the recruitment by activated FXR of chromatin modifying enzymes coactivator-associated arginine methyltransferase 1 (CARM1) and arginine methyltransferase, PRMT1, with a subsequent chromatin remodeling is essential for full potentiation of BSEP stimulation by bile acids (103,114,115). Taken together, these findings indicate that FXR mediated induction of BSEP is an essential adaptive response to the accumulation of hydrophobic bile acids in the hepatocytes (104). Indeed, BSEP expression was found to be increased in patients with stage III primary biliary cirrhosis as a compensatory mechanism to overcome the injury of associated cholestasis (56). On the other hand, impaired function or expression has detrimental effects leading or predisposing to cholestasis (104).

Inhibitors that are known to directly bind BSEP on the canalicular membrane such as glybenclamide and troglitazone cause drug-induced cholestasis (104). Reduced expression of BSEP on the canalicular membrane upon an induction in its internalization by taurolithocholic acid may also be involved in drug-induced cholestasis (116,117). The decrease in BSEP expression in patients with inflammation-induced cholestasis further indicates the possible role of BSEP in the pathology of sepsis-associated cholestasis (57).

In addition to the acquired BSEP derangement of function and expression, several mutations have been identified in BSEP gene leading to inherited cholestatic disorder termed as progressive familial intrahepatic cholestasis type 2, PFIC2 (24,103,104) (Table II). The disease starts during infancy and is characterized by severe jaundice, hepatomegaly, failure to thrive and pruritus (103). Patients have high levels of serum bile acids and aminotransferases but normal level of gamma glutamyl transpeptidase (103). The disease is also associated with extremely low concentration of biliary bile acids (<1% of normal) (118). A wide diversity of mutations in BSEP gene have been reported leading to premature truncations in the protein or missense alteration in structural domains essential for its function (103,104). Recent studies have introduced some of missense mutations into rat Bsep and demonstrated either altered membrane targeting or increased degradation of the protein by proteosomal pathway when expressed in Madin–Darby canine kidney MDCK cells (119). PFIC2 disease has a rapid progressive course and lead to cirrhosis and liver failure within the first decade of life (120). PFIC2 is cured by liver transplantation (103).

Type 1 of the familial intrahepatic cholestasis (PFIC1) or Byler's disease (Table II) results from mutations in the *FIC1* gene or *ATP8B1* that encode for P-type ATPase (121). The clinical features of PFIC1 are similar to those of PFIC2, however, patients suffer from diarrhea and malabsorption (122). FIC1 has been shown to function as aminophospholipid flippase and is predominantly expressed in the intestine (123). FIC1 is also expressed in the liver and cholangiocytes (123). Since patients with PFIC1 have low biliary concentration of bile acids, it is speculated that FIC1 is indirectly involved in canalicular bile acid secretion (103). This may be attributed to its role in maintaining, as a flippase, an asymmetrical distribution of aminophospholipids between the inner and the outer leaflets of the canalicular membrane (24). Intriguingly, ileal bile acid transporter (ASBT) is upregulated in patients with PFIC1 whereas IBABP is down regulated (103). The exact molecular mechanism by which mutated FIC1 alters the expression and the function of bile acid transporters is not known (103). Recent studies showed that the attenuation of endogenous FIC1 expression in human intestinal Caco2 cells led to an increase in ASBT promoter activity and inhibition in the promoter activity of BSEP (124). Surprisingly, FXR-DNA binding activity was reduced in nuclear but not cytoplasmic extracts in response to FIC1 attenuated expression in Caco-2 cells (124). These studies indicated that FIC1 is necessary for the nuclear translocation of FXR (103,124). Mice deficient in FIC1 demonstrated normal bile acid excretion and accumulated bile acids only when fed diet-enriched with bile acids mostly due to abnormal regulation of their intestinal absorption (6). Overall, the pathobiology of PFIC1 appears to be a result of over load of bile acids from increased intestinal absorption and decreased canalicular secretion secondary to a defect in FXR pathway (103). Although BSEP function and canalicular secretion of bile acids may also be affected in other types of inherited cholestasis (24), full discussion of these disorders is beyond the scope of this review.

BSEP regulation by posttranscriptional mechanisms has also been reported. These adaptive responses allow for rapid modulations that occur in normal physiological conditions such as postprandial alteration or during cholestasis (58,104). The recruitment of BSEP to the canalicular membrane by vesicular targeting from intracellular pool is induced in hypoosmolarity, in response to cAMP and bile acids (125,126). The vesicular trafficking of BSEP appears to be dependent on microtubule cytoskeleton (58). Several cellular kinases and signal transduction molecules such protein kinase C and p38 MAP kinases are involved in BSEP regulation by membrane trafficking (127). Also, mouse Bsep was shown to be a phosphoprotein that is activated by PKCα-mediated phosphorylation. In contrast, the phosphorylation of mouse Bsep could be inhibited by the activation of PKCε leading to a reduction in its function (128). These mechanisms of regulation by various signal transduction pathways should be taken into consideration when explaining defective BSEP function or membrane expression during diseases such as drug-induced cholestasis (58,104).

Multidrug Resistance Protein MRP2

MRP2 is another ABC transporter involved in canalicular transport of bile acids (6). Human *MRP2* gene is mapped to chromosome 10q24 and its mouse ortholog is located on chromosome 19D2 (129,130). MRP2 polypeptide is localized to the canalicular membrane of the hepatocytes and the apical membrane of renal proximal tubule epithelial cells and enterocytes of the duodenum and jejunum (131). MRP2 mediates the export of bilirubin conjugates and its substrate profile includes wide range of organic substrates such as glutathione, glucuronide and sulfate conjugates and also

some unconjugated drugs, such as pravastatin, fluvastatin, methotrexate, ampicillin, ceftriaxone, irinotecan and temocaprilat (131–133). With respect to bile acids, MRP2 mediates the transport of divalent bile acids such as sulfated tauro and glycolithocholate (6). On the other hand, MRP2 does not have the capacity to transport monovalent bile acids (6,134). Interestingly, substitutions of single cationic amino acid in the transmembrane domains 11 and 14 render rat MRP2 capable of transporting even monovalent bile acids (134).

Mutations in MRP2 lead to Dubin–Johnson syndrome (Table II) that is a rare and benign disorder characterized by a chronic conjugated hyperbilirubinemia with elevated level of serum gamma glutamyl transpeptidase (4,6,24). On the other hand, the canalicular transport of bile acids and their levels in the serum remains unaltered in patients with Dubin–Johnson syndrome (135). Several mutations have been identified in MRP2 that are associated with Dubin–Johnson syndrome including missense, nonsense mutations, premature stop codons and deletions (135). Rats that are naturally deficient in MRP2, such as transport deficient (TR−), Groningen Yellow (GY) rats of the Wistar strain or Eisa hyperbilirubinemic (EHBR) rat of Sprague–Dawley strain, provided great models to investigate the function and to facilitate cloning of MRP2 gene (6,135).

INTESTINAL TRANSPORT OF BILE ACIDS

Bile acids in the intestinal lumen undergo extensive enterohepatic circulation to return to the liver for their re-excretion in the bile (2,4,6). An efficient intestinal reabsorption of bile acids and delivery to portal blood represents the second major component of the enterohepatic circulation (Fig. 1). Several studies in the past utilizing *in situ* perfusion of the whole gut with radio labeled bile acids, cell culture models, primary enterocytes as well as purified intestinal apical and basolateral membrane fractions have enriched our understanding of mechanisms underlying intestinal bile acid transport (19,136–140). The bile acid transport in the enterocytes basically consists of three components: (1) Apical uptake of bile acids in the enterocytes largely facilitated by uptake of conjugated bile acids in the terminal ileum via a Na^+-dependent mechanism; (2) intracellular bile acid transport in the enterocytes mediated via cytosolic intestinal bile acid-binding protein (IBABP) and (3) an anion exchange mechanism for the basolateral efflux of bile acids from enterocytes. The ileal bile acid transport system with its remarkable substrate specificity and various regulatory mechanisms offers enormous potential as a valuable target for cholesterol lowering therapy by controlling enterohepatic circulation with specific inhibitors. The following section describes current knowledge of the bile acid transport mechanisms, structure/activity relationships of bile acids for intestinal absorption and molecular regulation of these transporters that is essential for the rational design of putative inhibitors to be used as enticing pharmaceutical tools.

Apical Transport of Bile Acids

Apical uptake of bile acids in the enterocytes occurs via (1) passive diffusion of unconjugated bile acids in small and large intestine; (2) uptake of conjugated bile acids in the terminal ileum via a Na^+-dependent mechanism and (3) Na^+-independent anion exchange mechanism in proximal rat jejunum.

Passive Diffusion

Bile acids with a low pKa (~5.0) are negatively charged molecules. Conjugation with glycine or taurine in the intestinal lumen further reduces the pKa of bile acids to 3.8 or 2.0 making them impermeable to cell membranes and allowing high concentrations to persist in bile and intestinal lumen. Deconjugation by the intestinal microflora increases their pKa rendering these uncharged. The unconjugated bile acids can be rapidly transported into the enterocytes via passive diffusion, which occurs throughout the intestine (19,141). Since bile acids also undergo other modification such as dehydroxylation by the intestinal bacteria, these secondary bile acids can easily partition across the lipid bilayers due to their increased hydrophobicity (19). The passive mechanism involves non-carrier mediated mechanisms and accounts for a small fraction of intestinal bile acid conservation (19).

Apical Sodium Dependent Uptake of Bile Acids

*A*pical *S*odium *D*ependent *B*ile *A*cid *T*ransporter (ASBT; SLC10A2), the ileal counterpart of hepatic NTCP (35,36), represents a highly efficient bile acid conservation mechanism via uptake of bile acids across the luminal membrane in the terminal ileum.

Structure and Expression. ASBT has also been identified in the apical membranes of proximal renal tubular cells and cholangiocytes. Originally cloned from a hamster intestinal cDNA library (142), ASBT was subsequently cloned from human (143), rat (144), rabbit (145) and mouse ileum (146). ASBT is a 48-kDa glycoprotein consisting of 348 amino acids and is encoded by a major 4.0-kb transcript (6). The ASBT gene is localized on chromosome 13q33 in human and on 16q12 and 8A1 in rat and mouse, respectively, (6,147). The expression of ASBT has been shown to increase markedly during the third postnatal week of rat development, correlating with a functional bile acid transport at that time (148).

The structural aspects of ASBT and their specific relation to function and ligand recognition are slowly beginning to unfold. The exact membrane topology of ASBT remains controversial. Previous studies based on the bioinformatics predictions and experimental models suggested ASBT as a transmembrane protein (seven or nine helices) containing extracellular N-terminus and a cytoplasmic C-terminus (35). Membrane insertion scanning of the human ASBT supported a model of hASBT with nine integrated membrane segments (149). In contrast, recent studies utilizing N-glycosylation-scanning-mutagenesis showed that human ASBT is composed of seven transmembrane-spanning segments with a cytosolic carboxy terminus and an extracellular amino-terminal bearing an N-linked glycosylation site (150).

The cytoplasmic tail of ASBT has been shown to play an important role with respect to sorting of ASBT on the apical plasma membrane (151,152). Truncation of the 40 C-terminal

amino acid residues of ASBT eliminated its apical sorting polarity (151). Further, a 14-amino acid sorting signal on the c-terminus (aa 335–348) with two potential phosphorylation residues S^{335} and T^{339} has been identified as main determinant of apical targeting of ASBT (152). Site-directed mutagenesis studies revealed that cysteine residues (C^{51}, C^{74}, C^{105}, C^{106}, C^{132}, C^{144} and C^{225}) are important for ASBT transport function (36,153). Additionally, several mutations and polymorphisms have been identified in ASBT such as L243P, T262M and P290S, which reduces the bile acid transport function (36). Although, the ligand binding site for bile acids for ASBT is not very clearly defined; a computer generated 3D structure model has identified E^{282} and L^{283} as being involved in hydrogen bond formation with 12α-hydroxy group of bile acids (36,154). In an attempt to localize the ligand binding site experimentally, Kramer *et al.* (155,156) utilizing a photoaffinity labeling approach showed that 7-OH group of bile acid attaches to 67–56 C-terminal amino acids down stream of position 280 or 291 of rabbit ASBT. More recent evidence has implicated an important role of transmembrane segment seven (TM7) of hASBT in substrate translocation (157). E^{282} in human ASBT is the potential candidate for the extracellular sodium sensor for the Na^+/bile acid co-transport (154).

Function. The human ASBT efficiently transports conjugated and unconjugated bile acids with a preference for the taurine and glycine conjugates over the unconjugated forms (36,50). Further, ASBT exhibits a higher affinity for dihydroxy bile acids (Chenodeoxycholate CDCA) and deoxycholate compared to trihydroxy bile acids such as cholate, taurocholate and glycocholate (50). On the contrary, ASBT affinity for dihydroxy bile acid tauroursodeoxycholate was found to be lower than for taurocholate (50). The ASBT mediated transport is electrogenic with 2:1 Na^+/bile acid coupling stoichiometry (34). The essential role of ASBT in intestinal bile acid absorption is evident by the fact that ASBT knockout mice show intestinal bile acid malabsorption and elimination of enterohepatic circulation of bile acids (158). Since bile acids in the enterohepatic circulation exert a negative feedback effect on their hepatic biosynthesis from cholesterol, the size of circulating pool of bile acids is an essential factor influencing lipid and cholesterol homeostasis (2,12). Therefore, impairment of ASBT function by mutation or inhibitors has generated considerable interest pharmaceutically to lower plasma cholesterol levels and prevent atherosclerosis. In this regard, several studies in animal models have supported the feasibility of treating hypercholesterolemia with specific ASBT inhibitors (159). Several classes of ASBT inhibitors have been developed. A detailed structure, site of their action and effect has been extensively reviewed elsewhere (36). Recent studies have documented a novel non-systemic inhibitor of ileal apical Na^+-dependent bile acid transporter that efficiently reduces serum cholesterol levels in hamsters and monkeys (160).

Pathophysiological Implication. Pharmacological inhibition of ASBT has important therapeutic implications, however, dysfunction of ASBT has been associated with significant diseases in human. A dysfunctional ASBT missense mutation was first identified while cloning human ASBT cDNA from an ileal library constructed from a patient with Crohn's disease (143). Subsequently, single stranded conformation polymorphism (SSCP) was utilized to screen for the inherited mutations in a family with primary bile acid malabsorption (PBAM) (161). PBAM (Table II) is an idiopathic congenital intestinal disorder disease associated with interrupted enterohepatic circulation, infantile diarrhea, fat malabsorption and reduced level of plasma cholesterol (161). SSCP and direct sequence analysis established that mutations in ASBT lead to PBAM, thus emphasizing the role of ASBT in intestinal reclamation of bile acids. Dysregulation of ASBT is also associated with various ileal diseases such as radiation enteritis, Crohn's disease and intestinal inflammation. Further, bile acid malabsorption is well described in HIV and cystic fibrosis (162).

Regulation. Since ASBT plays a central role in a number of physiological and pathophysiological processes, it is of importance to understand the regulation of ASBT at the cellular and molecular level.

Regulation by Transcription Factors. Transcriptional regulation of ASBT in the intestine has been studied utilizing several approaches including *in vitro* transcription of the gene, promoter analysis and characterization *in vitro*, DNA protein interactions and utilizing transgenic animals (4). Several lines of evidence suggest that ASBT gene expression is subject to extensive transcriptional regulation (Table I). HNF1-α (encoded by TCF1), a homeodomain-containing transcription factor is important for diverse metabolic functions (86). $TCF^{-/-}$ knock out mice lacked ASBT expression in the intestine and kidney indicating that ASBT gene expression is HNF1-α dependent (86). These mice also showed a defect in bile acid transport (162). In contrast, $FXR^{-/-}$ knock out mice do not show any apparent alterations in ASBT expression (102). The human ASBT gene is also critically dependent on HNF1α for baseline promoter activity and this promoter activity is induced by the peroxisome proliferator-activated receptor α (PPAR α) (163). The rat ASBT gene promoter contains an AP-1 element that binds to c-jun and c-Fos (164). Co-expression of c-jun has been shown to enhance ASBT promoter activity (165).

Regulation by Sterols. It is well established that blocking the intestinal absorption of bile acids results in low levels of plasma cholesterol (2). Pharmacological inhibition of ASBT also causes similar effect on plasma cholesterol (36). Recent studies have increased our understanding of the molecular mechanism(s) underlying the coordination between intestinal ASBT function and cholesterol metabolism (138,166). We have shown that sterols such as 25-hydroxycholesterol inhibit the ASBT activity in human intestinal epithelial cells, which was associated with a decrease in ASBT mRNA and promoter activity (138). The inhibitory effect of cholesterol on ASBT function has been further supported by *in vivo* and *ex vivo* studies of Thomas *et al.* (166) and was shown to be mediated via SREBP-2 and HNF-1alpha transcription factors (Fig. 3). SREBPs belong to a family of transcription factors that contain a basic helix-loop-helix-zip domain and consists of three isoforms designated as SREBP-1a, SREBP-1c, and SREBP-2, which play different roles in the pathways of

Table I. Molecular Regulation of Bile Acid Transporters

Transcription Factor	Ligands	Effects on Transporters	Physiological Implications	References
Farnesoid-X Receptor (FXR)	Bile acids	IBABP (+) BSEP (+) OATP1B3 (+) OSTα/β (+)	Increased bile acid efflux	(195,196) (92) (94) (208)
Small Heterodimer Partner (SHP)	–	NTCP (−) ASBT (−)	Decreased bile acid transport into the cell	(60) (173,174)
Peroxisome proliferator activated receptor-alpha ($PPAR-\alpha$)	Fatty Acids Fibrates	ASBT (+) IBABP (+)	Increased intestinal bile acid absorption	(163) (200)
Liver X receptor (LXR)	Oxysterols	IBABP (+) OSTα/β (+)	?	(199) (209)
Hepatic Nuclear Factor 1-alpha (HNF1-α)	–	NTCP Oatp1a1 Oatp1a4 ASBT	Important for basal promoter activity	(61) (86)
Hepatic Nuclear Factor 4-alpha (HNF4-α)	–	OATP1	Important for basal promoter activity	(87)
Pregnane X receptor (PXR)	Xenobiotics Rifampin	Oatp1a4 (+)	Increased transport of organic anions and xenobiotics	(91)
Glucocorticoid Receptor (GR)	Glucocorticoid	ASBT (+) NTCP (+)	Increased Na^+ dependent bile acid uptake	(179) (65)
Retinoic Acid Receptor (RAR)	Retinoids	ASBT (+) NTCP (+)	Increased Na^+ dependent bile acid uptake	(173) (60)
Vitamin D Receptor (VDR)	Vitamin D	ASBT (+)	Increased ileal Na^+ dependent bile acid uptake	(180)

(+) denotes an increase and (−) denotes a decrease in the expression.

cholesterol and lipid synthesis (167). The transcriptional activity of SREBPs is influenced by cholesterol by proteolytic modifications (167). Low levels of cholesterol result in a cleavage of the NH2-terminus of inactive SREBPs, producing the mature transcription factor, which translocates to the nucleus to affect gene expression (168). This proteolytic process is inhibited by high levels of cholesterol (Fig. 3). Our studies as shown in Fig. 3 suggest that the modulation of hASBT by cholesterol could be an adaptive response to high cholesterol. The function and the expression of intestinal hASBT will be reduced in response to high cholesterol leading to an inhibition in intestinal absorption of bile acids and a subsequent reduction in the size of their circulating pool. This, in turn, will limit the negative feedback effect of bile acids on their hepatic biosynthesis from cholesterol and, therefore, enhance cholesterol catabolism and lower its plasma level.

Intriguingly, humans exhibit variations in responses to cholesterol enriched diet as some develop hypercholesterolemia and others are resistant (169,170). In this regard, the differential expression of regulatory pathways such as SREBP2 in some individuals might underlie the discrepancy in their responses to high-cholesterol diet and the development of hypercholesterolemia. Delineation of the molecular mechanism(s) by which 25-HCH regulates hASBT is, therefore, of great significance, because such pathways would be the target of pharmacological upregulation as a modality for the treatment of cholesterol-related disorders. These studies further underscore the critical participation of ASBT in the maintenance of body cholesterol homeostasis.

Regulation by Bile Acids. The bile acid responsiveness of the ASBT gene varies depending upon the cell line or species under investigation. For example, the ileal expression of the ASBT in the rat is unaffected by bile salts (171), however in the mouse, it is under negative feedback regulation (172). Similarly, bile acids were shown to induce a negative feedback regulation of the human ASBT (173). Recent advances in elucidation of the molecular mechanisms underlying bile acid responsiveness have provided insights into the apparent differences observed in bile acid responsiveness in different species. In the mice, bile acid induced regulation of ASBT gene is mediated by farnesoid X receptor (FXR)-dependent upregulation of the short heterodimer partner (SHP) expression (Fig. 2) (174). SHP in turn represses (LRH-1)-dependent activation of ASBT expression (174). The presence of LRH-1 cis and trans acting elements

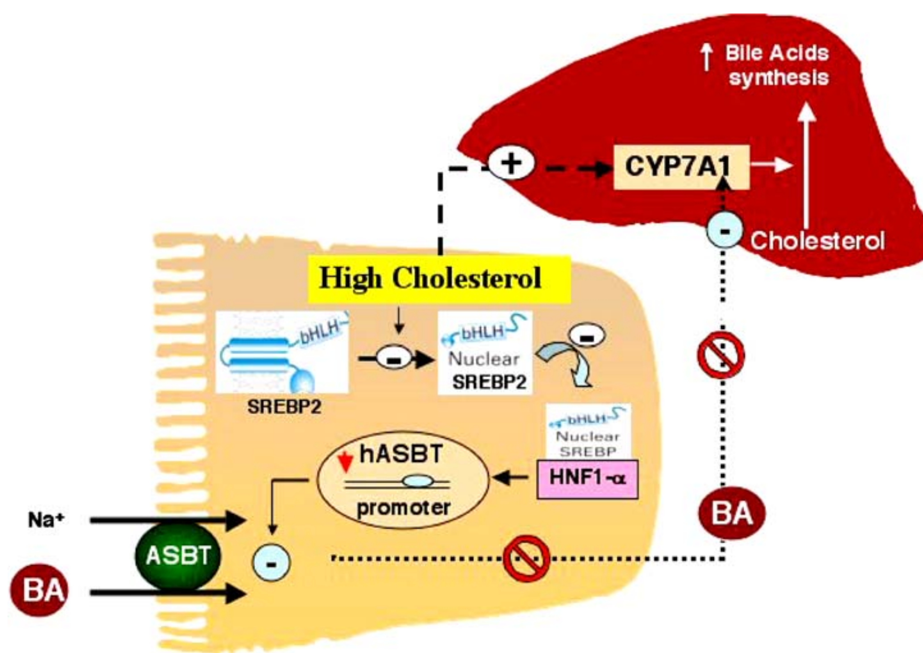

Fig. 3. Regulation of ASBT by cholesterol and its physiological implications. High level of cholesterol suppresses the cleavage of Sterol Response Element Binding Protein-2 (*SREBP2*) into a mature transcription factor leading to a reduction in the promoter activity of ASBT and a decrease in its expression. The down regulation of ASBT expression and the parallel decrease in intestinal bile acid absorption reduces the circulating pool of bile acids and attenuates their negative feed back effect on cytochrome P450 (CYP7A1) enzyme, the rate limiting step in bile acid synthesis and cholesterol catabolism. This in turn leads to an increase in bile acid synthesis and cholesterol catabolism.

in the mouse but not the rat promoter, therefore, correlates with the cell line and bile acid responsiveness (174). Similarly in the human, effects of bile acids are mediated via an FXR and SHP-mediated repression of RAR/RXR induced activation of ASBT (173). Interestingly, NTCP in the liver undergoes similar negative feed back regulation by bile acids suggesting a coordinated interplay between the processes of bile acid and cholesterol homeostasis.

Regulation by Cytokines. Ileal inflammation or resection has been associated with pathologic bile acid malabsorption that potentiates the associated diarrhea (162). Extensive malabsorption in these settings can lead to a severe depletion of the bile acid pool and subsequent development of gall stones and steatorrhea (175). ASBT expression or function has been found to be decreased in various animal models of experimentally induced ileitis (162,176). A decrease in ASBT protein expression has also been reported in pinch biopsy specimens from the ileum of adults with Crohn's disease (162). Since intestinal inflammation is associated with increased levels of cytokines, several studies have attempted to delineate the transcriptional mechanisms involved in cytokine-mediated repression of ASBT. The proinflammatory cytokines interleukin-1β and tumor necrosis factor have been shown to repress the rat ASBT promoter in Caco-2 and IEC-6 cells (175). This repression was mediated via upregulation and nuclear translocation of c-fos and binding of the downstream AP-1 element to the c-jun/c-fos heterodimer. Similarly, proinflammatory cytokines decreased both mouse and human ASBT activity along with induction of c-jun and c-fos expression (165). Also, lack of C-fos *in vivo* (c-fos$^{-/-}$) was associated with activation of ASBT and enhanced the ileal inflammation in response to indomethacin (165). These studies suggested that c-fos is a critical mediator involved in repression of ASBT by proinflammatory cytokines. However, extensive studies in other models of inflammation are needed to fully understand the ASBT responsiveness to intestinal inflammation.

Regulation by Hormones and Vitamins. Given a central role of glucocorticoids (GC) in the treatment of Crohn's disease, examining the pharmacological effect of glucocorticoids on ileal bile acid transport has been an area of interest. Glucocorticoids have been shown to increase the activity of ASBT both in rat and rabbit ileum (177). Conversely, adrenalectomy performed in adult rats reduced active ileal taurocholate transport (178). Human ASBT is also shown to be induced by glucocorticoids both *in vitro* and *in vivo*. Studies done in healthy male volunteers showed that ASBT protein expression was increased after 21 days intake of budesonide (179). Also, reporter constructs of the human ASBT promoter were strongly activated by coexpression of the glucocorticoid receptor (GR) and exposure to the GR ligands dexamethasone or budesonide (179). Two glucocorticoid response elements in the ASBT promoter (IR3 elements) conferred inducibility by GR and dexamethasone (179). Therefore, ASBT appears as a novel target of glucocorticoid controlled gene regulation in the human intestine (179).

ASBT gene is also regulated by 1alpha,25-dihydroxyvitaminD (180). The rat ASBT gene expression was activated by 1,25(OH)(2)D(3)] by specific binding to the vitamin D receptor response element VDRE and was associated with enhanced ileal bile acid transport (180). Human ABST

mRNA and promoter activity have also been observed to increase in Caco-2 cells treated with 1,25(OH)(2)D(3), suggesting a physiological role of VDR in human ileal bile acid homeostasis (180).

Ontogenic Developmental Regulation of ASBT. ASBT regulation has also been investigated during developmental stages. Na^+-dependent TC uptake has been demonstrated in the small intestine of an 8-month child but not in the intestine of neonate or a fetus (181). In the rats, ASBT mRNA, protein and activity are expressed in the fetal tissue, which disappears completely in the early neonatal life and reappears abruptly at 17d of age during weaning (181). A role of endogenous thyroxine but not corticosterone has been described in the regulation of developmentally timed appearance of ASBT (181).

Expression of ASBT, TC transport activity in ileal BBMVs, and TC absorption in ileal loops are all significantly increased in postpartum rats (182). The ASBT protein expression in the proximal ileum was significantly increased and was maximal at days 12–21 of lactation. Interestingly, this increased expression was preferentially observed in the proximal region of the ileum where constitutive expression of the transporter is low, but not in the most distal region that normally exhibits the highest constitutive expression of ASBT. Neither intravenous administration of prolactin to ovariectomized rats nor administration of GLP-2 to normal rats increased expression of ASBT. Also. ASBT mRNA levels were not affected in lactating rats suggesting posttranscriptional regulation (182). Further exhaustive studies on posttranslational modifications are necessary to confirm the exact mechanisms involved.

Regulation by Post-translational Modifications. Previous studies have demonstrated the involvement of various signal transduction molecules and membrane trafficking events in the acute modulation of ASBT function and membrane expression. For example, a role of cAMP-dependent pathways and MAP kinases has been demonstrated in the alteration of active bile acid absorption in both rat ileum and renal proximal tubular cells (183,184). Also, secretin stimulated the activity of ASBT by shuttling ASBT from sub-apical endosomes to the apical membrane and thus increasing its surface membrane expression in rat cholangiocytes (185). ASBT in cholangiocytes has also been shown to undergo ubiquitin-proteosome degradation under basal conditions (186). Xia *et al.* presented evidence that rapid IL-1β dependent reduction of ASBT in cholangiocytes occurs through increased ASBT disposal through the ubiquitin-proteasome pathway and requires phosphorylation of ASBT (186). Information regarding posttranslational regulation of ASBT gene in the human ileum is very limited. Our recent studies have indicated that ASBT is also subject to acute regulation by enteropathogenic *E. coli*, EPEC and can contribute to the pathophysiology of EPEC associated diarrhea. Enteropathogenic *E. coli* (EPEC), a food-borne enteric pathogen is a major cause of infantile diarrhea world-wide leading to high morbidity and mortality (187). Our studies utilizing human intestinal Caco-2 cells as an experimental model demonstrated that short-term EPEC infection (15–60 min) significantly inhibited ASBT activity (187). This EPEC-induced inhibition of ASBT function was dependent on an intact type III secretion system (TTSS), through which bacteria delivers its proteins into the host cell (187). These studies warrant further investigation both *in vitro* and *in vivo* models in terms of role of EPEC virulence genes and signaling or membrane trafficking events to fully understand the mechanistic basis for microbial/ epithelial bile acid transport interactions that contribute to the development of diarrhea. Another important aspect that increased our understanding of ASBT regulation comes from our demonstration that human ileal ASBT exists in specialized compartments of plasma membranes known as lipid rafts (188). The association of ASBT with lipid rafts not only dictates its specific regulation by protein kinases but might also explain the rapid adaptive changes of ASBT to the varying intestinal milieu.

Na^+ Independent Bile Acid Transporter

The Na^+ independent bile acid transporter Oatp1a5 (Slco1a5A7) was originally cloned from retina and is 80–82% identical to Oatp1a1 and Oatp1a4 (189). Oatp1a5 was also cloned from rat and was found to be expressed in brain, lung, kidney and brush border membrane of jejunal enteorcytes (6). The uptake of bile acids into jejunum brush border membrane vesicles has been characterized and occurs via in-to-out HCO_3^- gradient; however whether this mechanism also pertains to Oatp1a5 is unclear (190). The functional expression of bile acid transporting OATP has not been established in the human intestine, although OATP1A2 has been suggested as the human Oatp1a5 ortholog (5,189).

Intracellular Transport of Bile Acids

The intestinal bile acid binding protein (IBABP) belongs to a family of intracellular lipid-binding proteins that bind fatty acids, retinoids, cholesterol, and bile acids (6). The IBABP gene has been cloned and characterized from mouse and rabbit (6). IBABP is 14–15 kDa cytoplasmic protein that reversibly binds the bile acids once they enter the cell (6). The expression of IBABP is restricted to the ileum with low expression found in the cholangiocytes and functionally has been implicated in transcellular trafficking and enterohepatic circulation of bile salts (191). Human I-BABP has been shown to binds two molecules of glycocholate (GCA) with low intrinsic affinity but high degree of positive cooperativity (192). Besides, human I-BABP demonstrates a high degree of site selectivity in its interactions with the two major bile salts in humans i.e GCA and glycochenodeoxycholate (GCDA) (193). Further studies including the generation of *I-BABP*-null mice, are needed to elucidate the biological functions of I-BABP.

Regulation. IBABP gene is under tight regulation by different sterol sensors (194). However, unlike ASBT, which is under negative bile acid feed back regulation, I-BABP gene expression is up-regulated by bile acids through the activation of the nuclear FXR (195,196). This regulation is mediated via binding of an FXR/RXR heterodimer to a bile acid responsive element (IR1) identified on promoter region of human IBABP (196). Consistently, FXR null mice show a

decrease in the expression levels of IBABP (197). The functional association of I-BABP has been described with FXR in the nucleus and with ASBT on the apical membrane (198). The concerted action of ASBT and I-BABP in response to reciprocal regulation by bile acids thus helps abolish the cytotoxic effects of bile acids in the ileum while promoting their biological functions (Fig. 2). Cholesterol enriched diet has also been shown to induce IBABP gene expression (194). Further, IBABP gene has been shown to be under direct regulation by LXR, which involves binding of an LXR/RXR heterodimer to the FXR responsive element in the IBABP promoter (199). The physiological implication of downregulation of IBABP by cholesterol is not clear. PPAR has been shown to regulate the expression of IBABP gene in a species-specific manner (200). The PPARα-PPARβ/δ agonist bezafibrate induced the endogenous IBABP gene expression in human intestinal Caco-2 cells (200). A functional PPAR response element was identified in the proximal region of human IBABP promoter (200). In contrast, mouse IBABP promoter lacks a conserved PPRE and thus was unresponsive to bezafibrate in inducing IBABP expression (200). Recent studies by Halpern *et al.* (201) suggested that dysregulation of IBABP may be involved in the pathophysiology of necotic enterocolitis (NEC) as evident in a rat model.

Basolateral Efflux of Bile Acids

Until recently, the mechanisms by which bile acids are transported across the basolateral membranes of the enterocytes to enter portal blood were not well defined. The bile acid transport in rat ileal basolateral membrane vesicles was demonstrated to occur via sodium-independent exchange mechanism that was trans-stimulated by sulfate, bicarbonate ions and p-aminohippurate (137). An ATP-dependent bile acid transporter activity has also been demonstrated (202). Subcellular fraction studies and affinity labeling using bile acid photoprobes implicated a 54–59 kDa protein (with no particular identity) enriched in the basolateral membranes of ileal epithelial cells (203,204). In this regard, alternatively spliced form of ASBT (t-ASBT) and the multidrug resistance-associated protein (Mrp3) were also proposed as potential candidates for the basolateral bile acid transporter; however, their specific contributions to efflux of bile acids is minor or uncertain (6). More recently, transcriptional profiling of wild type and SLC10A2 null mice identified a new candidate basolateral bile acid carrier, the heteromeric organic solute transporter Ostα-Ostβ (139).

OSTα-OSTβ

Structure, Expression and Function. OStα-OSTβ were originally cloned from the liver cDNA library of an evolutionarily ancient vertebrate, skate *Leucoraja erinacea* (205). Subsequently human and mouse orthologues were also identified (206). OSTα/OSTβ mediated transport was found to be Na^+-independent, saturable and inhibited by organic anions and steroids (205,206). The transport activity requires the co-expression of two distinct gene products: a 340 amino acid, seven transmembrane domain protein (OSTα) and a putative 128-amino acid single transmembrane domain ancillary polypeptide (OSTβ:(207). The substrates for Ostα-Ostβ mediated transport include taurocholate, estrone-3-sulfate, digoxin, PGE2 and dehydroepiandrosterone sulfate (DHEAS) (205,206). OSTα/OSTβ is expressed at relatively high levels in the small intestine and kidneys of human, mice and rats with both OSTα and OSTβ localized to the basolateral surface of ileal enterocytes (139,207). The basolateral localization further supports its role as major bile acid efflux transporter in the ileum.

Regulation. The information regarding molecular regulation of OSTα/OSTβ has slowly beginning to emerge. Recent studies demonstrated that in human ileal biopsies exposed to bile acid, chenodeoxycholic acid, the levels of human OSTα/OSTβ mRNA were induced (208). Also, OSTα/OSTβ promoters were transactivated by FXR in the presence of ligand (208). These studied identified OSTα/OSTβ as a target for FXR-mediated gene regulation similar to intestinal IBABP. OSTα/OSTβ is functionally analogous to the hepatocyte BSEP in terms of being a major bile acid efflux pump that is also transactivated by FXR. Therefore, bile acids probably are able to induce their own clearance by feed forward induction of the efflux pumps, OSTα/OSTβ and BSEP from hepatocytes and enterocytes, respectively, (Fig. 2).

Interestingly, Ostα/Ostβ mRNA levels have been shown to be increased in the cecum and proximal colon but decreased in ileum of $ASBT^{-/-}$ knock out mice (139). This could be explained by reduced uptake of bile acids in the ileal enterocytes of ASBT knock out mice resulting in decreased intracellular levels of the FXR ligands and thus subsequent decrease in Ostα/Ostβ expression. More recent studies demonstrated the coordinated regulation of both mouse Ostα and Ostβ by FXR/RXRα and LXRα/RXRα via functional FXREs/LXREs (IR-1 elements) in their promoter region (209). Further, this regulation of Ostα but not Ostβ was enhanced by HNF-4α (209). However, the physiological implication of this regulation by LXR remains elusive.

Table II. Disorders Associated with Bile Acid Transporters

Disease	Abbreviation	Impairment in Transporter	Symptoms	Reference
Progressive Familial Intrahepatic Cholestasis Type I	PFIC Type 1	FIC1	Cholestasis, diarrhea	(121)
Progressive Familial Intrahepatic Cholestasis Type 2	PFIC Type 2	BSEP	Cholestasis	(103)
Dubin–Johnson Syndrome	DJ	MRP2	Jaundice	(135)
Primary Bile Acid Malabsorption	PBAM	ASBT	Diarrhea, fat malabsorption	(161)

BILE ACID TRANSPORTERS IN CHOLANGIOCYTES AND RENAL EPITHELIAL CELLS

Besides their expression in hepatocytes and enterocytes, bile acid transporters are also expressed in other epithelial cells such as cholangiocytes and renal proximal tubule cells (4,6,35). Bile acids are actively absorbed by cholangiocytes lining bile ducts to recycle back to the hepatocytes for their re-secretion (6,210). The circulation of bile acids between the cholangiocytes and hepatocytes is known as choleheptic shunt pathway (6,210). In cases such as chronic cholestasis associated with secondary to extrahepatic obstruction in which bile is blocked from reaching the small intestine, the cholehepatic shunt appears to be the major pathway for the absorption and recycling of bile acids from the bile back to the liver compensating for the ileal absorption (210). Another possible role for the uptake of bile acids into the cholangiocytes is to coordinately trigger adaptive intracellular responses in the cholangiocytes in response to bile acid content of the bile (210). Vectorial transport of bile acids across the cholangiocytes is mediated with similar set of transporters found in the terminal ileum. In the cholangiocytes, ASBT mediates Na^+-dependent bile acids transport across the apical membrane, IBABP facilitates their transcellular movement and Ostα/Ostβ heterodimer of transporters or MRP3 or a putative t-ASBT (truncated ASBT) are potentially responsible for bile acid efflux from the basolateral membrane (6,210). The function and regulation of bile acid transporters in cholangiocytes have been recently reviewed in detail elsewhere (210).

Active bile acid transport has also been reported in renal proximal tubule (6). The re-absorption of bile acids from the glomerular filterate ensures the conservation of bile acids that escape hepatic clearance and spill to the systemic blood (184,211). Under normal conditions, the urinary excretion of bile acids is smaller compared to the amount of bile acids in the gomerular filterate due to almost complete renal reabsorption (184,211). Interestingly, urinary secretion of bile acids is significantly increased during the early stages of obstructive cholestasis (211). These findings suggested the presence of adaptive mechanisms that decrease active renal reabsorption of bile acids stimulating their renal excretion (211). With respect to bile acid transporters, ASBT, MRP4 and MRP2 were found to be localized on the apical membrane renal epithelial cells (4,6). Moreover, the recently identified OSTα/OSTβ heterodimer is also expressed on the basolateral membrane of proximal tubule suggesting its involvement in bile acid efflux from renal epithelial cells (207).

SUMMARY

The enterohepatic circulation of bile acids has attracted considerable interest due to its essential role in bile formation and maintenance of lipid and cholesterol homeostasis. However, a greater leap in our understanding of physiology of enterohepatic circulation occurred only within the last two decades when the cloning of bile acid transporters was made possible by advances in molecular biology. Further, identification of mutations in bile acid transporters such as BSEP and ASBT unraveled the basic pathobiology of several related inherited diseases as type 2 familial intrahepatic cholestasis (FIC2) and primary bile acid malabsoprtion (PBAM), respectively. Knockout mice lacking the expression of certain bile acid transporters provided indispensable models to better evaluate the function of these transporters and the associated diseases such as cholestasis in BSEP knockout mice. Likewise, determining structure-function relationship of bile acid transporters and examining their substrate and inhibitor profiles enhanced our understanding of the pathophysiology of drug-induced cholestasis. The development of ASBT-specific inhibitors is an attractive area for the development of cholesterol-lowering drugs. Certain bile acid transporters may also be considered as drug-carriers taking advantage of their restricted tissue expression such as NTCP expression in the liver and its ability to mediate the transport of bile acid-conjugated chlorambucil. Elucidating the molecular regulation of the expression of bile acid transporters and the signal transduction pathways that modulate their function have broadened the potentials in designing drug therapy for related-disorders. For example, altering hepatic expression of OAPTs modulates the clearance of certain drugs influencing their bioavailability. Inducing hepatic intracellular Ca^{+2} may improve cholestatic conditions by stimulating the function of NTCP and increasing its plasma membrane expression. Delineating the molecular regulation of bile acid transporters is still, however, a fertile area for investigative research. The exact mechanisms coordinating the function of the intracellular and plasma membrane bile acid transporters are not fully understood. Finally, the generation of knockout mice for other bile acid transporters, such as NTCP, may pave the way to identify additional pathways for bile acid transport and may help determine the contribution of several known pathways in bile acid transport.

ACKNOWLEDGMENTS

The authors are thankful to Drs Pradeep K. Dudeja, K. Ramaswamy for their invaluable suggestions and critical reading of the manuscript. Thanks are also due to Dr. Fadi Annaba for his help in the preparation of this review. Studies in the authors laboratory are supported by NIDDK grant DK71596 (WAA).

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