Molecular aspects of bile formation and cholestasis

Marco Arrese[1] and Michael Trauner[2]

[1]Departmento de Gastroenterología, Facultad de Medicina, Pontificia Universidad Católica de Chile, Marcoleta 347, 8320000 Santiago, Chile
[2]Medizinische Universitätsklinik Graz, Auenbruggerplatz 15, A-8036 Graz, Austria

Recent insights into the cellular and molecular mechanisms that control the function and regulation of hepatobiliary transport have led to a greater understanding of the physiological significance of bile secretion. Individual carriers for bile acids and other organic anions in both liver and intestine have now been cloned from several species. In addition, complex networks of signals that regulate key enzymes and membrane transporters located in cells that participate in the metabolism or transport of biliary constituents are being unraveled. This knowledge has major implications for the pathogenesis of cholestatic liver diseases. Here, we review recent information on molecular aspects of hepatobiliary secretory function and its regulation in cholestasis. Potential implications of this knowledge for the design of new therapies of cholestatic disorders are also discussed.

Bile secretion plays a pivotal role in liver physiology because it serves as an important excretory route for many endo- and xeno-biotics, and participates in absorption of lipids from the intestinal lumen [1]. Recently, major advances have been made in the molecular identification of membrane transport proteins in liver and intestine that control bile formation [2–4]. Information on the regulation of these transport systems under physiological and pathophysiological conditions have unraveled a complex network of signals controlling bile salt (BS) synthesis and transport, as well as cholesterol, lipoprotein and drug metabolism [4,5]. Thus, new concepts have emerged for the normal regulation of organic solute transporters and the adaptive responses of the hepatocyte to intracellular accumulation of potentially toxic compounds [4]. This information has led to a better understanding of the control of normal bile flow and, hence, the processes involved in the pathogenesis of several forms of cholestatic liver diseases. Moreover, transport proteins and their regulators represent novel potential pharmacological targets for therapeutic intervention in cholestatic diseases and drug-induced liver damage [5–7]. This review summarizes recent data on the molecular determinants of bile formation and their regulation in cholestasis.

Basic concepts of bile formation

Bile formation results from the active secretion of osmotically active compounds by hepatocytes into the canalicular space, followed by the passive movement of water through the tight junctions. Bile salts are the main solutes in bile and are considered to be the major osmotic driving force in the generation of bile flow, although BS-independent processes also contribute to bile production [1,8]. Transport of biliary constituents from blood to bile is a vectorial process that includes uptake from sinusoidal blood, intracellular transport through the hepatocyte (with or without metabolic modifications) and canalicular secretion against steep concentration gradients that require energy-dependent active transport [4,8]. Although the main determinant of overall bile flow is the volume of water generated at the canalicular level, further modifications of bile composition occur along the biliary tree [9]. In addition, BS – the major biliary solutes – undergo enterohepatic cycling as a result of the presence of an active transport mechanism located in the apical pole of enterocytes at terminal ileum [4,10]. This enables retrieval of BS from the intestinal lumen and transport to the portal circulation and, ultimately, to the liver for uptake and re-secretion.

Molecular basis of hepatobiliary transport

The main membrane transporters that primarily determine hepatic bile production are now largely characterized at the molecular level. Most of them have been cloned from both human and rodent tissues. Information on the localization, nomenclature and function of hepatobiliary transporters is listed in Table 1. Some of these transporters are also expressed in tissues other than the liver, where they play specific physiological roles. Owing to space limitations, only the basic concepts of hepatic transporters involved in bile formation (Figure 1) are reviewed here. More comprehensive reviews are available elsewhere [4,11,12]. The following sections describe individual transport processes according to their localization in the plasma membrane of liver cells.

Sinusoidal uptake of biliary solutes

The sinusoidal membrane of hepatocytes contains several carrier proteins that facilitate entry into the liver of BS and other lipid-soluble organic substances, including physiological substrates (i.e. bilirubin), as well as drugs

Corresponding author: Marco Arrese (marrese@med.puc.cl).

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Table 1. Localization, nomenclature and function of major membrane transporters relevant for bile formation[a,b]

Transporter/gene nomenclature[c]	Location	Main function
NTCPs/SLC10A1	Basolateral membrane of hepatocytes	Main carrier for Na^+-dependent uptake of conjugated bile salt from portal blood.
OATPs/SLC2IA	Basolateral membrane of hepatocytes	Na^+-independent uptake of unconjugated bile salts and other organic anions. Polyspecific transporters with overlapping substrate affinity that are able to uptake endo- and xeno-biotics.
OCT/SLC22A	Basolateral membrane of hepatocytes	Hepatic uptake of hydrophilic organic cations. Relevant for drug transport.
OATs/SLC22A	Basolateral membrane of hepatocytes	Na^+-independent transport of *para*-aminohippurate, salicylate, acetylsalicylate and methotrexate.
MRP 3/ABCC3	Basolateral membrane of hepatocytes and cholangiocytes	Basolateral efflux of biliary constituents including non-sulfated and sulfated bile salts. Preferentially transports glucuronides but not glutathione, S-conjugates or free glutathione. Might play a role in the removal of bile acids from the liver in cholestasis.
MRP 4/ABCC4	Basolateral membrane of hepatocytes and cholangiocytes	Mediates glutathione efflux from hepatocytes into blood by co-transport with monoanionic bile salts. Might also function as an overflow pathway during cholestasis. In bile duct cells, might facilitate the return of bile salts from the obstructed bile ducts to the systemic circulation.
MDR1/ABCBI	Canalicular membrane of hepatocytes	ATP-dependent excretion of bulky organic cations into bile.
MDR3/ABCB4	Canalicular membrane of hepatocytes	Translocation of phosphatidylcholine from inner to outer leaflet of the membrane bilayer. Crucial for biliary phospholipid secretion.
MRP2/ABCC2	Canalicular membrane of hepatocytes	Canalicular conjugate export pump previously known as cMOAT. Transports bilirubin diglucuronide, sulfates, glutathione conjugates and various organic anions into bile in an ATP-dependent manner.
BSEP/ABCB11	Canalicular membrane of hepatocytes	Mediates ATP-dependent bile salt transport into bile.
ABCG5/ABCG8	Canalicular membrane of hepatocytes	'Half ABC transporters'[d] that function as heterodimers to transport sterols into bile. They might also partially mediate biliary cholesterol secretion.
BCRP/ABCG2	Canalicular membrane of hepatocytes	'Half ABC transporter'[d] that mediates cellular extrusion of sulfated conjugates.
AE2/SLC4A2	Canalicular membrane of hepatocytes and apical membrane of cholangiocytes	Facilitates bicarbonate secretion into bile and contributes to bile-salt-independent bile flow.
ABST/SLCI0A2	Apical membrane of cholangiocytes	Identical to the ileal bile salt transporter[b]. Might function as an uptake mechanism for bile salts, removing them from bile.
FIC1/ATP8B1	Canalicular membrane of hepatocytes and apical membrane of cholangiocytes	Member of the Type IV P-type ATPase family, which functions as an ATP-dependent aminophospholipid translocase. However, FIC1 function is not yet clearly defined. It is mutated in two different disorders: PFIC1 and BRIC.

[a]Abbreviations: ABC, ATP-binding cassette; ABST, apical Na^+-dependent bile salt transporter; AE2, chloride-bicarbonate anion exchanger isoform 2; BCRP, breast cancer related protein; BRIC, benign recurrent intrahepatic cholestasis; BSEP, bile salt export pump; cMOAT, multi-specific organic-anion transporter; FIC1, familial intrahepatic cholestasis 1; MDR, multidrug resistance; MRP, multidrug-resistance-associated protein; NTCP, Na^+ taurocholate co-transporting polypeptide; OATs, Organic anion transporters; OATPs, organic anion transporting polypeptides; OCT, Organic cation transporter; PFIC1, progressive familial intrahepatic cholestasis type 1.
[b]Some of these transporters are also expressed in tissues other than the liver (intestine and kidney) where they play specific physiological roles. These were excluded from the Table owing to space limitations. Full details of all transport proteins can be found in Ref. [4].
[c]Nomenclature schemes using a 'stem' (or 'root') symbol for members of a gene family or grouping, with a hierarchical numbering system to distinguish individual members has been developed by the Human Genome Nomenclature Committee. Guidelines are available at: http://www.gene.ucl.ac.uk/nomenclature/guidelines.html.
[d]'Half ABC transporters' indicates transporters that, in contrast to typical ABC transporters, contain only six instead of twelve transmembrane domains and one instead of two ATP-binding sites.

and xenobiotics. Bile salt uptake is mediated by Na^+-dependent and -independent mechanisms. The Na^+ taurocholate co-transporting polypeptide (NTCP) and a growing family of multi-specific organic anion transporters are the major proteins involved in this step of bile formation. The NTCP is exclusively expressed in liver, strictly localized on the basolateral membrane of hepatocytes, and is the predominant Na^+-dependent BS transporter of the hepatocyte [11]. The NTCP transports mainly conjugated BS, taking advantage of the membrane inwardly directed Na^+ gradient, which is maintained by the Na^+/K^+-ATPase of the liver cell [8,11].

Sinusoidal Na^+-independent transport of BS and organic anions is mediated by the organic anion transporting polypeptides (OATPs). The OATPs are a family of polyspecific transporters with overlapping substrate affinity that mediate the Na^+-independent uptake of unconjugated species of BS [12]. In addition, OATPs mediate the uptake of a large number of other compounds with differing charge and structure, including some bilirubin conjugates, thyroid hormones, neutral steroids and numerous drugs and xenobiotics [11,12]. An increasing number of OATPs have been identified both in rat and humans. In contrast to NTCP, OATPs are expressed in extrahepatic tissues, particularly the intestine, kidney and brain, underscoring their role in the overall disposition of amphipatic compounds [12]. Although some human OATPs are not true orthologs of the mouse or rat gene product, those that are predominantly or exclusively expressed in the liver have similar functions to mouse or rat Oatps*. Uptake function of some members of the OATP family appears to involve anion exchange with reduced glutathione and bicarbonate [11]. Moreover, some OATPs might work as bi-directional transporters at high intracellular substrate concentrations, functioning, when needed, as an extrusion system of potentially dangerous compounds [13].

Additional transport proteins, such as the liver homologs of the kidney organic anion transporters (OATs) and polyspecific organic cation transporters (OCTs), have

* By convention, the names of human hepatobiliary transporter genes are capitalized, whereas rodent genes and their products are written in lower case with capitalization of the first letter. In addition, transporter genes are set in italics, whereas gene products are set in roman.

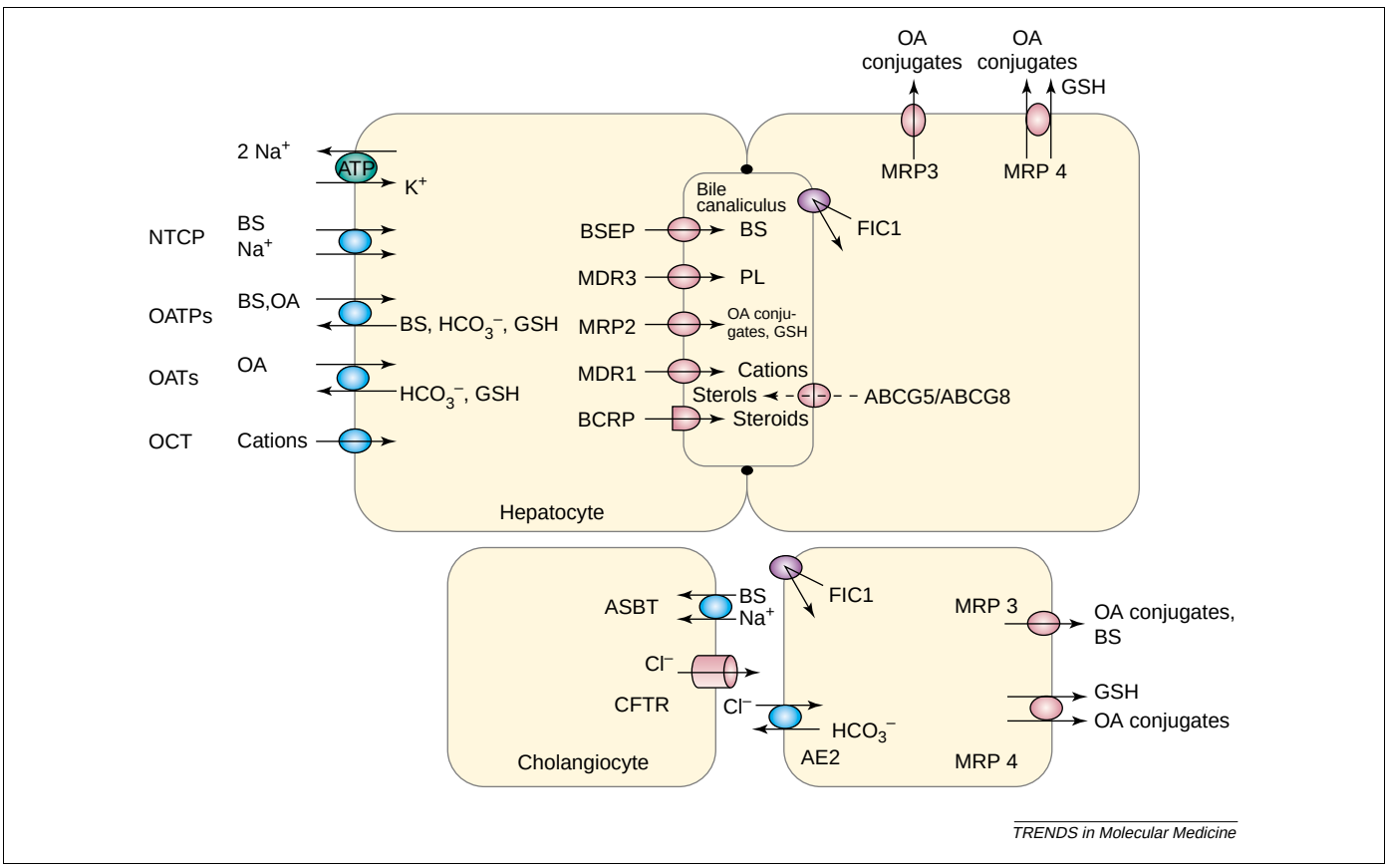

Figure 1. Hepatobiliary transport proteins involved in bile formation. Transport proteins are shown as circles with arrows showing the direction of transport. Transporters depicted in blue correspond to proteins belonging to the solute carrier (SLC) family, whereas those depicted in pink belong to the ATP-binding cassette (ABC) transporter family. The 'half ABC transporters' are depicted as semi-circles. The cystic fibrosis transmembrane conductance regulator (CFTR), which functions as a Cl^- channel, is shown as a cylinder. It should be noted that channels are not transporters. The hepatic Na^+/K^+-ATPase is shown as a green circle. The Na^+ taurocholate co-transporter (NTCP) at the basolateral membrane of hepatocytes mediates bile salt (BS) uptake, whereas a family of Na^+-independent organic anion transporting polypeptides (OATPs) transports not only BS but also various (non-BS) organic anions (OA). Organic cations (OC) are transported by an OC transporter (OCT), and certain organic anions (e.g. drugs) are transported by a family of OA transporters (OATs). Bile salts are excreted through a canalicular BS export pump (BSEP). Glutathione (GSH) and (non-BS) OAs (e.g. conjugated bilirubin) are excreted through a canalicular conjugate export pump (multidrug-resistance-associated protein 2; MRP2). In addition, the canalicular membrane contains export pumps for organic cations [multidrug-resistance 1 (MDR1) gene product], phospholipids (PL) (MDR3), and sulfate conjugates (breast cancer related protein; BCRP). The twinned canalicular half-transporters ABCG5 and ABCG8 transport sterols into bile and seem to play an important role in the regulation of biliary cholesterol secretion. MRP3 and MRP4 are expressed in the basolateral domain of hepatocytes and might facilitate extrusion of biliary constituents as they accumulate in the cytoplasm. Bile duct epithelial cells contain the CFTR, which is a Cl^- channel that drives bicarbonate (HCO_3^-) excretion through a $Cl^- - HCO_3^-$ anion exchanger (AE2). In addition, bile duct epithelial cells contain the apical Na^+-dependent BS transporter (ASBT) and MRP3 for reabsorption and cholehepatic cycling of BS. The familial intrahepatic cholestasis 1 (FIC1) protein (purple circle) is a P-type ATPase, the function of which is not yet fully understood (it is thought to translocate aminophospholipids within the canalicular membrane). However, it seems to be crucial for bile formation because mutations of this transporter are present in certain forms of genetic cholestasis.

recently been identified at the sinusoidal domain of hepatocytes [14,15]. These transporters could play a role in drug uptake by the liver.

Canalicular transport of biliary solutes

After intracellular transport, which might involve binding, sequestration, biotransformation or conjugation, colephilic substances are secreted across the canalicular membrane of the hepatocyte. This process provides the primary driving force for generation of bile flow and is crucial for the excretory function of the liver (e.g. body disposal of endo- and xeno-biotics, including drugs). The so-called ATP-binding cassette (ABC) transporters that function as unidirectional ATP-dependent export pumps are key proteins in this step of bile formation [16]. ABC transporters present in the canalicular membrane include: (i) the BS export pump (BSEP), which mediates BS secretion into the canaliculus; (ii) the multidrug-resistant-associated protein 2 (MRP2), which transports anionic conjugates of many lipophilic substances and reduced glutathione; (iii) the multidrug-resistant 1 (MDR1) gene product, which acts as a transporter of bulky cationic compounds and steroids; and (iv) the MDR3 gene product, which acts as a phospholipid translocator. Three additional 'half ABC transporters', which contain only six of the twelve transmembrane domains typical of ABC transporters [16], have been recently identified in the canalicular membrane. These include the twinned sterol half-transporters ABCG5 and ABCG8, which seem to play an important role in the regulation of biliary cholesterol secretion [17] and ABCG2 (also known as breast cancer related protein; BCRP), which preferentially transports sulfate conjugates [18]. In addition to these ABC transporters, the canalicular membrane contains an anion exchanger that excretes bicarbonate into bile, and a P-type ATPase (familial intrahepatic cholestasis 1; FIC1) thought to participate in the transport of aminophospholipids from the outer to the inner leaflet of liver or biliary cell membranes (Table 1). FIC1 might be relevant to the regulation of bile acid transport or the maintenance of the

lipid composition of the canalicular membrane, but this assumption remains speculative [4].

Basolateral efflux of biliary constituents

Sinusoidal BS efflux might be mediated by certain OATPs or by ABC transporters of the basolateral membrane. MRP-3 and -4 transport several physiological substrates, including non-sulfated and sulfated BS [16]. Although Mrp3 is minimally expressed in normal livers, it is inducible [19]. MRP4 is expressed in the liver to a greater degree and functions as an ATP-dependent co-transporter of reduced glutathione, together with monoanionic BS [20]. Given the vectorial nature of hepatobiliary transport, the export to the portal blood of compounds normally excreted into bile might serve as an alternative pathway for elimination of biliary constituents, limiting the accumulation of toxic biliary constituents when the canalicular secretory pathway is disrupted (see later).

Transport systems in cholangiocytes

Bile can be significantly modified by an array of absorptive mechanisms on the apical membrane of cholangiocytes. A wealth of information is accumulating on the biology of cholangiocytes from rodent and human liver [4,9]. Specific transport proteins are located in biliary epithelia. These transporters are listed in Table 1.

Molecular mechanisms in cholestasis

Cholestasis is defined as the impairment of normal bile flow resulting either from a functional defect at the level of the hepatocyte or from obstruction at the bile duct level. Cholestasis can result from infections, the use of certain drugs, and autoimmune, metabolic or genetic disorders [21]. Identification of the transport proteins involved in bile formation led to the concept that altered expression and/or function of membrane transporters might underlie some forms of cholestasis. Moreover, recognition of mutations in progressive familial intrahepatic cholestasis, in addition to genetic defects responsible for cystic fibrosis and Alagille's syndrome, established the molecular basis of a clinically important group of pediatric cholestatic disorders, and provided compelling evidence of the importance of hepatic transporters in bile formation. Full details on inherited cholestatic disorders are reviewed elsewhere [2,3,22]. Here, we focus on changes in hepatocellular transporter expression observed in experimental and human cholestatic conditions. In this setting, alterations in transporter expression are mainly secondary (rather than primary or causative); nevertheless, these alterations might explain and maintain the ongoing functional impairment of bile secretion in cholestasis [4,23].

Expression of hepatocyte transporters has been investigated in several models of cholestasis as well as in certain human cholestatic diseases. Current information on changes in the expression of hepatic transporters seen in cholestasis is summarized later. Owing to space limitations, we do not discuss modifications of transporter expression in extrahepatic tissues (i.e. intestine and kidney) that might facilitate the extrahepatic excretion of retained biliary constituents [4].

Hepatocellular changes

Cholestatic injury in rodents results in a marked reduction of mRNA and protein levels of Ntcp and other organic anion-transporting proteins (i.e. Oatp1 and Mrp2), and – to a lesser extent – of Bsep. Decreased transporter expression might explain the impaired hepatocellular uptake and canalicular excretion of BS and other organic anions (e.g. bilirubin diglucuronide) seen in cholestasis. Expression of the canalicular Bsep initially decreases and partially recovers with prolonged cholestasis [23]. Thus, Bsep is more stably expressed than the other transporters (Ntcp, Oatp1 and Mrp2) and might continue to excrete BS into bile even under cholestatic conditions [4,23,24]. In contrast to the downregulation of some of the organic anion transporters, expression of Mdr1 at the canalicular membrane, and isoforms of Mrp (Mrp1, Mrp3 and Mrp4) at the basolateral membrane increases following cholestatic injury [25–28]. In addition, two other liver Oatp isoforms, Oatp2 and Oatp4, are relatively well-preserved under cholestatic conditions [4].

From a teleological viewpoint, alterations in transporter expression are thought to represent a compensatory (anti-cholestatic) response that aims to limit hepatocellular accumulation of potentially toxic biliary constituents and provide alternative excretory routes for accumulating cholephils in cholestasis. As canalicular transport represents the rate-limiting step in bile secretion, alterations of canalicular transporter expression can be considered to be the primary events in hepatocellular forms of cholestasis. By contrast, alterations of basolateral transport systems might be secondary, with the aim of limiting further uptake and protecting the hepatocyte from overload with potentially toxic compounds. For example, maintained expression of Oatp2 and Oatp4 under cholestatic conditions could facilitate efflux of BS in the opposite direction (i.e. from the hepatocyte into the sinusoidal blood) by virtue of their function as anion exchangers [13]. The same interpretation has been given to the observed upregulation of basolateral Mrps in cholestasis. Notably, Mrp3 and Mrp4 transport sulfated BS, which are increased in cholestasis [28,29]. Upregulation of basolateral Mrp3 might also explain the appearance of conjugated bilirubin in plasma and urine during cholestasis. [30]. Finally, under cholestatic conditions, adaptive changes of organic anion transporters also occur in the kidney, favoring the shift towards renal excretion of BS and other biliary constituents [23,31].

Changes in transporter expression in human cholestatic liver diseases [32,33] are consistent with concepts derived from experimental animal models. Expression of NTCP and OATP2, as well as BSEP and MRP2, is reduced in patients with acute inflammation-induced cholestasis [32]. In primary biliary cirrhosis (PBC), changes in transporter expression evolve in a stage-dependent manner [33]. In early PBC stages, no changes in BS or organic anion transporters are seen. With disease progression, OATP2 and – to a lesser degree – NTCP are downregulated, whereas MRP3 and MDR1 expression is upregulated. The inverse changes in NTCP (downregulation) and MRP3 expression (upregulation) are most predominant in the periportal area, where hepatocytes

are exposed to the highest BS levels under normal and cholestatic conditions. This spatially coordinated down-regulation of basolateral uptake systems, and overexpression of potentially compensatory efflux pumps (MRP3) might protect the hepatocyte from further accumulation of toxic BS and other biliary constituents in chronic cholestasis [34]. Canalicular BSEP and MRP2 increases in stage III PBC before returning to normal levels in stage IV. This transient induction of MRP2 and BSEP could be interpreted as a compensatory attempt to overcome the cholestatic injury [33]. Similar observations have been made in primary sclerosing cholangitis [35,36].

Cholangiocellular changes

Similar to hepatocytes, cholangiocellular transport systems might also undergo compensatory changes or become the target of the initial cholestatic injury [4,31]. However, information on this topic is limited. Increase in uptake of BS from bile owing to the presence of the apical Na^+-dependent BS transporter and Mrp3 (promoting efflux of BS into peribiliary circulation) might facilitate the return of BS from the obstructed bile ducts to the systemic circulation. Bile salt reabsorption from bile might prevent or limit BS-induced bile duct injury in obstructive cholestasis.

Molecular mechanisms of regulation of hepatic transporters in cholestasis

The mechanisms involved in modulation of transporter expression during cholestasis are only partially known. Multiple factors such as BS, proinflammatory cytokines, oxidative stress, retinoids, drugs and hormones are at play [4]. Regulation of mRNA transcription initiation is recognized as the predominant mechanism governing transporter gene expression [4,5]. An expanding role of ligand-activated transcription factors and members of the nuclear hormone receptor (NHR) superfamily in the regulation of transporter expression has emerged [5–7,37–40]. In particular, the farnesoid X receptor (FXR) that acts as an intracellular BS sensor regulates basal expression of *Bsep* and participates in its upregulation upon a BS load [41–43]. In addition, through activation of another NHR termed short heterodimer partner (SHP), FXR participates in Ntcp downregulation in cholestasis [40]. SHP might suppress rat *NTCP* transcription by competing with co-activators for binding to ligand-activated retinoid X receptor, a master partner of NHR [44,45]. FXR is also involved in the transcriptional regulation of MRP2 [46] and control of BS synthesis through regulation of 7-α-hydroxylase (CYP7A1). Studies in FXR-null mice suggest that increased levels of BS inside the hepatocyte elicit an FXR-mediated response that reduces BS import (by reducing Ntcp expression) and BS synthesis (by suppressing CYP7A1 expression), and increases canalicular BS export by activating Bsep expression. These regulatory changes also seem to be at play in cholestasis.

In addition to FXR, two other members of the NHR superfamily regulate hepatobiliary transporters: the pregnane X receptor (PXR) and the constitutive androstane receptor (CAR). The pregnane X receptor stimulates expression of Oatp2, which is involved in BS and organic anion or cation transport, and Cyp3a, which is involved in BS hydroxylation and detoxification [47,48], whereas CAR is involved in the regulation of hepatic Mrp2 and Mrp3 expression [38,46]. Finally, another transcription factor, hepatocyte nuclear factor 1α (HNF1α), appears to play a key role in mediating BS suppression of *OATP2* [49]. Thus, changes in transporter expression during cholestasis appear to be mediated by biliary constituents (mainly BS) and can be explained by changes in a set of NHRs including FXR, PXR and CAR, as well as HNF1α. In addition, proinflammatory cytokines that mediate decreased gene transcription of hepatobiliary transporters in cholestasis (such as tumor necrosis factor-α and interleukin-1β) affect mRNA levels of several NHRs and HNF1α [50–52], which could explain the reduced transcription rates of dependent or corresponding genes.

Finally, in addition to transcriptional events, post-transcriptional and/or post-translational changes might also play a role in transporter changes in cholestasis, although information regarding this is limited [53].

Therapeutic perspectives

Treatment options for cholestatic diseases are limited [54]. Stimulation of defective transporter expression and function might be of therapeutic benefit in cholestasis. Treatment strategies could also be directed to support and stimulate rescue pathways such as alternative detoxification and elimination routes.

The anti-cholestatic drug ursodeoxycholic acid (UDCA) acts, at least in part, by stimulation of transporter expression or function [55]. UDCA stimulates vesicular exocytosis and insertion of canalicular transporters, and increases Bsep, Mrp2, Mrp3 and Mrp4 protein levels in experimental animals [55–59]. More recently, UDCA has also been shown to be capable of stimulating Cyp3A4 expression, a relevant detoxification pathway for BS [60].

An additional example of stimulation of transporter expression or function is the beneficial effects of corticosteroids in the treatment of cholestatic jaundice (‘steroid whitewash’). Corticosteroids have been shown to stimulate Bsep and Mrp2 expression [57,61] in hepatocytes, and cholangiocelluar transporter expression [62].

With the exception of UDCA, other treatment options for cholestasis have not proven to be beneficial. Thus, the search for more specific and effective therapies of cholestatic disorders continues. Ligand-activated transcriptions factors known to regulate transport and metabolism of biliary constituents represent attractive targets for drug design [7,63,64]. Given its effects on BS transport and metabolism in both hepatic and extrahepatic tissues, FXR is an amenable candidate for pharmacological manipulation. A potent gene-specific FXR-agonist should be able to reduce intracellular BS levels and promote renal elimination of retained BS, thereby decreasing liver injury. It might also be possible to use PXR as a drug target. Clinical evidence on the effect of rifampicin in cholestasis suggests that more potent and efficacious PXR agonists might be useful [65]. PXR agonists are expected to reduce the accumulation of toxic BS in the liver, thereby promoting their metabolism and excretion. Finally, the

combined use of FXR and PXR agonists is an exciting possibility that might prove to be useful in the treatment of cholestatic disorders [65,66].

Concluding remarks

Recent insights into the mechanisms of bile formation have established that this function of the liver depends of a concerted action of membrane transporters. Molecular regulation of these hepatobiliary transport systems in cholestasis seems to be part of an adaptive response aiming to minimize the extent of cholestatic injury. A better understanding of the molecular mechanisms involved in cholestasis, particularly the role of NHRs in transcriptional regulation of hepatic transporter expression, will open new horizons for therapeutic intervention.

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