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REVIEW

TRANSPORT OF BILE ACIDS IN HEPATIC AND NON-HEPATIC TISSUES

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Summary

Bile acids are steroidal amphipathic molecules derived from the catabolism of cholesterol. They modulate bile flow and lipid secretion, are essential for the absorption of dietary fats and vitamins, and have been implicated in the regulation of all the key enzymes involved in cholesterol homeostasis. Bile acids recirculate through the liver, bile ducts, small intestine and portal vein to form an enterohepatic circuit. They exist as anions at physiological pH and, consequently, require a carrier for transport across the membranes of the enterohepatic tissues. Individual bile acid carriers have now been cloned from several species. Na^+-dependent transporters that mediate uptake into hepatocytes and reabsorption from the intestine and biliary epithelium and an ATP-dependent transporter that pumps bile acids into bile comprise the classes of transporter that are specific for bile acids. In addition, at least four human and five rat genes that code for Na^+-independent organic anion carriers with broad multi-substrate specificities that include bile acids have been discovered. Studies concerning the regulation of these carriers have permitted identification of molecular signals that dictate eventual changes in the uptake or excretion of bile acids, which in turn have profound physiological implications. This overview summarizes and compares all known bile acid transporters and highlights findings that have identified diseases linked to molecular defects in these carriers. Recent advances that have fostered a more complete appreciation for the elaborate disposition of bile acids in humans are emphasized.

Key words: bile acid, liver, cholesterol, transporter, organic anion transport, carrier proteins, bile.

Introduction

Bile acids are amphipathic steroidal compounds derived from the enzymatic catabolism of cholesterol (Fig. 1). They perform essential roles in gastrointestinal physiology (Hofmann, 1994). As the major solute in bile, they are a primary determinant of bile flow and biliary lipid secretion. The catabolism of cholesterol to bile acids is an important route for the elimination of cholesterol from the body, accounting for approximately 50 % of cholesterol eliminated daily. In the intestine, bile acids regulate pancreatic secretions and the release of gastrointestinal peptides (Koop et al., 1996; Riepl et al., 1996) and activate carboxyl ester lipase, which is required for the absorption of lipid-soluble vitamins. The detergent properties of bile acids aid in the solubilization of cholesterol in bile and of dietary fats and cholesterol in intestinal fluid, a prerequisite for their intestinal absorption. Bile acids are also implicated in signal transduction pathways that regulate apoptosis, mucin secretion and biliary ductular secretion (Alpini et al., 1997a; Dray-Charier et al., 1997; Jones et al., 1997). The full physiological importance of bile acids may still not be fully appreciated.

Conjugated bile acids, which carry a negative charge at physiological pH, require carrier-mediated transport to cross membranes. The cloning of cDNAs for individual carriers, first from rodent then from human tissues, has permitted an increasing number of diseases and clinical syndromes to be attributed to specific defects in the regulation and expression of bile acid transporters (Balistreri, 1999; Jacquemin, 1999; Jansen et al., 1999). Bile acid carriers operating at the basolateral and apical surfaces of several cell types are diverse in their structure, in their driving forces and in their substrate specificities. This review gives a current account of the regulated biosynthesis of bile acids and of the identity, distribution and characteristics of transporters that mediate their disposition within the liver, biliary tree, intestine and kidney in humans. Reference is made to rodent tissues for comparison and when information from humans is lacking. The gene symbols associated with each carrier are consistent with the Human Gene Nomenclature Committee Data Base and are given in parentheses (Table 1; Fig. 2).

Biosynthesis of bile acids

The major biosynthetic pathway begins in the liver with 7-

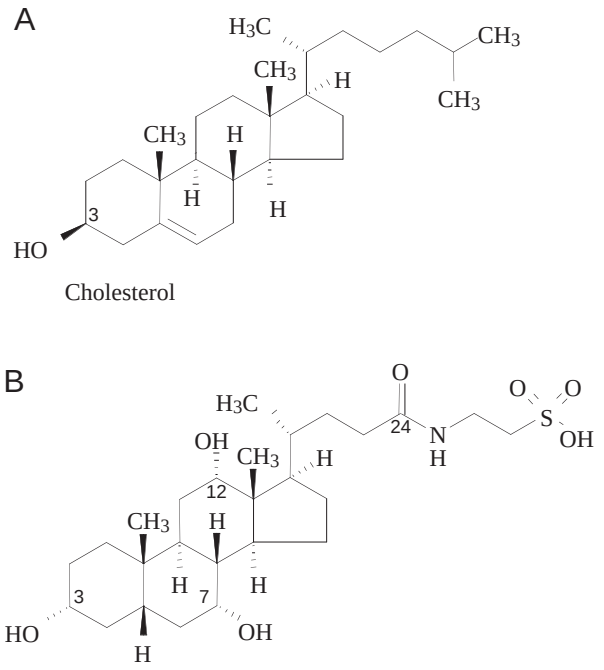

Fig. 1. Structure of cholesterol (A) and a bile acid amidated with taurine (taurocholic acid) (B). Cholesterol, a C_{27} hydrophobic compound, is converted to the more hydrophilic, trihydroxylated (3αOH,7αOH,12αOH) C_{24} bile acid. Amidation with taurine (or glycine) lowers the pKa of the terminal acidic group, such that bile acids are negatively charged at physiological pH and exist in their anionic salt form.

α hydroxylation of cholesterol catalyzed by the rate-limiting, microsomal cholesterol 7-α-hydroxylase (CYP7A1) (Jelinek et al., 1990). Additional hydroxylation and oxido-reduction steps form the dihydroxylated (3αOH,7αOH) chenodeoxycholic acid or the more hydrophilic trihydroxylated (3αOH,7αOH,12αOH) cholic acid (Fig. 1), which are the predominant bile acids in man. An alternative extra-hepatic pathway begins with sequential oxidation reactions at C_{27} of cholesterol by sterol 27-hydroxylase (CYP27) (Cali and Russell, 1991; Martin et al., 1993), a mitochondrial enzyme that is expressed in hepatic and in most extra-hepatic tissues including vascular endothelium and macrophages (Bjorkhem et al., 1994; Reiss et al., 1997). Oxidation products of cholesterol formed extra-hepatically by CYP27 are transported to the liver for conversion to chenodeoxycholic acid (Pikuleva et al., 1997). Mutations in the human *CYP27* gene underlie a recessive sterol storage disease, cerebrotendinous xanthomatosis, characterized by the accumulation of cholesterol and cholestanol in the tissues (Cali et al., 1991). Atherosclerosis is an additional finding, consistent with a role for CYP27 in promoting the flux and removal of cholesterol from extra-hepatic sources (Bjorkhem et al., 1994; Lund et al., 1996).

Bile acids are conjugated at the terminal (C_{24}) carboxyl group with the amino acids taurine and glycine by bile acid-CoA:amino acid *N*-acyltransferase (Falany et al., 1994). This amidation increases their amphipathic character and decreases their ionization constants, rendering them more hydrophilic and more readily excretable into bile. The glycoconjugates, which predominate in man, and tauroconjugates (Fig. 1), which predominate in rodents, have pKa values of approximately 4 and 2, respectively, and exist in their anionic salt form at physiological pH (Hofmann, 1994). Bile acids are excreted into the bile for eventual discharge into the small intestine (approximately 30 g day^{-1}) then undergo reabsorption, creating a cycle of enterohepatic circulation. In the intestinal lumen, bacterial enzymes dehydroxylate chenodeoxycholic acid to lithocholic acid and cholic acid to deoxycholic acid. A proportion of these secondary bile acids is absorbed from the intestine and recirculates entero-hepatically (Hofmann, 1994).

Regulation of biosynthesis

The synthesis of bile acids in man is highly regulated (Chiang, 1998). Measures that decrease the return of bile acids to the portal circulation, such as biliary diversion in animals or intake of binding resins, increase the conversion of cholesterol into bile acid (Pandak et al., 1991; Xu et al., 1999). Biosynthesis is also regulated by dietary intake of cholesterol, although the response is species-specific: whereas an increase in bile acid production is an important response in mice and rats (Nguyen et al., 1999), only a modest compensatory increase occurs in man (Duane, 1993).

The transcription of *CYP7A1* is subject to both feedforward and feedback regulation (Stroup et al., 1997). A host of transcription factor binding sites have now been identified on the *CYP7A1* promoter to explain the nature of its activation and repression. A nuclear receptor, the liver X receptor α (LXRα), for which ligands are hydroxylated cholesterol metabolites, dimerizes with a second nuclear receptor, the retinoid X receptor (RXR), and binds to its response element on the *Cyp7a1* promoter, thereby mediating transcriptional activation. Knockout LXRα(–/–) mice fail to upregulate *Cyp7A1* in response to dietary cholesterol (Peet et al., 1998). In humans, ligand-activated LXRα/RXR binds less avidly to the *CYP7A1* promoter, consistent with minor transcriptional upregulation (Repa and Mangelsdorf, 2000). Transcription of *CYP7A1* is downregulated by the farnesoid X nuclear receptor (FXR) *via* a complex molecular mechanism that involves the coordinated regulation of several liver-enriched nuclear receptors. Bile acids are physiological ligands of FXR (Makishima et al., 1999; Parks et al., 1999). Bile acid-activated FXR dimerizes with RXR and transcriptionally activates its target nuclear receptor, short heterodimer partner (SHP). SHP represses *CYP7A1* indirectly through its association with yet another nuclear receptor, liver receptor homolog-1 (LRH-1) (Lu et al., 2000).

CYP27 is also transcriptionally repressed by bile acids. One transcription factor identified is hepatocyte nuclear factor-1α (HNF1α), whose binding to the *CYP27* promoter is reduced by bile acids (Rao et al., 1999).

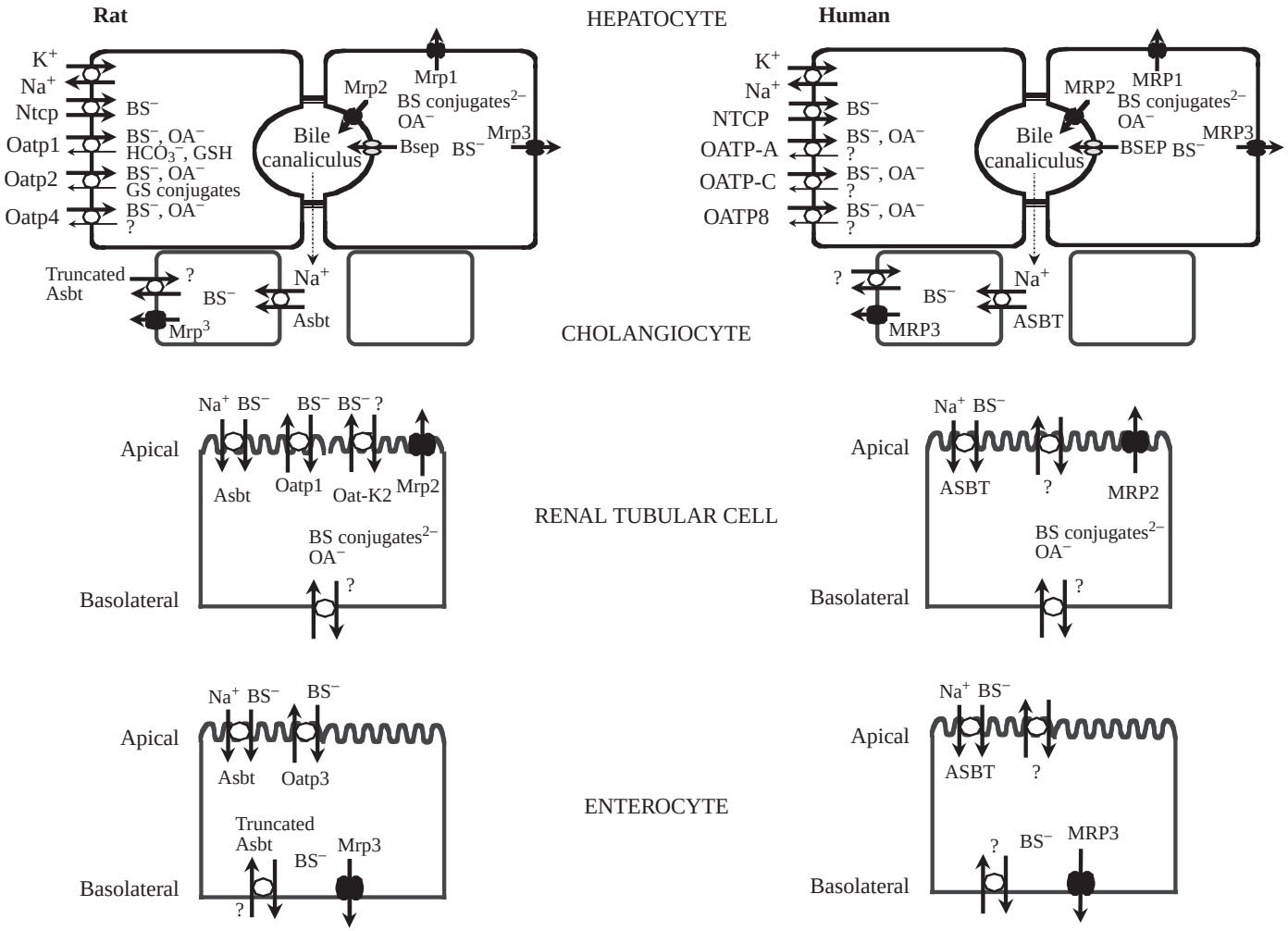

Fig. 2. Bile salt carriers in rat and human hepatocytes, cholangiocytes, renal tubular cells and enterocytes. At the basolateral membrane, the Na$^+$–taurocholate cotransporting polypeptides Ntcp (rat) and NTCP (human) mediate uptake of conjugated bile salts (BS$^-$). In rat liver, Na$^+$-independent transport is mediated by the organic anion-transporting polypeptides Oatp1, Oatp2 and Oatp4, which are carriers of BS$^-$ and other organic anions (OA$^-$). In human liver, Na$^+$-independent BS$^-$ uptake is predominantly mediated by OATP-C. OATP-A is of very low abundance and may play only a minor role. The affinity of OATP8 for bile acids is uncertain. At the canalicular membrane, monovalent BS$^-$ is pumped into bile *via* the bile salt export pump Bsep (rat)/BSEP (human), which is a member of the ATP-binding cassette (ABC) superfamily. Excretion of divalent sulphated or glucuronidated bile salts into bile is mediated by the multidrug resistance protein 2, Mrp2 (rat)/MRP2 (human). Levels of Mrp1/MRP1 and Mrp3/MRP3 are very low or undetectable at the basolateral membrane of hepatocytes, but are increased when Mrp2/MRP2 is absent or its expression is reduced to maintain ongoing organic anion (OA$^-$) efflux from the hepatocyte. Within the bile ducts, conjugated BS$^-$ can be absorbed by the apical sodium-dependent bile acid transporter, Asbt (rat)/ASBT (human), localized at the apical cholangiocyte membrane. Bile salts then exit at the basolateral surface into the hepatic arterial circulation, *via* the ATP-dependent MRP3/ Mrp3 and possibly through a proposed mechanism of basolateral anion exchange. A truncated Asbt carrier may mediate this efflux in the rat. BS$^-$ is reabsorbed into renal tubular cells at the apical membrane by Asbt/ASBT. The multispecific Oatp1 is also localized to the rat apical membrane, as is the organic anion transporter Oat-K2. It is unclear whether Oat-K2 mediates uptake or secretion of BS$^-$. Divalent conjugated BS$^-$ can be pumped out by Mrp2/MRP2. Other members of the Oatp/OATP family have not been definitely localized to basolateral or apical membranes. In the enterocyte, BS$^-$ is reabsorbed by Asbt/ASBT. The multispecific Oatp3 has also been localized to the apical membrane in the rat. BS$^-$ then exits at the basolateral surface *via* the ATP-dependent Mrp3/MRP3. The truncated Asbt carrier may also mediate this efflux in the rat. GS, glutathione; GSH, reduced glutathione.

Bile acid carriers within the enterohepatic circulation

Influx of bile acids at the basolateral surface of hepatocytes

The entry of bile acids into hepatocytes at the basolateral surface (in contact with the blood sinusoids) occurs by two processes. The major uptake system is Na$^+$-dependent and driven by the transmembrane Na$^+$ gradient maintained by Na$^+$/K$^+$-ATPase. A second Na$^+$-independent uptake system is mediated by several members of the Organic Anion-Transporting Polypeptide (OATP) family of transporters, which function as anion exchangers.

Table 1. *Bile acid transporters in human and rat tissues*

Cell type	Localization	Carrier[a]	Accession number	Function	References
Hepatocyte					
Human	Basolateral	NTCP (*SLC10A1*)	NM_003049	Na^+-dependent entry of bile acids; K_m taurocholate=6 μmol l^{-1} [b]	Hagenbuch and Meier, 1994
Rat	Basolateral	Ntcp (*Slc10a1*)	M77479	K_m=30 μmol l^{-1}	Hagenbuch et al., 1991
Hepatocyte					
Human	Basolateral? (low expression)	OATP-A (*SLC21A3*)	NM_005075	Multi-specific; Na^+-independent entry of bile acids and organic anions; K_m=60 μmol l^{-1}	Kullak-Ublick et al., 1995
	Basolateral (high expression)	OATP-C (*SLC21A6*)	NM_006446	K_m=14 μmol l^{-1}	Konig et al., 2000a; Abe et al., 1999
	Basolateral (high expression)	OATP8 (*SLC21A8*)	AJ251506	Multi-specific; Na^+-independent entry of bile acids and organic anions	Konig et al., 2000a; Kullak-Ublick et al., 2001
Rat	Basolateral (even acinar distribution)	Oatp1 (*Slc21a1*)	L19031	Multi-specific; Na^+-independent entry of bile acids and organic anions. K_m=20-50 μmol l^{-1} [b]	Jacquemin et al., 1994
	Basolateral (perivenous acinar distribution)	Oatp2 (*Slc21a5*)	U88036	K_m=35 μmol l^{-1}	Noe et al., 1997
	Basolateral	Oatp4 (*Slc21a10*)	AJ271682	K_m=26 μmol l^{-1}	Cattori et al., 2000a
Hepatocyte					
Human	Apical (canalicular)	BSEP (*ABCB11*)	NM_003742	ATP-dependent export of bile acids	Strautnieks et al., 1998
Rat	Apical	Bsep (*Abcb11*)	U69487	K_m=5 μmol l^{-1}	Gerloff et al., 1998
Human	Apical	MRP2 (*ABCC2*)	NM_000392	Multi-specific, ATP-dependent export of organic anions including di-anionic conjugated bile acids	Cui et al., 1999; Evers et al., 1998; Paulusma et al., 1997
Rat	Apical	Mrp2 (*Abcc2*)	L49379		Paulusma et al., 1996; Stieger et al., 2000
Hepatocyte					
Human	Basolateral (low expression but induced in Dubin–Johnson syndrome and cholestasis)	MRP3 (*ABCC3*)	NM_003786	Multi-specific; ATP-dependent export of organic anions and conjugated bile acids; K_m glycocholate=248 μmol l^{-1}	Konig et al., 1999b; Kool et al., 1999; Zeng et al., 2000
Rat	Basolateral (low expression but induced in EHBR[c] and experimental cholestasis)	Mrp3 (*Abcc3*)	AB010467	K_m=16 μmol l^{-1}	Hirohashi et al., 2000b; Ogawa et al., 2000
Hepatocyte					
Human	Basolateral (very low expression, except in proliferating cells)	MRP1 (*ABCC1*)	NM_004996	Multi-specific; ATP-dependent export of organic anions and di-anionic bile salts	Roelofsen et al., 1997
Rat	Basolateral (very low expression, but induced in endotoxaemia)	Mrp1 (*Abcc1*)	AF022908		Vos et al., 1998
Cholangiocyte					
Human	Apical	ASBT (*SLC10A2*)	NM_000452	Na^+-dependent uptake of conjugated bile acids	Que et al., 1999

Na^+/taurocholate cotransporting polypeptide (NTCP/Ntcp)

Ntcp (*Slc10a1*), a glycoprotein of 362 amino acid residues, whose cDNA was isolated from rat liver (Hagenbuch et al., 1991), was the first bile acid carrier identified at both the molecular and functional levels. It is expressed exclusively in hepatocytes and is localized at the basolateral membrane

Table 1. *Continued*

Cell type	Localization	Carrier[a]	Accession number	Function	References
Rat	Apical	Asbt (*Slc10a2*)	U07183	K_m=46 µmol l^{-1}	Alpini et al., 1997b; Lazaridis et al., 1997
Cholangiocyte					
Human	Basolateral	MRP3 (*ABCC3*)	NM_003786	Multi-specific; ATP-dependent efflux of organic anions and conjugated bile acids	Kool et al., 1999)
Rat	Basolateral	Mrp3 (*Abcc3*)	AB010467		Hirohashi et al., 2000b
Cholangiocyte					
Rat	Basolateral	Truncated Asbt		Na^+-independent efflux of conjugated bile acids; K_m=35 µmol l^{-1}	Lazaridis et al., 2000
Enterocyte					
Human	Apical	ASBT (*SLC10A2*)	NM_000452	Na^+-dependent uptake of bile acids; K_m=13 µmol l^{-1}	Wong et al., 1995; Craddock et al., 1998
Rat	Apical	Asbt (*Slc10a2*)	U07183	K_m=50 µmol l^{-1}	Christie et al., 1996; Moyer et al., 1986
Enterocyte					
Rat	Apical	Oatp3 (*Slc21a7*)	AF083469	Multispecific; Na^+-independent uptake of organic anions and bile acids; K_m=20 µmol l^{-1}	Walters et al., 2000
Enterocyte					
Human	Basolateral	MRP3 (*ABCC3*)	NM_003786	Multi-specific; ATP-dependent export of organic anions and conjugated bile acids	Hirohashi et al., 2000a; Hirohashi et al., 2000b
Enterocyte					
Rat	Basolateral?	Truncated Asbt		Na^+-independent efflux of conjugated bile acids	Lazaridis et al., 2000
Renal tubules					
Human	Apical	ASBT (*SLC10A2*)	NM_000452	Na^+-dependent re-uptake of conjugated and unconjugated bile acids	Craddock et al., 1998
Rat	Apical	Asbt (*Slc10a2*)	U07183	K_m=40–70 µmol l^{-1}	Christie et al., 1996
Human	Apical	MRP2 (*ABCC2*)	NM_000392	Multi-specific; ATP-dependent export of di-anionic conjugated bile acids	Schaub et al., 1999
Rat	Apical	Mrp2 (*Abcc2*)	L49379		Schaub et al., 1997
Rat	Apical; S3 segment of the proximal straight tubule	Oatp1 (*Slc21a1*)	NM_013797	Multi-specific; Na^+-independent re-uptake? of organic anions and bile acids	Bergwerk et al., 1996
	Apical?; expression is kidney-specific	Oat-K2 (*Slc21a4*)	AB012662	K_m=10 µmol l^{-1}	Masuda et al., 1999

All specific and multi-specific carriers for bile acids are included. Where uncertainty exists in the location or function of a carrier, a question mark is added.

[a]Gene symbols consistent with the Human Gene Nomenclature Committee Data Base are given in parentheses.

[b]Reported K_m values for the transport of taurocholate, where available. Since data from several experimental methods are combined, a range is given where possible.

[c]EHBR, Eisai hyperbilirubinaemic rats with a defect in Mrp2.

(Stieger et al., 1994) (Fig. 2). Its human counterpart, NTCP (*SLC10A1*), a 349 amino acid residue glycoprotein, exhibits high affinity (K_m≈6 µmol l^{-1}) for taurocholate (Hagenbuch and Meier, 1994), commensurate with fasting serum bile acid

levels (range 5–20 μmol l^{-1}) in healthy subjects. The coupled transport mediated by NTCP/Nctp is electrogenic, with a Na^+:bile acid stoichiometry of 2:1 (Weinman, 1997).

Physiological and pathophysiological stimuli, such as pregnancy and cholestasis, modulate Ntcp. To explain these changes, the Ntcp promoter and transcription factors were identified (Karpen et al., 1996). In the *postpartum* state, prolactin facilitates the binding of signal transducer and activator of transcription 5 (STAT5) to consensus sites on the promoter, leading to transcriptional activation and upregulation of Ntcp (Ganguly et al., 1997). Decreased expression of Ntcp occurs in rat models of experimental cholestasis such as bile duct ligation, ethinyloestradiol and endotoxin treatment (Gartung et al., 1996; Green et al., 1996; Kupferschmidt et al., 1994). Endotoxin-induced downregulation was investigated by measuring nuclear binding of several transactivators of the Ntcp promoter (Trauner et al., 1998). A selective decrease in the nuclear binding of HNF-1 and the Footprint-B-binding protein was associated with a decrease in Ntcp mRNA levels. The identification of Footprint-B-binding protein as a retinoid receptor heterodimer led to the finding that retinoids activate the Ntcp promoter, and this in turn is inhibited by cytokine-mediated signalling (Denson et al., 2000).

Organic anion-transporting polypeptide (OATP/Oatp)

The OATP/Oatp carriers are members of a growing family that mediate the Na^+-independent uptake of a host of compounds, including conjugated and unconjugated bile acids. Other substrates include cardiac glycosides (Noe et al., 1997), steroids (Bossuyt et al., 1996; Eckhardt et al., 1999), peptides (Gao et al., 2000; Ziegler et al., 1991) and selected organic cations (van Montfoort et al., 1999). Certain members are predominantly or exclusively expressed in extra-hepatic tissues, such as brain (Gao et al., 1999), intestine (Walters et al., 2000), lung and retina (Abe et al., 1998).

In the rat, there are now three Oatps localized in the liver and for which bile salts are substrates (Fig. 2, Table 1). Oatp1 (*Slc21a1*) is a 80 kDa protein expressed mainly at the basolateral membrane of hepatocytes, but also in kidney and brain (Angeletti et al., 1997; Jacquemin et al., 1994). Conjugated and unconjugated bile acids are substrates, albeit with lower affinities than Ntcp (Eckhardt et al., 1999). Oatp1 probably works as an exchanger, and recent studies have proposed different physiological counter-anions such as HCO_3^- (Satlin et al., 1997) or glutathione (Li et al., 1998). A second member, Oatp2 (*Slc21a5*), was cloned from the rat brain, is 77 % identical with Oatp1 and is also expressed basolaterally in hepatocytes (Noe et al., 1997; Reichel et al., 1999). Further comparisons of Oatp1 and Oatp2 showed overlapping substrate specificities with respect to bile salts, but that the acinar localization of the Oatp1 and Oatp2 proteins along the liver sinusoid differed (Eckhardt et al., 1999; Reichel et al., 1999). Whereas Oatp1 showed a homogeneous distribution, Oatp2 was predominantly expressed in perivenous hepatocytes, excluding the innermost 1–2 cell layers surrounding the central vein (Kakyo et al., 1999; Reichel et al., 1999). Since the major uptake of bile salts occurs in periportal hepatocytes (Buscher et al., 1993; Groothuis et al., 1982), Oatp1 is implicated in the Na^+-independent uptake of bile salts under normal conditions. However, in models of cholestasis such as treatment with oestrogen, an event associated with downregulation of both Ntcp and Oatp1 (Simon et al., 1996), hepatocytes in zones 2 and 3 (pericentral) of the sinusoids are recruited for bile salt transport (Buscher et al., 1993). Therefore, Oatp2 may assume a more important role in situations in which the expression or activity of Ntcp and/or Oatp1 is compromised. A third family member, Oatp4 (*Slc21a10*), is expressed exclusively at the basolateral membrane of rat liver (Cattori et al., 2000b) and shares 43–44 % identities with Oatp1 and Oatp2 (Cattori et al., 2000a). Its substrates include taurocholate and amphipathic organic anions such as oestradiol-17β-glucuronide and dehydroepiandrosterone sulphate (DHEAS) (Cattori et al., 1999).

Three human OATP orthologs, expressed in the liver and whose substrates include bile salts, have now been cloned (Fig. 2, Table 1). OATP-C (*SLC21A6*) is selectively expressed at the basolateral membrane of hepatocytes (Abe et al., 1999; Hsiang et al., 1999; Konig et al., 2000b) and exhibits the highest (64 %) amino acid identity with rat Oatp4. Accordingly, the substrate specificities of OATP-C and Oatp4 are comparable, although it remains uncertain whether the two proteins represent truly orthologous gene products. OATP-C transports taurocholate (K_m≈14–34 μmol l^{-1}) with slightly lower affinity than does NTCP and, in keeping with the nature of Oatp carriers as multispecific organic anion carriers, its substrates also include bilirubin monoglucuronide (Konig et al., 2000b), conjugated steroids, eicosanoids, thyroid hormones and peptides (Kullak-Ublick et al., 2000). The available data suggest that OATP-C represents an important Na^+-independent bile salt uptake system in human liver.

The recently cloned OATP8 (*SLC21A8*) is 80 % identical with OATP-C and shares the same basolateral expression in human hepatocytes (Konig et al., 2000a). Substrates for this multispecific carrier include bromosulphophthalein and digoxin (Kullak-Ublick et al., 2000). In contrast to OATP-C, bile salts were not transported when OATP8 was expressed in mammalian cells (Konig et al., 2000a) but were identified as substrates in the oocyte expression system (Kullak-Ublick et al., 2000).

OATP-A (*SLC21A3*), the first human Na^+-independent, multispecific organic anion transporter cloned, was isolated from human liver, although it is predominantly expressed in brain and also occurs in lung and kidney (Kullak-Ublick et al., 1995). It is 44 % identical with OATP-C, 40 % identical with OATP8, and its closest rat ortholog is Oatp2 (73 % identity). Although postulated, a basolateral localization for OATP-A has not yet been definitely demonstrated in human hepatocytes. Given its relatively low affinity for bile acids compared with that of other Oatp/OATP family members (K_m for taurocholate=60 μmol l^{-1}) (Kullak-Ublick et al., 1995) and its high affinity for the steroid precursor DHEAS (K_m≈6 μmol l^{-1})

(Kullak-Ublick et al., 1998), OATP-A may play only a minor role in bile acid transport.

Efflux of bile acids at the apical surface of hepatocytes

At the apical surface of hepatocytes, conjugated bile acids are actively extruded into the canalicular space against a 1000-fold concentration gradient. Transport *in vitro* by isolated membrane vesicles was shown to be saturable, ATP-dependent and to have a high affinity for conjugated bile acids (Nishida et al., 1991; Stieger et al., 1992). This transport activity is now ascribed to a 160 kDa protein cloned from rat liver (Gerloff et al., 1998), which is a member of the ATP-binding cassette (ABC) superfamily of proteins (Higgins, 1995). ABC proteins share common structural motifs including a conserved intracellular domain that binds ATP and couples primary active, unidirectional export of a broad range of compounds to ATP hydrolysis.

Bile salt export pump (BSEP/Bsep)

Originally named sister of P-glycoprotein (spgp) because of its homology to the family of P-glycoproteins (49–50 % identity) that confer multidrug resistance in a large number of cell types (Childs et al., 1995; Childs et al., 1998), this ABC protein was renamed the bile salt export pump (Bsep) (*Abcb11*) to emphasize ATP-dependent bile salt transport (Gerloff et al., 1998). The rat Bsep is a glycoprotein with an apparent molecular mass of 160 kDa. The kinetics of taurocholate transport, as measured in membrane vesicles from insect Sf9 cells expressing Bsep, gave a K_m range of 2–5 μmol l^{-1} for taurine-conjugated bile salts (Gerloff et al., 1998). The human BSEP (*ABCB11*) carrier is approximately 80 % identical with its rodent ortholog (Strautnieks et al., 1998), but has yet to be functionally expressed and characterized. The human *BSEP* gene locus on chromosome 2q24 is linked to progressive familial intra-hepatic cholestasis type 2 (PFIC2) (Strautnieks et al., 1998), a progressive liver disease characterized by biliary bile salt concentrations of less than 1 % of normal and an absence of BSEP from the canalicular membrane. Mutations in the *BSEP* gene have been identified in these patients (Jansen et al., 1999).

Immunogold labelling studies of rat liver demonstrated selective localization of Bsep at the canalicular microvilli and at subcanalicular smooth membrane vesicles (Gerloff et al., 1998). In rats, the maximum secretory capacity of the liver is rapidly upregulated after a bile salt load (Boyer and Soroka, 1995). An increase in intracellular trafficking of Bsep has been postulated as a means of acute regulation to increase the recruitment of a subcanalicular pool of bile salt transporters for insertion into the plasma membrane (Misra et al., 1998). Phosphoinositide lipids phosphorylated by the enzyme phosphatidylinositol-3-kinase appear to be directly involved in the recruitment of Bsep to the canalicular membrane and in modulating its transport activity (Misra et al., 1999). The transcriptional and post-transcriptional regulation of Bsep have also been studied in rat and mouse models. Both hypo-osmolarity and glucocorticoids induce Bsep mRNA levels, whereas hyperosmolarity has the opposite effect (Warskulat et al., 1999). In rodent models of oestrogen- and endotoxin-induced cholestasis, which are both associated with reduced ATP-dependent canalicular bile salt secretion (Bolder et al., 1997; Bossard et al., 1993), the mRNA of murine Bsep was downregulated (Green et al., 2000), whereas in the rat, post-transcriptional changes leading to a decrease in protein levels were more important (Lee et al., 2000). The molecular mechanisms underlying these changes have yet to be unravelled.

Efflux of di-anionic conjugated bile acids at the apical surface of hepatocytes

Conjugation of bile acids at the C_3 position with sulphate or glucuronide in addition to amidation at the COOH group at C_{24} produces 3-sulphate or 3-glucuronide di-anionic derivatives. This is an important metabolic pathway for the secondary bile acid lithocholic acid, a cytotoxic compound that is normally a minor constituent of the bile acid pool and is secreted in bile as a di-anionic, sulphated glyco- or tauro-conjugate (Cowen et al., 1975). In cholestatic conditions, such as extra-hepatic biliary obstruction and primary biliary cirrhosis, associated with an impaired excretory capacity of the liver, sulphated and glucuronidated derivatives of lithocholate and of chenodeoxycolate accumulate in the blood (Stiehl et al., 1985). The di-anionic conjugated bile salts are not substrates of Bsep (Stieger et al., 2000) but rather of other ABC proteins that belong to the multidrug resistance protein (MRP) family (Keppler et al., 1997).

Multidrug resistance proteins (MRP/Mrp)

The first member of the MRP family, MRP1 (*ABCC1*), was cloned from a multidrug-resistant human lung cancer cell line (Cole et al., 1992). Since then, at least five further members have been identified (Kool et al., 1997). MRP2 (*ABCC2*) exhibits 50 % identity with MRP1 and is localized at the apical domain of hepatocytes (Paulusma et al., 1997) (Fig. 2). A mutation in the *MRP2* gene is the basis for Dubin–Johnson syndrome, a liver disorder characterized by chronic conjugated hyperbilirubinaemia. MRP2 exports a range of organic anions, among which are bilirubin glucuronides, glutathione-*S*-conjugates and mainly di-anionic xenobiotics (Cui et al., 1999; Konig et al., 1999a). Bile salts bearing two negative charges, such as sulphated tauro- or glycolithocholate (Keppler et al., 1997), are also exported by MRP2, thereby providing hepatocytes with a mechanism for the elimination of lithocholic acid metabolites. Despite a lack of functional MRP2, Dubin–Johnson patients do not experience hepatoxicity from an accumulation of lithocholic acid metabolites, so hepatocytes must have an alternative mechanism for the export of di-anionic bile salts.

Efflux of bile acids at the basolateral surface of hepatocytes

A third MRP member, Mrp3(*Abcc3*)/MRP3(*ABCC3*), bears 58 % and 47 % identity with MRP1 and MRP2, respectively. Both MRP1 and MRP3 are expressed at the basolateral surface

of normal hepatocytes, but at very low or undetectable levels (Konig et al., 1999b; Kool et al., 1999; Mayer et al., 1995). However, Mrp1 is upregulated in endotoxaemic rat liver when the canalicular transporters Mrp2 and Bsep are downregulated (Vos et al., 1998). MRP3 is induced in patients with Dubin–Johnson syndrome and in primary biliary cirrhosis (Konig et al., 1999b). Rat Mrp3 is similarly induced in livers of Eisai hyperbilirubinaemic (EHBR) rats, which have an equivalent Mrp2 defect, and in experimental cholestasis (Ogawa et al., 2000). Like Mrp2/MRP2, Mrp1/MRP1 and Mrp3/MRP3 are ATP-dependent pumps whose spectrum of substrates include glucuronide and glutathione conjugates of endogenous and exogenous compounds (Konig et al., 1999a). Rat Mrp1 and Mrp3 and human MRP1 export di-anionic bile salts such as sulphated taurolithocholate and taurochenodeoxycholate. Mrp3 can also export monovalent bile acids, such as tauro- and glycocholate (Hirohashi et al., 2000b). Human MRP3 transports glycocholate with low affinity (K_m=248 μmol l^{-1}), but transport of taurocholate was not detected (Zeng et al., 2000). The inducible nature of MRP1 and MRP3 and their localization at the basolateral surface of hepatocytes may explain the shift towards renal excretion as the major mechanism for bile acid elimination in patients with some forms of cholestasis (Raedsch et al., 1981).

Members of the Oatp/OATP family remain candidates for the efflux of bile salts at the basolateral membrane when accumulation occurs in hepatobiliary disease. Studies in *Xenopus laevis* oocytes show that Oatp1 and Oatp2 are able to operate as bi-directional exchangers in this expression system (Li et al., 2000). However, experimental data are still lacking to show the bi-directional movement of bile acids across the hepatocyte surface.

Intestinal reabsorption of bile acids

An efficient intestinal re-uptake of bile salts and delivery to the portal blood for re-entry at the sinusoidal surface of hepatocytes maintains the enterohepatic recirculation. Reabsorption mechanisms exist in discrete portions of the small intestine. The distal ileum expresses a Na^+-dependent carrier for the re-uptake of taurine/glycine-conjugated bile salts (Craddock et al., 1998). In the more proximal jejunum of rat intestine, the transport of conjugated bile salts driven by anion exchange has been demonstrated in brush-border membrane vesicles (Amelsberg et al., 1999). A candidate Oatp member has since been identified (Walters et al., 2000). In man, some degree of passive absorption may occur for certain unconjugated bile acids in the colon (Mekhjian et al., 1979).

Apical sodium-dependent bile acid transporter (ASBT/Asbt)

ASBT/Asbt (*SLC10A2/Slc10a2*), also referred to as the ileal sodium-dependent bile acid transporter (ISBT and IBAT), is an electrogenic bile acid carrier, coupled with Na^+ in a 2:1 Na^+:bile acid stoichiometry (Weinman et al., 1998). It was first cloned from hamster (Wong et al., 1994), then from human (Wong et al., 1995) and rat (Shneider et al., 1995). Its size and topology are comparable with those of the liver NTCP, and the two Na^+-dependent bile acid transporters share 35 % amino acid identity (Dawson and Oelkers, 1995; Hallen et al., 1999). Unlike NTCP, which is expressed basolaterally in hepatocytes, ASBT is expressed at the apical surface in the ileum (Fig. 2). An additional distinction is that bile salts are the sole substrates for ASBT, whereas the liver NTCP can transport at least one other organic anion, the C_3OH–sulphate conjugate of oestrone (Craddock et al., 1998). Both primary and secondary conjugated and unconjugated bile acids are substrates for ASBT. The highest affinities reported are for the conjugated dihydroxy bile acids (Craddock et al., 1998). Mutations in the cDNA of ASBT that result in a syndrome of primary bile acid malabsorption have been identified (Oelkers et al., 1997). The clinical phenotype includes severe diarrhoea, malabsorption of fat and malnutrition. There is evidence for transcriptional regulation of ASBT by bile acids, but the nature and extent appear to be species-dependent. Negative feedback regulation was reported in the guinea pig (Lillienau et al., 1993), positive feedback in biliary-diverted rats (Stravitz et al., 1997) and no regulatory changes in rats after common bile duct ligation and intestinal sequestration of bile acids (Arrese et al., 1998). The response in humans to perturbations of bile acid homeostasis, such as occur during cholestasis or bile acid sequestrant therapy, is uncertain (Hofmann, 1999).

Organic anion-transporting polypeptide-3 (Oatp3)

A fourth, multispecific member of the rat Oatp family, Oatp3 (*Slc21a7*), which is 80–82 % identical to Oatp1 and Oatp2, was first cloned from a retina cDNA library and its transcripts were then localized to the brain, liver and kidney (Abe et al., 1998). Further work suggested a different pattern of expression: in the brain, lung, retina and intestine (Walters et al., 2000). Oatp3 mRNA was detected along the entire length of the small intestine, but the protein was primarily localized to the apical surface of jejunal epithelial cells. Its transport properties are similar to those of Oatp2, with a range of amphipathic anions among its substrates (Cattori et al., 2000b), including bile acids (K_m for taurocholate=30 μmol l^{-1}) (Walters et al., 2000). The localization of Oatp3 is consistent with a role in Na^+-independent uptake of bile acids from intestinal brush-border membranes. However, the relative importance of Oatp3 in intestinal bile acid uptake has yet to be investigated. Asbt is expressed more abundantly than Oatp3, and Oatp3 may have higher affinity towards certain non-bile acid substrates (Cattori et al., 2000b), which may in turn compete with bile acid uptake.

OATP-A has been proposed as the human ortholog of Oatp3 on the basis of amino acid identity, sequence identity of the 3′-untranslated region and chromosomal localization (Walters et al., 2000). However, OATP-A has not been detected in intestine, and the identity and importance of bile acid uptake systems distinct from ASBT in man await confirmation.

Efflux of bile acids at the basolateral surface of enterocytes

The secretion of bile salts from the basolateral surface of enterocytes into the splanchnic circulation has been attributed to an anion-exchange protein (Weinberg et al., 1986), yet the

carrier is unknown. Most recently, an alternatively spliced truncated form of Asbt (t-Asbt) was isolated from a rat cholangiocyte cDNA library, and its expression has also been confirmed in rat ileum (Lazaridis et al., 2000). In contrast to the full-length Asbt, functional studies in oocytes predict that t-Asbt is an efflux carrier. It is located at the basolateral rather than the apical membrane, at least in rat biliary epithelium. At the mRNA level, t-Asbt is twice as abundant as full-length Asbt (Lazaridis et al., 2000). However, the subcellular localization of t-Asbt within enterocytes must first be confirmed before proposing that this carrier mediates the basolateral efflux of bile acids. There is also evidence to support a role for MRP3 in pumping out bile acids at this site since it is expressed in intestine and in the human adenocarcinoma Caco-2 cell line that retains many characteristics of normal enterocytes (Hirohashi et al., 2000a; Kiuchi et al., 1998). Preliminary data have also located MRP3 at the basolateral surface of the human ileum (Hirohashi et al., 2000b).

Cholehepatic circulation of bile acids

The intra-hepatic biliary epithelium consists of at least two subpopulations of cholangiocytes that line the small and large intra-hepatic bile ducts and participate in secretory and absorptive processes (Alpini et al., 1997a). To some extent, bile acids undergo cholehepatic recycling, i.e. from the lumen of bile ductules, through the apical and basolateral surfaces of cholangiocytes into the periductular capillary plexus and back into the sinusoids; passive reabsorption of the un-ionized form had been implicated as the sole mechanism (Hofmann, 1994). It has since been demonstrated that Na^+-dependent transport of bile salts occurs in polarized rat cholangiocytes in culture, in apical cholangiocyte vesicles (Lazaridis et al., 1997) and in a human cholangiocyte cell line (Que et al., 1999). The presence of Asbt, identical to that expressed in the rat ileum, has been convincingly demonstrated at the apical surface of large but not small cholangiocytes (Alpini et al., 1997b).

Efflux of bile acids at the basolateral membrane of rat cholangiocytes has been functionally characterized, and a Na^+-independent exchange mechanism was implicated (Benedetti et al., 1997). The alternatively spliced truncated form of Asbt (t-Asbt) has been localized to the basolateral surface of cholangiocytes (Lazaridis et al., 2000). This provides a mechanism for the vectorial apical-to-basolateral transfer of bile acids in rat cholangiocytes by two isoforms of Asbt. No driving force for this carrier has yet been identified (Lazaridis et al., 2000). It is now evident that additional mechanisms are in place to export bile acids from cholangiocytes. In man, MRP3 is also present in normal liver at the basolateral membrane of cholangiocytes (Kool et al., 1999).

The cholehepatic circulation of bile salts is more complex than previously thought. Its importance in bile physiology may be linked in part to the conservation of bile acids and the generation of a hypercholeretic bile flow (Gurantz et al., 1991; Yeh et al., 1997). However, the relative abundance of Asbt in rat biliary epithelia is some sevenfold lower than that in the ileum brush border (Lazaridis et al., 1997). Moreover, the heterogeneous distribution of Asbt in large, but not small, cholangiocytes coincides with the stimulatory effects of taurocholate on cell proliferation and secretory activity within this same subpopulation of cholangiocytes (Alpini et al., 1997a). These findings are consistent with a role for bile acids as signalling molecules that regulate secretory and proliferative events within the biliary tree (Alpini et al., 1999).

Bile acid transport in renal tubular cells

Normally, low concentrations of bile acids escape into the systemic circulation, but this can increase markedly in cholestasis (Raedsch et al., 1981). After filtration through the glomerulus, reabsorption of bile acids occurs from the apical surface of the proximal tubular cells *via* a Na^+-gradient-driven process (Wilson et al., 1981). Expression of the ileal Na^+/bile acid cotransporter (Asbt), at both the messenger RNA and protein levels, has been demonstrated in rat kidney (Christie et al., 1996) and human kidney (Craddock et al., 1998). Unconjugated as well as conjugated di- and trihydroxy bile acids are substrates for this salvage mechanism. However, the renal reabsorption of bile acids remains selective, since sulphate conjugates of chenodeoxycholate and taurolithocholate, which reach high systemic concentrations in hepatobiliary disease (Nittono et al., 1986), are not transported by ASBT. This promotes their urinary excretion.

At least two non-Na^+-dependent transport systems for conjugated bile salts have been identified in rodent kidney (Fig. 2). The organic anion transporter Oat-K2 (*Slc21a4*), with a high affinity for taurocholate (K_m=10 μmol l^{-1}), was cloned from rat kidney, and RNA transcripts were localized to the proximal tubules and collecting ducts (Masuda et al., 1999). Although the subcellular distribution of Oat-K2 was not determined, an apical localization was inferred by analogy with its homolog Oat-K1, which is expressed in kidney brush-border membranes (Masuda et al., 1997). Moreover, MDCK transfectants exhibited enhanced uptake and efflux of taurocholate across the apical surface, suggesting that Oat-K2 may mediate either the secretion or the re-uptake of bile acids at the luminal surface of renal tubular cells (Masuda et al., 1999). The Oatp family member Oatp1 (see above) is also expressed in rat kidney, where it is confined to the S3 segment of the proximal straight tubule (Bergwerk et al., 1996) and, in contrast to its basolateral localization in hepatocytes (Eckhardt et al., 1999), is localized at the apical membrane. The multispecific organic anion-transporting polypeptide-3 (Oatp3) (*Slc21a7*) was reportedly expressed in rat kidney (Abe et al., 1998). However, in the light of recent work that emphasizes the degree of sequence identity among Oatp isoforms and the cross-reactivity of antibodies, the presence of Oatp3 in the kidney requires further study (Walters et al., 2000).

Oatp1 and Oat-K2 are both multispecific organic anion transporters whose substrate list includes bile acids among other steroidal, hormonal and exogenous compounds. Given that Asbt/ASBT is also positioned at the luminal surface of the tubular cells, Oatp1/Oat-K2 may not play critical roles in the re-uptake of bile acid. It is uncertain whether bile acids, which are normally 50–90 % protein-bound (Aldini et al., 1982), are secreted as anions by tubular cells but, to account for their urinary excretion in hepatobiliary disease, a component of tubular secretion is likely. An active export mechanism does exist for the di-anionic bile salts, since Mrp2/MRP2 is located at the apical brush border of proximal tubules in rat and humans (Schaub et al., 1997; Schaub et al., 1999).

The human multi-specific carrier OATP-A is also expressed in the kidney, but at low levels, and has not been further localized (Kullak-Ublick et al., 1995). OATP-A exhibits 53 % identity with rat Oat-K2.

The entry of bile acids into tubular cells at the basolateral membrane has not been specifically studied. A newly defined organic ion-transporter family (Oat), which now has three members in the rat, operates as an anion/dicarboxylate exchange system. Oat1, the kidney-specific isoform, is expressed at the basolateral membrane of proximal tubular cells (Sekine et al., 1997), and Oat2 is expressed in both liver and kidney (Sekine et al., 1998). Neither has been specifically implicated in bile acid transport, but cholic acid can inhibit the Oat2-mediated uptake of salicylate. The precise mechanisms of renal handling of bile acids are unknown.

Bile acid carriers in the placenta

Bile acids are synthesized by the foetal liver *in utero* (Nakagawa and Setchell, 1990), but undergo minimal biliary excretion because of the immature excretory capacity of the foetal liver. Instead, they undergo vectorial translocation from the foetal to the maternal circulation for elimination (Marin et al., 1990). Distinct bile salt transport systems have been localized to the basolateral membrane (foetal-facing) and apical brush-border membrane (maternal-facing) of the trophoblasts but have been described only on a functional basis. Bile salt uptake into basolateral trophoblast vesicles is Na^+-independent, bi-directional and *trans*-stimulatable by bicarbonate (Marin et al., 1990). Both ATP-independent and ATP-dependent bile acid transport across the apical membrane of human trophoblasts have also been demonstrated (Bravo et al., 1995), with the ATP-independent mechanism predominating (Serrano et al., 1998). Partial transcripts of OATP-A have been identified in term placenta (St-Pierre et al., 2000a); however, more detailed characterization and localization of members of this family in the placenta is needed. BSEP remains a candidate transporter for the ATP-dependent transport at the apical surface of the trophoblast since partial transcripts of Bsep/BSEP have been identified in the rat and human placenta, respectively (St-Pierre et al., 2000a). Recent studies have localized MRP3 primarily to the foetal blood endothelia of term placenta, with some additional evidence for expression in the syncytiotrophoblast layer (St-Pierre et al., 2000b). This suggests that MRP3 is well placed to extrude foetal bile salts through both the endothelial and syncytiotrophoblast cellular barriers.

Bile acid carriers in yeast

An unexpected discovery was the transport of mammalian C_{24} bile acids into organelles of lower eukaryotes. The vacuoles and secretory vesicles of the budding yeast *Saccharomyces cerevisiae* accumulate bile acids in a concentrative and ATP-dependent manner (St-Pierre et al., 1994). Subsequent isolation of the cDNA for this yeast bile acid transporter (Bat1) identified it as a member of the MRP family of transporters (Ortiz et al., 1997). It is one of a total of 16 ABC transporters in the yeast proteome (Goffeau, 1998). Bat1 shares 32 % identity with the mammalian transporters MRP1 and MRP3. Its apparent affinity for taurocholate (K_m=60 μmol l^{-1}) is lower than that of the rat Bsep (Gerloff et al., 1998) or rat Mrp3 (Hirohashi et al., 2000b). This is not surprising, given that C_{24} bile acids are not endogenous to yeast. Decades ago, it was proposed that an acidic lipid with physicochemical properties similar to those of bile acids could inhibit ergosterol biosynthesis in yeast, specifically at the reduction step of the conversion of HMG-CoA (β-hydroxy-β-methylglutonyl-CoA) to mevalonate, a pathway common to both yeast and man (Kawaguchi, 1970). The endogenous yeast acids were purified and shown directly to inhibit conversion of acetate to mevalonate (Hatanaka et al., 1972). This led to the hypothesis that a fundamental mechanism has been conserved throughout evolution whereby endogenous catabolic products of sterols exert negative feedback control on the biosynthesis of cholesterol or ergosterol (Ortiz et al., 1997).

Concluding remarks

The cloning of individual bile acid carriers has had a great impact. There is now a greater appreciation of the elaborate disposition of bile acids in humans, from their biosynthesis from cholesterol to their excretion into the bile and their reabsorption from the apical surfaces of cells in the biliary tract, intestine and kidney. Key genetic studies have made it possible to identify defects at the molecular level, with the attendant recognition of diseases, clinical syndromes and new possibilities for therapy. In view of the profound physiological implications of abnormal bile acid disposition in man and its inextricable link to cholesterol homeostasis, research into bile acid transport and its regulation should continue at an accelerated pace.

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References

Abe, T., Kakyo, M., Sakagami, H., Tokui, T., Nishio, T., Tanemoto, M., Nomura, H., Hebert, S. C., Matsuno, S., Kondo, H. and Yawo, H. (1998). Molecular characterization and tissue distribution of a new organic anion transporter subtype (oatp3) that transports thyroid hormones and taurocholate and comparison with oatp2. *J. Biol. Chem.* **273**, 22395–22401.

Abe, T., Kakyo, M., Tokui, T., Nakagomi, R., Nishio, T., Nakai, D., Nomura, H., Unno, M., Suzuki, M., Naitoh, T., Matsuno, S. and Yawo, H. (1999). Identification of a novel gene family encoding human liver-specific organic anion transporter LST-1. *J. Biol. Chem.* **274**, 17159–17163.

Aldini, R., Roda, A., Labate, A. M., Cappelleri, G., Roda, E. and Barbara, L. (1982). Hepatic bile acid uptake: effect of conjugation, hydroxyl and keto groups and albumin binding. *J. Lipid Res.* **23**, 1167–1173.

Alpini, G., Glaser, S., Robertson, W., Phinizy, J. L., Rodgers, R. E., Caligiuri, A. and LeSage, G. (1997a). Bile acids stimulate proliferative and secretory events in large but not small cholangiocytes. *Am. J. Physiol.* **273**, G518–G529.

Alpini, G., Glaser, S. S., Rodgers, R., Phinizy, J. L., Robertson, W. E., Lasater, J., Caligiuri, A., Tretjak, Z. and LeSage, G. D. (1997b). Functional expression of the apical Na^+-dependent bile acid transporter in large but not small rat cholangiocytes. *Gastroenterology* **113**, 1734–1740.

Alpini, G., Glaser, S. S., Ueno, Y., Rodgers, R., Phinizy, J. L., Francis, H., Baiocchi, L., Holcomb, L. A., Caligiuri, A. and LeSage, G. D. (1999). Bile acid feeding induces cholangiocyte proliferation and secretion: evidence for bile acid-regulated ductal secretion. *Gastroenterology* **116**, 179–186.

Amelsberg, A., Jochims, C., Richter, C. P., Nitsche, R. and Folsch, U. R. (1999). Evidence for an anion exchange mechanism for uptake of conjugated bile acid from the rat jejunum. *Am. J. Physiol.* **276**, G737–G742.

Angeletti, R. H., Novikoff, P. M., Juvvadi, S. R., Fritschy, J. M., Meier, P. J. and Wolkoff, A. W. (1997). The choroid plexus epithelium is the site of the organic anion transport protein in the brain. *Proc. Natl. Acad. Sci. USA* **94**, 283–286.

Arrese, M., Trauner, M., Sacchiero, R. J., Crossman, M. W. and Shneider, B. L. (1998). Neither intestinal sequestration of bile acids nor common bile duct ligation modulate the expression and function of the rat ileal bile acid transporter. *Hepatology* **28**, 1081–1087.

Balistreri, W. F. (1999). Inborn errors of bile acid biosynthesis and transport. Novel forms of metabolic liver disease. *Gastroenterol. Clin. North Am.* **28**, 145–172.

Benedetti, A., Di Sario, A., Marucci, L., Svegliati-Baroni, G., Schteingart, C. D., Ton-Nu, H. T. and Hofmann, A. F. (1997). Carrier-mediated transport of conjugated bile acids across the basolateral membrane of biliary epithelial cells. *Am. J. Physiol.* **272**, G1416–G1424.

Bergwerk, A. J., Shi, X., Ford, A. C., Kanai, N., Jacquemin, E., Burk, R. D., Bai, S., Novikoff, P. M., Stieger, B., Meier, P. J., Schuster, V. L. and Wolkoff, A. W. (1996). Immunologic distribution of an organic anion transport protein in rat liver and kidney. *Am. J. Physiol.* **271**, G231–G238.

Bjorkhem, I., Andersson, O., Diczfalusy, U., Sevastik, B., Xiu, R. J., Duan, C. and Lund, E. (1994). Atherosclerosis and sterol 27-hydroxylase: evidence for a role of this enzyme in elimination of cholesterol from human macrophages. *Proc. Natl. Acad. Sci. USA* **91**, 8592–8596.

Bolder, U., Ton-Nu, H. T., Schteingart, C. D., Frick, E. and Hofmann, A. F. (1997). Hepatocyte transport of bile acids and organic anions in endotoxemic rats: impaired uptake and secretion. *Gastroenterology* **112**, 214–225.

Bossard, R., Stieger, B. B. O. N., Fricker, G. and Meier, P. J. (1993). Ethinylestradiol treatment induces multiple canalicular membrane transport alterations in rat liver. *J. Clin. Invest.* **91**, 2714–2720.

Bossuyt, X., Muller, M., Hagenbuch, B. and Meier, P. J. (1996). Polyspecific drug and steroid clearance by an organic anion transporter of mammalian liver. *J. Pharmac. Exp. Ther.* **276**, 891–896.

Boyer, J. L. and Soroka, C. J. (1995). Vesicle targeting to the apical domain regulates bile excretory function in isolated rat hepatocyte couplets. *Gastroenterology* **109**, 1600–1611.

Bravo, P., Marin, J. J., Beveridge, M. J. and Novak, D. A. (1995). Reconstitution and characterization of ATP-dependent bile acid transport in human and rat placenta. *Biochem. J.* **311**, 479–485.

Buscher, H. P., Meder, I., MacNelly, S. and Gerok, W. (1993). Zonal changes of hepatobiliary taurocholate transport in intra-hepatic cholestasis induced by 17 alpha-ethinyl estradiol: a histoautoradiographic study in rats. *Hepatology* **17**, 494–499.

Cali, J. J., Hsieh, C. L., Francke, U. and Russell, D. W. (1991). Mutations in the bile acid biosynthetic enzyme sterol 27-hydroxylase underlie cerebrotendinous xanthomatosis. *J. Biol. Chem.* **266**, 7779–7783.

Cali, J. J. and Russell, D. W. (1991). Characterization of human sterol 27-hydroxylase. A mitochondrial cytochrome P-450 that catalyzes multiple oxidation reaction in bile acid biosynthesis. *J. Biol. Chem.* **266**, 7774–7748.

Cattori, V., Eckhardt, U. and Hagenbuch, B. (1999). Molecular cloning and functional characterization of two alternatively spliced Ntcp isoforms from mouse liver. *Biochim. Biophys. Acta* **1445**, 154–159.

Cattori, V., Hagenbuch, B., Hagenbuch, N., Stieger, B., Winterhalter, K. E. and Meier, P. J. (2000a). Identification of a novel rat organic anion transporting polypeptide (Oatp4) as a full-length isoform of the liver-specific transporter-1 (rlst-1). *FEBS Lett.* **474**, 242–245.

Cattori, V., Hagenbuch, B., Stieger, B., Landmann, L., Winterhalter, K. H. and Meier, P. J. (2000b). Characterization of the rat liver organic anion transporting polypeptide 4 (Oatp4) and its comparison with Oatp3. *Hepatology* **32**, 437A.

Chiang, J. Y. L. (1998). Regulation of bile acid synthesis. *Front. Biosci.* **15**, D176–D193.

Childs, S., Yeh, R. L., Georges, E. and Ling, V. (1995). Identification of a sister gene to P-glycoprotein. *Cancer Res.* **55**, 2029–2034.

Childs, S., Yeh, R. L., Hui, D. and Ling, V. (1998). Taxol resistance mediated by transfection of the liver-specific sister gene of P-glycoprotein. *Cancer Res.* **58**, 4160–4167.

Christie, D. M., Dawson, P. A., Thevananther, S. and Shneider, B. L. (1996). Comparative analysis of the ontogeny of a sodium-dependent bile acid transporter in rat kidney and ileum. *Am. J. Physiol.* **271**, G377–G385.

Cole, S. P., Bhardwaj, G., Gerlach, J. H., Mackie, J. E., Grant, C. E., Almquist, K. C., Stewart, A. J., Kurz, E. U., Duncan, A. M. and Deeley, R. G. (1992). Overexpression of a transporter gene in a multidrug-resistant human lung cancer cell line. *Science* **258**, 1650–1654.

Cowen, A. E., Korman, M. G., Hofmann, A. F. and Cass, O. W. (1975). Metabolism of lethocholate in healthy man. I. Biotransformation and biliary excretion of intravenously administered lithocholate, lithocholylglycine and their sulfates. *Gastroenterology* **69**, 59–66.

Craddock, A. L., Love, M. W., Daniel, R. W., Kirby, L. C., Walters, H. C., Wong, M. H. and Dawson, P. A. (1998). Expression and transport properties of the human ileal and renal sodium-dependent bile acid transporter. *Am. J. Physiol.* **274**, G157–G169.

Cui, Y., König, J., Buchholz, U., Spring, H., Leier, I. and Keppler, D. (1999). Drug resistance and ATP-dependent conjugate transport mediated by the apical multidrug resistance protein, MRP2, permanently expressed in human and canine cells. *Mol. Pharmac.* **55**, 929–937.

Dawson, P. A. and Oelkers, P. (1995). Bile acid transporters. *Curr. Opin. Lipidol.* **6**, 109–114.

Denson, L. A., Auld, K. L., Schiek, D. S., McClure, M. H., Mangelsdorf, D. J. and Karpen, S. J. (2000). Interleukin-1beta suppresses retinoid transactivation of two hepatic transporter genes involved in bile formation. *J. Biol. Chem.* **275**, 8835–8843.

Dray-Charier, N., Paul, A., Combettes, L., Bouin, M., Mergey, M., Balladur, P., Capeau, J. and Housset, C. (1997). Regulation of mucin secretion in human gallbladder epithelial cells: predominant role of calcium and protein kinase C. *Gastroenterology* **112**, 978–990.

Duane, W. C. (1993). Effects of lovastatin and dietary cholesterol on sterol homeostasis in healthy human subjects. *J. Clin. Invest.* **92**, 911–918.

Eckhardt, U., Schroeder, A., Stieger, B., Höchli, M., Landmann, L., Tynes, R., Meier, P. J. and Hagenbuch, B. (1999). Polyspecific substrate uptake by the hepatic organic anion transporter oatp1 in stably transfected CHO cells. *Am. J. Physiol.* **276**, G1037–G1042.

Evers, R., Kool, M., van Deemter, L., Janssen, H., Calafat, J., Oomen, L. C., Paulusma, C. C., Oude Elferink, R. P., Baas, F., Schinkel, A. H. and Borst, P. (1998). Drug export activity of the human canalicular multispecific organic anion transporter in polarized kidney MDCK cells expressing cMOAT (MRP2) cDNA. *J. Clin. Invest.* **101**, 1310–1319.

Falany, C. N., Johnson, M. R., Barnes, S. and Diasio, R. B. (1994). Glycine and taurine conjugation of bile acids by a single enzyme. Molecular cloning and expression of human liver bile acid CoA:amino acid N-acyltransferase. *J. Biol. Chem.* **269**, 19375–19379.

Ganguly, T. C., O'Brien, M. L., Karpen, S. J., Hyde, J. F., Suchy, F. J. and Vore, M. (1997). Regulation of the rat liver sodium-dependent bile acid cotransporter gene by prolactin – Mediation of transcriptional activation by Stat5. *J. Clin. Invest.* **99**, 2906–2914.

Gao, B., Hagenbuch, B., Kullak-Ublick, G. A., Benke, D., Aguzzi, A. and

Meier, P. J. (2000). Organic anion transporting polypeptides mediate transport of opioid peptides across the blood–brain barrier. *J. Pharmac. Exp. Ther.* **294**, 73–79.

Gao, B., Stieger, B., Noe, B., Fritschy, J. M. and Meier, P. J. (1999). Localization of the organic anion transporting polypeptide 2 (Oatp2) in capillary endothelium and choroid plexus epithelium of rat brain. *J. Histochem. Cytochem.* **47**, 1255–1264.

Gartung, C., Ananthanarayanan, M., Rahman, M. A., Schuele, S., Nundy, S., Soroka, C. J., Stolz, A., Suchy, F. J. and Boyer, J. L. (1996). Downregulation of expression and function of the rat liver Na^+/bile acid cotransporter in extra-hepatic cholestasis. *Gastroenterology* **110**, 199–209.

Gerloff, T., Stieger, B., Hagenbuch, B., Madon, J., Landmann, L., Roth, J., Hofmann, A. F. and Meier, P. J. (1998). The sister of P-glycoprotein represents the canalicular bile salt export pump of mammalian liver. *J. Biol. Chem.* **273**, 10046–10050.

Goffeau, A. (1998). The inventory of all ion and drug ATPases encoded by the yeast genome. *Acta Physiol. Scand.* (Suppl.) **643**, 297–300.

Green, R. M., Beier, D. and Gollan, J. L. (1996). Regulation of hepatocyte bile salt transporters by endotoxin and inflammatory cytokines in rodents. *Gastroenterology* **111**, 193–198.

Green, R. M., Hoda, F. and Ward, K. L. (2000). Molecular cloning and characterization of the murine bile salt export pump. *Gene* **241**, 117–123.

Groothuis, G. M., Hardonk, M. J., Keulemans, K. P., Nieuwenhuis, P. and Meijer, D. K. (1982). Autoradiographic and kinetic demonstration of acinar heterogeneity of taurocholate transport. *Am. J. Physiol.* **243**, G455–G462.

Gurantz, D., Schteingart, C. D., Hagey, L. R., Steinbach, J. H., Grotmol, T. and Hofmann, A. F. (1991). Hypercholeresis induced by unconjugated bile acid infusion correlates with recovery in bile of unconjugated bile acids. *Hepatology* **13**, 540–550.

Hagenbuch, B. and Meier, P. J. (1994). Molecular cloning, chromosomal localization and functional characterization of a human liver Na^+/bile acid cotransporter. *J. Clin. Invest.* **93**, 1326–1331.

Hagenbuch, B., Stieger, B., Foguet, M., Lubbert, H. and Meier, P. J. (1991). Functional expression cloning and characterization of the hepatocyte Na^+/bile acid cotransport system. *Proc. Natl. Acad. Sci. USA* **88**, 10629–10633.

Hallen, S., Branden, M., Dawson, P. A. and Sachs, G. (1999). Membrane insertion scanning of the human ileal sodium/bile acid co-transporter. *Biochemistry* **38**, 11379–11388.

Hatanaka, H., Kawaguchi, A., Hayakawa, S. and Katsuki, H. (1972). Structural specificity of bile acid for inhibition of sterol synthesis in cell-free extracts of yeast. *Biochim. Biophys. Acta* **270**, 397–406.

Higgins, C. F. (1995). The ABC of channel regulation. *Cell* **82**, 693–696.

Hirohashi, T., Suzuki, H., Chu, X. Y., Tamai, I., Tsuji, A. and Sugiyama, Y. (2000a). Function and expression of multidrug resistance-associated protein family in human colon adenocarcinoma cells (Caco-2). *J. Pharmac. Exp. Ther.* **292**, 265–270.

Hirohashi, T., Suzuki, H., Takikawa, H. and Sugiyama, Y. (2000b). ATP-dependent transport of bile salts by rat multidrug resistance-associated protein 3 (Mrp3). *J. Biol. Chem.* **275**, 2905–2910.

Hofmann, A. F. (1994). Bile acids. In *The Liver: Biology and Pathobiology* (ed. I. M. Arias, J. L. Boyer, N. Fausto, W. B. Jakoby, D. A. Schachter and D. A. Shafritz), pp. 678–710. New York: Raven Press, Ltd.

Hofmann, A. F. (1999). Regulation of ileal bile acid transport: desirability of measuring transport function as well as transporter activity. *Hepatology* **29**, 1335–1337.

Hsiang, B., Zhu, Y., Wang, Z., Wu, Y., Sasseville, V., Yang, W. P. and Kirchgessner, T. G. (1999). A novel human hepatic organic anion transporting polypeptide (OATP2). Identification of a liver-specific human organic anion transporting polypeptide and identification of rat and human hydroxymethylglutaryl-CoA reductase inhibitor transporters. *J. Biol. Chem.* **274**, 37161–37168.

Jacquemin, E. (1999). Progressive familial intra-hepatic cholestasis. *J. Gastroenterol. Hepatol.* **14**, 594–599.

Jacquemin, E., Hagenbuch, B., Stieger, B., Wolkoff, A. W. and Meier, P. J. (1994). Expression cloning of a rat liver Na^+-independent organic anion transporter. *Proc. Natl. Acad. Sci. USA* **91**, 133–137.

Jansen, P. L., Strautnieks, S. S., Jacquemin, E., Hadchouel, M., Sokal, E. M., Hooiveld, G. J., Koning, J. H., De Jager-Krikken, A., Kuipers, F., Stellaard, F., Bijleveld, C. M., Gouw, A., Van Goor, H., Thompson, R. J. and Muller, M. (1999). Hepatocanalicular bile salt export pump deficiency in patients with progressive familial intra-hepatic cholestasis. *Gastroenterology* **117**, 1370–1379.

Jelinek, D. F., Andersson, S., Slaughter, C. A. and Russell, D. W. (1990). Cloning and regulation of cholesterol 7 alpha-hydroxylase, the rate-limiting enzyme in bile acid biosynthesis. *J. Biol. Chem.* **265**, 8190–8197.

Jones, B. A., Rao, Y. P., Stravitz, R. T. and Gores, G. J. (1997). Bile salt-induced apoptosis of hepatocytes involves activation of protein kinase C. *Am. J. Physiol.* **272**, G1109–G1115.

Kakyo, M., Sakagami, H., Nishio, T., Nakai, D., Nakagomi, R., Tokui, T., Naitoh, T., Matsuno, S., Abe, T. and Yawo, H. (1999). Immunohistochemical distribution and functional characterization of an organic anion transporting polypeptide 2 (oatp2). *FEBS Lett.* **445**, 343–346.

Karpen, S. J., Sun, A. Q., Kudish, B., Hagenbuch, B., Meier, P. J., Ananthanarayanan, M. and Suchy, F. J. (1996). Multiple factors regulate the rat liver basolateral sodium-dependent bile acid cotransporter gene promoter. *J. Biol. Chem.* **271**, 15211–15221.

Kawaguchi, A. (1970). Control of ergosterol biosynthesis in yeast. Existence of lipid inhibitors. *J. Biochem.* **67**, 219–227.

Keppler, D., Leier, I. and Jedlitschky, G. (1997). Transport of glutathione conjugates and glucuronides by the multidrug resistance proteins MRP1 and MRP2. *Biol. Chem.* **378**, 787–791.

Kiuchi, Y., Suzuki, H., Hirohashi, T., Tyson, C. A. and Sugiyama, Y. (1998). cDNA cloning and inducible expression of human multidrug resistance associated protein 3 (Mrp3). *FEBS Lett.* **433**, 149–152.

Konig, J., Cui, Y., Nies, A. T. and Keppler, D. (2000a). Localization and genomic organization of a new hepatocellular organic anion transporting polypeptide. *J. Biol. Chem.* **275**, 23161–23168.

Konig, J., Cui, Y., Nies, A. T. and Keppler, D. (2000b). A novel human organic anion transporting polypeptide localized to the basolateral hepatocyte membrane. *Am. J. Physiol.* **278**, G156–G164.

Konig, J., Nies, A. T., Cui, Y., Leier, I. and Keppler, D. (1999a). Conjugate export pumps of the multidrug resistance protein (MRP) family: localization, substrate specificity and MRP2-mediated drug resistance. *Biochim. Biophys. Acta* **1461**, 377–394.

Konig, J., Rost, D., Cui, Y. and Keppler, D. (1999b). Characterization of the human multidrug resistance protein isoform MRP3 localized to the basolateral hepatocyte membrane. *Hepatology* **29**, 1156–1163.

Kool, M., de Haas, M., Scheffer, G. L., Scheper, R. J., van Eijk, M. J., Juijn, J. A., Baas, F. and Borst, P. (1997). Analysis of expression of cMOAT (MRP2), MRP3, MRP4 and MRP5, homologues of the multidrug resistance-associated protein gene (MRP1), in human cancer cell lines. *Cancer Res.* **57**, 3537–3547.

Kool, M., van der Linden, M., de Haas, M., Scheffer, G. L., de Vree, J. M., Smith, A. J., Jansen, G., Peters, G. J., Ponne, N., Scheper, R. J., Elferink, R. P., Baas, F. and Borst, P. (1999). MRP3, an organic anion transporter able to transport anti-cancer drugs. *Proc. Natl. Acad. Sci. USA* **96**, 6914–6919.

Koop, I., Schindler, M., Bosshammer, A., Scheibner, J., Stange, E. and Koop, H. (1996). Physiological control of cholecystokinin release and pancreatic enzyme secretion by intraduodenal bile acids. *Gut* **39**, 661–667.

Kullak-Ublick, G. A., Fisch, T., Oswald, M., Hagenbuch, B., Meier, P. J., Beuers, U. and Paumgartner, G. (1998). Dehydroepiandrosterone sulfate (DHEAS): identification of a carrier protein in human liver and brain. *FEBS Lett.* **424**, 173–176.

Kullak-Ublick, G. A., Hagenbuch, B., Stieger, B., Schteingart, C. D., Hofmann, A. F., Wolkoff, A. W. and Meier, P. J. (1995). Molecular and functional characterization of an organic anion transporting polypeptide cloned from human liver. *Gastroenterology* **109**, 1274–1282.

Kullak-Ublick, G. A., Ismair, M. G., Stieger, B., Landmann, L., Huber, R., Pizzagalli, F., Fattinger, K., Meier, P. J. and Hagenbuch, B. (2001). Organic anion transporting polypeptide B (OATP-B) and its functional comparison with three other OATPS of human liver. *Gastroenterology* **120**, 525–533.

Kupferschmidt, H., Hagenbuch, B., Stieger, B., Kraehenbühl, S. and Meier, P. J. (1994). Ethinylestradiol induces differential effects on various transporter mRNA and protein levels in rat liver. *Hepatology* **20**, 175A.

Lazaridis, K. N., Pham, L., Tietz, P., Marinelli, R. A., deGroen, P. C., Levine, S., Dawson, P. A. and LaRusso, N. F. (1997). Rat cholangiocytes absorb bile acids at their apical domain *via* the ileal sodium-dependent bile acid transporter. *J. Clin. Invest.* **100**, 2714–2721.

Lazaridis, K. N., Tietz, P., Wu, T., Kip, S., Dawson, P. A. and LaRusso, N. F. (2000). Alternative splicing of the rat sodium/bile acid transporter changes its cellular localization and transport properties. *Proc. Natl. Acad. Sci. USA* **97**, 11092–11097.

Lee, J. M., Trauner, M., Soroka, C. J., Stieger, B., Meier, P. J. and Boyer, J. L. (2000). Expression of the bile salt export pump is maintained after chronic cholestasis in the rat. *Gastroenterology* **118**, 163–172.

Li, L., Lee, T. K., Meier, P. J. and Ballatori, N. (1998). Identification of glutathione as a driving force and leukotriene C4 as a substrate for oatp1, the hepatic sinusoidal organic solute transporter. *J. Biol. Chem.* **273**, 16184–16191.

Li, L., Meier, P. J. and Ballatori, N. (2000). Oatp2 mediates bidirectional organic solute transport: a role for intracellular glutathione. *Mol. Pharmac.* **58**, 335–340.

Lillienau, J., Crombie, D. L., Munoz, J., Longmire-Cook, S. J., Hagey, L. R. and Hofmann, A. F. (1993). Negative feedback regulation of the ileal bile acid transport system in rodents. *Gastroenterology* **104**, 38–46.

Lu, T. T., Makishima, M., Repa, J. J., Schoonjans, K., Kerr, T. A., Auwerx, J. and Mangelsdorf, D. J. (2000). Molecular basis for feedback regulation of bile acid synthesis by nuclear receptors. *Mol. Cell* **6**, 507–515.

Lund, E., Andersson, O., Zhang, J., Babiker, A., Ahlborg, G., Diczfalusy, U., Einarsson, K., Sjövall, J. and Björkhem, I. (1996). Importance of a novel oxidative mechanism for elimination of intracellular cholesterol in humans. *Arterioscler. Thromb. Vasc. Biol.* **16**, 208–212.

Makishima, M., Okamoto, A. Y., Repa, J. J., Tu, H., Learned, R. M., Luk, A., Hull, M. V., Lustig, K. D., Mangelsdorf, D. J. and Shan, B. (1999). Identification of a nuclear receptor for bile acids. *Science* **284**, 1362–1365.

Marin, J. J., Serrano, M. A., el-Mir, M. Y., Eleno, N. and Boyd, C. A. (1990). Bile acid transport by basal membrane vesicles of human term placental trophoblast. *Gastroenterology* **99**, 1431–1438.

Martin, K. O., Budai, K. and Javitt, N. B. (1993). Cholesterol and 27-hydroxycholesterol 7 alpha-hydroxylation: evidence for two different enzymes. *J. Lipid Res.* **34**, 581–588.

Masuda, S., Ibaramoto, K., Takeuchi, A., Saito, H., Hashimoto, Y. and Inui, K. I. (1999). Cloning and functional characterization of a new multispecific organic anion transporter, OAT-K2, in rat kidney. *Mol. Pharmac.* **55**, 743–752.

Masuda, S., Saito, H., Nonoguchi, H., Tomita, K. and Inui, K. (1997). mRNA distribution and membrane localization of the Oat-K1 organic anion transporter in rat renal tubules. *FEBS Lett.* **407**, 127–131.

Mayer, R., Kartenbeck, J., Buchler, M., Jedlitschky, G., Leier, I. and Keppler, D. (1995). Expression of the MRP gene-encoded conjugate export pump in liver and its selective absence from the canalicular membrane in transport-deficient mutant hepatocytes. *J. Cell Biol.* **131**, 137–150.

Mekhjian, H. S., Phillips, S. F. and Hofmann, A. F. (1979). Colonic absorption of unconjugated bile acids: perfusion studies in man. *Dig. Dis. Sci.* **24**, 545–550.

Misra, S., Ujhazy, P., Gatmaitan, Z., Varticovski, L. and Arias, I. M. (1998). The role of phosphoinositide 3-kinase in taurocholate-induced trafficking of ATP-dependent canalicular transporters in rat liver. *J. Biol. Chem.* **273**, 26638–26644.

Misra, S., Ujhazy, P., Varticovski, L. and Arias, I. M. (1999). Phosphoinositide 3-kinase lipid products regulate ATP-dependent transport by sister of P-glycoprotein and multidrug resistance associated protein 2 in bile canalicular membrane vesicles. *Proc. Natl. Acad. Sci. USA* **96**, 5814–5819.

Moyer, M. S., Heubi, J. E., Goodrich, A. L., Balistreri, W. F. and Suchy, F. J. (1986). Ontogeny of bile acid transport in brush border membrane vesicles from rat ileum. *Gastroenterology* **90**, 1188–1196.

Nakagawa, M. and Setchell, K. D. (1990). Bile acid metabolism in early life: studies of amniotic fluid. *J. Lipid Res.* **31**, 1089–1098.

Nguyen, L. B., Xu, G., Shefer, S., Tint, G. S., Batta, A. and Salen, G. (1999). Comparative regulation of hepatic sterol 27-hydroxylase and cholesterol 7alpha-hydroxylase activities in the rat, guinea pig and rabbit: effects of cholesterol and bile acids. *Metabolism* **48**, 1542–1548.

Nishida, T., Gatmaitan, Z., Che, M. and Arias, I. M. (1991). Rat liver canalicular membrane vesicles contain an ATP-dependent bile acid transport system. *Proc. Natl. Acad. Sci. USA* **88**, 6590–6594.

Nittono, H., Obinata, K., Nakatsu, N., Watanabe, T., Niijima, S., Sasaki, H., Arisaka, O., Kato, H., Yabuta, K. and Miyano, T. (1986). Sulfated and nonsulfated bile acids in urine of patients with biliary atresia: analysis of bile acids by high-performance liquid chromatography. *J. Pediatr. Gastroenterol. Nutr.* **5**, 23–29.

Noe, B., Hagenbuch, B., Stieger, B. and Meier, P. J. (1997). Isolation of a multispecific organic anion and cardiac glycoside transporter from rat brain. *Proc. Natl. Acad. Sci. USA* **94**, 10346–10350.

Oelkers, P., Kirby, L. C., Heubi, J. E. and Dawson, P. A. (1997). Primary bile acid malabsorption caused by mutations in the ileal sodium-dependent bile acid transporter gene (SLC10A2). *J. Clin. Invest.* **99**, 1880–1887.

Ogawa, K., Suzuki, H., Hirohashi, T., Ishikawa, T., Meier, P. J., Hirose, K., Akizawa, T., Yoshioka, M. and Sugiyama, Y. (2000). Characterization of inducible nature of MRP3 in rat liver. *Am. J. Physiol.* **278**, G438–G446.

Ortiz, D. F., St-Pierre, M. V., Abdulmessih, A. and Arias, I. M. (1997). A yeast ATP-binding cassette-type protein mediating ATP-dependent bile acid transport. *J. Biol. Chem.* **272**, 15358–15365.

Pandak, W. M., Li, Y. C., Chiang, J. Y., Studer, E. J., Gurley, E. C., Heuman, D. M., Vlahcevic, Z. R. and Hylemon, P. B. (1991). Regulation of cholesterol 7 alpha-hydroxylase mRNA and transcriptional activity by taurocholate and cholesterol in the chronic biliary diverted rat. *J. Biol. Chem.* **266**, 3416–3421.

Parks, D. J., Blanchard, S. G., Bledsoe, R. K., Chandra, G., Consler, T. G., Kliewer, S. A., Stimmel, J. B., Willson, T. M., Zavacki, A. M., Moore, D. D. and Lehmann, J. M. (1999). Bile acids: natural ligands for an orphan nuclear receptor. *Science* **284**, 1365–1368.

Paulusma, C. C., Bosma, P. J., Zaman, G. J. R., Bakker, C. T. M., Otter, M., Scheffer, G. L., Scheper, R. J., Borst, P. and Oude Elferink, R. P. J. (1996). Congenital jaundice in rats with a mutation in a multidrug resistance-associated protein gene. *Science* **271**, 1126–1128.

Paulusma, C. C., Kool, M., Bosma, P. J., Scheffer, G. L., ter Borg, F., Scheper, R. J., Tytgat, G. N., Borst, P., Baas, F. and Oude Elferink, R. P. (1997). A mutation in the human canalicular multispecific organic anion transporter gene causes the Dubin–Johnson syndrome. *Hepatology* **25**, 1539–1542.

Peet, D. J., Turley, S. D., Ma, W., Janowski, B. A., Lobaccaro, J. M., Hammer, R. E. and Mangelsdorf, D. J. (1998). Cholesterol and bile acid metabolism are impaired in mice lacking the nuclear oxysterol receptor LXR alpha. *Cell* **93**, 693–704.

Pikuleva, I. A., Bjorkhem, I. and Waterman, M. R. (1997). Expression, purification and enzymatic properties of recombinant human cytochrome P450c27 (CYP27). *Arch. Biochem. Biophys.* **343**, 123–130.

Que, F. G., Phan, V. A., Phan, V. H., LaRusso, N. F. and Gores, G. J. (1999). GUDC inhibits cytochrome c release from human cholangiocyte mitochondria. *J. Surg. Res.* **83**, 100–105.

Raedsch, R., Lauterburg, B. H. and Hofmann, A. F. (1981). Altered bile acid metabolism in primary biliary cirrhosis. *Dig. Dis. Sci.* **26**, 394–401.

Rao, Y. P., Vlahcevic, Z. R., Stravitz, R. T., Mallonee, D. H., Mullick, J., Avadhani, N. G. and Hylemon, P. B. (1999). Downregulation of the rat hepatic sterol 27-hydroxylase gene by bile acids in transfected primary hepatocytes: possible role of hepatic nuclear factor 1alpha. *J. Steroid Biochem. Mol. Biol.* **70**, 1–14.

Reichel, C., Gao, B., Van Montfoort, J., Cattori, V., Rahner, C., Hagenbuch, B., Stieger, B., Kamisako, T. and Meier, P. J. (1999). Localization and function of the organic anion-transporting polypeptide Oatp2 in rat liver. *Gastroenterology* **117**, 688–695.

Reiss, A. B., Martin, K. O., Rojer, D. E., Iyer, S., Grossi, E. A., Galloway, A. C. and Javitt, N. B. (1997). Sterol 27-hydroxylase: expression in human arterial endothelium. *J. Lipid Res.* **38**, 1254–1260.

Repa, J. J. and Mangelsdorf, D. J. (2000). The role of orphan nuclear receptors in the regulation of cholesterol homeostasis. *Annu. Rev. Cell Devl. Biol.* **16**, 459–481.

Riepl, R. L., Fiedler, F., Ernstberger, M., Teufel, J. and Lehnert, P. (1996). Effect of intraduodenal taurodeoxycholate and L-phenylalanine on pancreatic secretion and on gastroenteropancreatic peptide release in man. *Eur. J. Med. Res.* **1**, 499–505.

Roelofsen, H., Vos, T. A., Schippers, I. J., Kuipers, F., Koning, H., Moshage, H., Jansen, P. L. and Muller, M. (1997). Increased levels of the multidrug resistance protein in lateral membranes of proliferating hepatocyte-derived cells. *Gastroenterology* **112**, 511–521.

Satlin, L. M., Amin, V. and Wolkoff, A. W. (1997). Organic anion transporting polypeptide mediates organic anion/HCO_3^- exchange. *J. Biol. Chem.* **272**, 26340–26345.

Schaub, T. P., Kartenbeck, J., Konig, J., Spring, H., Dorsam, J., Staehler, G., Storkel, S., Thon, W. F. and Keppler, D. (1999). Expression of the MRP2 gene-encoded conjugate export pump in human kidney proximal tubules and in renal cell carcinoma. *J. Am. Soc. Nephrol.* **10**, 1159–1169.

Schaub, T. P., Kartenbeck, J., Konig, J., Vogel, O., Witzgall, R., Kriz, W. and Keppler, D. (1997). Expression of the conjugate export pump encoded by the mrp2 gene in the apical membrane of kidney proximal tubules. *J. Am. Soc. Nephrol.* **8**, 1213–12121.

Sekine, T., Cha, S. H., Tsuda, M., Apiwattanakul, N., Nakajima, N., Kanai, Y. and Endou, H. (1998). Identification of multispecific organic anion transporter 2 expressed predominantly in the liver. *FEBS Lett.* **429**, 179–182.

Sekine, T., Watanabe, N., Hosoyamada, M., Kanai, Y. and Endou, H.

(1997). Expression cloning and characterization of a novel multispecific organic anion transporter. *J. Biol. Chem.* **272**, 18526–18529.

Serrano, M. A., Brites, D., Larena, M. G., Monte, M. J., Bravo, M. P., Oliveira, N. and Marin, J. J. (1998). Beneficial effect of ursodeoxycholic acid on alterations induced by cholestasis of pregnancy in bile acid transport across the human placenta. *J. Hepatol.* **28**, 829–839.

Shneider, B. L., Dawson, P. A., Christie, D. M., Hardikar, W., Wong, M. H. and Suchy, F. J. (1995). Cloning and molecular characterization of the ontogeny of a rat ileal sodium-dependent bile acid transporter. *J. Clin. Invest.* **95**, 745–754.

Simon, F. R., Fortune, J., Iwahashi, M., Gartung, C., Wolkoff, A. and Sutherland, E. (1996). Ethinyl estradiol cholestasis involves alterations in expression of liver sinusoidal transporters. *Am. J. Physiol.* **271**, G1043–G1052.

St-Pierre, M. V., Ruetz, S., Epstein, L. F., Gros, P. and Arias, I. M. (1994). ATP-dependent transport of organic anions in secretory vesicles of *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA* **91**, 9476–9479.

St-Pierre, M. V., Serrano, M. A., Lauper, U., Marin, J. J. G. and Meier, P. J. (2000a). Identification of bile salt transporters in human and rat placenta. *Placenta* **20**, 64A.

St-Pierre, M. V., Serrano, M. A., Macias, R. I. R., Dubs, U., Hoechli, M., Lauper, U., Meier, P. J. and Marin, J. J. G. (2000b). Expression of members of the multidrug resistance protein family in human term placenta. *Am. J. Physiol.* **279**, R1495–R1503.

Stieger, B., Fattinger, K., Madon, J., Kullak-Ublick, G. A. and Meier, P. J. (2000). Drug- and estrogen-induced cholestasis through inhibition of the hepatocellular bile salt export pump (Bsep) of rat liver. *Gastroenterology* **118**, 422–430.

Stieger, B., Hagenbuch, B., Landmann, L., Hochli, M., Schroeder, A. and Meier, P. J. (1994). *In situ* localization of the hepatocytic Na^+/taurocholate cotransporting polypeptide in rat liver. *Gastroenterology* **107**, 1781–1787.

Stieger, B. B. O. N. and Meier, P. J. (1992). ATP-dependent bile-salt transport in canalicular rat liver plasma-membrane vesicles. *Biochem. J.* **284**, 67–74.

Stiehl, A., Raedsch, R., Rudolph, G., Gundert-Remy, U. and Senn, M. (1985). Biliary and urinary excretion of sulfated, glucuronidated and tetrahydroxylated bile acids in cirrhotic patients. *Hepatology* **5**, 492–495.

Strautnieks, S. S., Bull, L. N., Knisely, A. S., Kocoshis, S. A., Dahl, N., Arnell, H., Sokal, E., Dahan, K., Childs, S., Ling, V., Tanner, M. S., Kagalwalla, A. F., Nemeth, A., Pawlowska, J., Baker, A., Mieli-Vergani, G., Freimer, N. B., Gardiner, R. M. and Thompson, R. J. (1998). A gene encoding a liver-specific ABC transporter is mutated in progressive familial intra-hepatic cholestasis. *Nature Genetics* **20**, 233–238.

Stravitz, R. T., Sanyal, A. J., Pandak, W. M., Vlahcevic, Z. R., Beets, J. W. and Dawson, P. A. (1997). Induction of sodium-dependent bile acid transporter messenger RNA, protein and activity in rat ileum by cholic acid. *Gastroenterology* **113**, 1599–1608.

Stroup, D., Crestani, M. and Chiang, J. Y. (1997). Identification of a bile acid response element in the cholesterol 7 alpha-hydroxylase gene CYP7A. *Am. J. Physiol.* **273**, G508–G517.

Trauner, M., Arrese, M., Lee, H., Boyer, J. L. and Karpen, S. J. (1998). Endotoxin downregulates rat hepatic ntcp gene expression *via* decreased activity of critical transcription factors. *J. Clin. Invest.* **101**, 2092–2100.

van Montfoort, J. E., Hagenbuch, B., Fattinger, K. E., Muller, M., Groothuis, G. M., Meijer, D. K. and Meier, P. J. (1999). Polyspecific organic anion transporting polypeptides mediate hepatic uptake of amphipathic type II organic cations. *J. Pharmac. Exp. Ther.* **291**, 147–152.

Vos, T. A., Hooiveld, G. J., Koning, H., Childs, S., Meijer, D. K., Moshage, H., Jansen, P. L. and Muller, M. (1998). Upregulation of the multidrug resistance genes, Mrp1 and Mdr1b and downregulation of the organic anion transporter, Mrp2 and the bile salt transporter, Spgp, in endotoxemic rat liver. *Hepatology* **28**, 1637–1644.

Walters, H. C., Craddock, A. L., Fusegawa, H., Willingham, M. C. and Dawson, P. A. (2000). Expression, transport properties and chromosomal location of organic anion transporter subtype 3. *Am. J. Physiol.* **279**, G1188–G1200.

Warskulat, U., Kubitz, R., Wettstein, M., Stieger, B., Meier, P. J. and Haussinger, D. (1999). Regulation of bile salt export pump mRNA levels by dexamethasone and osmolarity in cultured rat hepatocytes. *Biol. Chem.* **380**, 1273–1279.

Weinberg, S. L., Burckhardt, G. and Wilson, F. A. (1986). Taurocholate transport by rat intestinal basolateral membrane vesicles. Evidence for the presence of an anion exchange transport system. *J. Clin. Invest.* **78**, 44–50.

Weinman, S. A. (1997). Electrogenicity of Na^+-coupled bile acid transporters. *Yale J. Biol. Med.* **70**, 331–340.

Weinman, S. A., Carruth, M. W. and Dawson, P. A. (1998). Bile acid uptake *via* the human apical sodium–bile acid cotransporter is electrogenic. *J. Biol. Chem.* **273**, 34691–34695.

Wilson, F. A., Burckhardt, G., Murer, H., Rumrich, G. and Ullrich, K. J. (1981). Sodium-coupled taurocholate transport in the proximal convolution of the rat kidney *in vivo* and *in vitro*. *J. Clin. Invest.* **67**, 1141–1150.

Wong, M. H., Oelkers, P., Craddock, A. L. and Dawson, P. A. (1994). Expression cloning and characterization of the hamster ileal sodium-dependent bile acid transporter. *J. Biol. Chem.* **269**, 1340–1347.

Wong, M. H., Oelkers, P. and Dawson, P. A. (1995). Identification of a mutation in the ileal sodium-dependent bile acid transporter gene that abolishes transport activity. *J. Biol. Chem.* **270**, 27228–27234.

Xu, G., Salen, G., Shefer, S., Tint, G. S., Nguyen, L. B., Chen, T. S. and Greenblatt, D. (1999). Increasing dietary cholesterol induces different regulation of classic and alternative bile acid synthesis. *J. Clin. Invest.* **103**, 89–95.

Yeh, H. Z., Schteingart, C. D., Hagey, L. R., Ton-Nu, H. T., Bolder, U., Gavrilkina, M. A., Steinbach, J. H. and Hofmann, A. F. (1997). Effect of side chain length on biotransformation, hepatic transport and choleretic properties of chenodeoxycholyl homologues in the rodent: studies with dinorchenodeoxycholic acid, norchenodeoxycholic acid and chenodeoxycholic acid. *Hepatology* **26**, 374–385.

Zeng, H., Liu, G., Rea, P. A. and Kruh, G. D. (2000). Transport of amphipathic anions by human multidrug resistance protein 3. *Cancer Res.* **60**, 4779–4784.

Ziegler, K., Lins, W. and Frimmer, M. (1991). Hepatocellular transport of cyclosomatostatins: evidence for a carrier system related to the multispecific bile acid transporter. *Biochim. Biophys. Acta* **1061**, 287–296.