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Regulation of hepatic ABCC transporters by xenobiotics and in disease states

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Abstract

The subfamily of ABCC transporters consists of 13 members in mammals, including the multidrug resistance-associated proteins (MRPs), sulfonyleurea receptors (SURs), and the cystic fibrosis transmembrane conductance regulator (CFTR). These proteins play roles in chemical detoxification, disposition, and normal cell physiology. ABCC transporters are expressed differentially in the liver and are regulated at the transcription and translation level. Their expression and function are also controlled by post-translational modification and membrane-trafficking events. These processes are tightly regulated. Information about alterations in the expression of hepatobiliary ABCC transporters could provide important insights into the pathogenesis of diseases and disposition of xenobiotics. In this review, we describe the regulation of hepatic ABCC transporters in humans and rodents by a variety of xenobiotics, under disease states and in genetically modified animal models deficient in transcription factors, transporters, and cell-signaling molecules.

Keywords

ABCC transporters; multidrug resistance-associated protein (MRP); sulfonyleurea receptor (SUR); cystic fibrosis transmembrane conductance regulator (CFTR); xenobiotics; gene regulation; liver diseases

Introduction

The ABCC transporter subfamily belongs to the ATP-binding cassette (ABC) transporter family, which is one of the largest transporter superfamilies found in a variety of organisms ranging from bacteria to mammalian in the evolution tree. Transporters are machines of cell membranes that facilitate the movement of substances, such as uptake of nutrients and export of toxic metabolites. Transporters also generate and maintain electrical and chemical concentration gradients across generally impermeable cell membranes. The ATP-binding cassette transporter superfamily belongs to P-P-bond-hydrolysis-driven transporters

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Declaration of interest

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subclass and primary active transporters class (Busch and Saier, 2002; Saier et al., 2009). The ATP-binding cassette transporters bind and hydrolyze ATP to power the translocation of a diverse assortment of substrates, ranging from ions to macromolecules, across membranes in a thermodynamically unfavorable direction (Saurin et al., 1999; Rees et al., 2009). The ABC transporters have a characteristic structure that minimally consists of four domains: two transmembrane domains (TMDs) that are embedded in the membrane bilayer and two ATP-binding cassettes (nucleotide-binding domains, NBDs) that are located in the cytoplasm. At the sequence level, the superfamily of ABC transporters is identified by a characteristic set of highly conserved motifs that are present in the nucleotide-binding domains. By contrast, the sequences and architectures of the transmembrane domains are variable, reflecting the chemical diversity of the translocated substrates. Beyond these four domains, additional elements can fuse to the transmembrane and/or nucleotide-binding domains and probably have regulatory functions. Conserved nucleotide-binding domain is responsible for the binding and lysis of nucleotides to provide energy for transport. Variable transmembrane domain(s) form a channel. Human ABC transporters have been identified and sequenced. More than 48 human ABC transporters are divided into seven distinct subfamilies of proteins, based on amino-acid sequence similarities and phylogeny. These seven subfamilies are represented in all eukaryotic genomes and are, therefore, of ancient origin. Many human ABC transporters are involved in membrane transport of drugs, xenobiotics, endogenous substances, or ions; thereby human ABC transporters play important roles in human physiology, toxicology, pharmacology, and disease. Complete characterization of all ABC transporters from the human genome and from model organisms will lead to important insights into the physiology and molecular basis of many human disorders (Dean et al., 2001; Borst and Elferink, 2002; Dean and Annilo, 2005; Szakacs et al., 2008; Toyoda et al., 2008).

The ABCC subfamily has 13 members in mammals and comprises three classes, including multidrug resistance-associated proteins (MRPs), sulfonyleurea receptors (SURs), and the cystic fibrosis transmembrane conductance regulator (CFTR). The largest group of ABCC transporters is MRPs, which contain 10 members, including MRP1/ABCC1, MRP2/ABCC2, MRP3/ABCC3, MRP4/ABCC4, MRP5/ABCC5, MRP6/ABCC6, MRP7/ABCC10, MRP8/ABCC11, MRP9/ABCC12, and MRP10/ABCC13. ABCC7, is the cystic fibrosis transmembrane conductance regulator (CFTR). ABCC8 and ABCC9 are the sulfonyleurea receptors that constitute the ATP-sensing subunits of a complex potassium channel. The extensive conservation of most ABCC genes suggests that the functions of the ABCC genes are critical to mammals. In addition to having relatively nonspecific roles in the removal of toxic metabolites and xenobiotics, the ABCC genes likely play important roles in normal cell physiology.

The ABCC transporters are expressed differentially in the liver, kidney, intestine, brain, and other tissues. These transporters are localized to the apical and/or basolateral membrane of the hepatocytes, enterocytes, renal proximal tubule cells, and endothelial cells of the blood-brain barrier. Thus, these transporters may have diverse functions that include ion transport, cell-surface receptors, and toxin-secretion activities. MRP members transport a structurally diverse array of important endogenous substances and xenobiotics and their metabolites with different substrate specificities and transport kinetics. Most MRP transporters are able to

export organic anions, such as drugs conjugated to glutathione, sulphate, or glucuronate. In addition, selected MRP/ABCC members may transport a variety of endogenous compounds, such as leukotriene C₄ (LTC₄) (MRP1/ABCC1), bilirubin glucuronides (MRP2/ABCC2 and MRP3/ABCC3), prostaglandins E₁ and E₂ (MRP4/ABCC4), cGMP (MRP4/ABCC4, MRP5/ABCC5, and MRP8/ABCC11), and several glucuronosyl or sulfatidyl steroids. The MRP transporters are associated with tumor resistance to natural product anticancer drugs and their conjugated metabolites and other anticancer drugs, such as platinum compounds, folate antimetabolites, nucleoside and nucleotide analogs, arsenical and antimonial oxyanions, peptide-based agents, and in concert with alterations in phase II conjugating or biosynthetic enzymes and alkylating agents (Borst et al., 2000; Bodo et al., 2003; Yu et al., 2007b; Zhou et al., 2008).

The activity of ABCC transporters is also subject to regulation by inhibition by a variety of compounds. Modulation of MRP function may represent a useful approach in the management of drug resistance and understanding diseases. A better understanding of specificity of substrates and inhibitors, as well as function and different mechanism of regulation of MRPs can help in the development of drugs for treatment of diseases, minimize and avoid drug toxicities, unfavorable drug-drug interactions, to overcome drug resistance, and promoting treatment of diseases (Yu et al., 2007b; Zhou et al., 2008).

Normal ABCC transporters are expressed through transcription, translation, post-translational modification, and traffic to membranes of polar cells, including cells in liver, to exert their efflux functions. The process is tightly regulated in a temporal- and spacial-dependent manner.

The localization and expression levels of ABCC transporters play an essential role in the regulation of function. Adaptive changes in transporter expression in the liver provide excretory pathways for substances important for biological process. Information about alterations in hepatobiliary transporter expression in diseases and exposure to xenobiotics could provide important insights into the pathogenesis of diseases and mechanism of detoxification and disposition of xenobiotics. In this review, we have described the regulation of hepatic ABCC transporters by xenobiotics and under disease states.

Regulation of hepatic ABCC1/MRP1 transporter by xenobiotics and in disease states

Function of MRP1/Mrp1

MRP1 (ABCC1) has a broad substrate specificity, including neutral hydrophobic compounds, and numerous glutathione, glucuronate, and sulfate conjugates. The substrate-binding sites in MRP1 protein permit both cooperativity and competition between various substrates. MRP1 can be found in the lung, testis, kidney, heart, and placenta at high levels, whereas its expression in the small intestine, colon, brain, and peripheral blood mononuclear cells is moderate to low. MRP1 is involved in various physiological and pharmacological functions, including defense against xenobiotics and endogenous toxic metabolites, leukotriene-mediated inflammatory responses, protection from toxic effect by oxidative stress, and efflux of clinical drugs. An overview of the considerable amount of knowledge

accumulated since the discovery of MRP1 in 1992 can be obtained elsewhere (Bakos and Homolya, 2007).

MRP1 is expressed in normal liver at very low levels, suggesting that MRP1 is not a major transporter in the liver for xenobiotic metabolism and detoxification. Below, we review the regulation of hepatic MRP1 gene expression by xenobiotics and under disease states.

MRP1/Mrp1 expression in normal liver

In the human liver, MRP1 mRNA and protein are relatively lowly expressed (Zaman et al., 1993; Hinoshita et al., 2001; Ros et al., 2003a). They are detected in the basolateral membrane of hepatocytes and the basolateral pole of bile ducts (Roelofsen et al., 1997; Payen et al., 2000; Vander Borgh et al., 2008b).

In the rat liver, Mrp1 mRNA and protein expression levels are also low (Ogawa et al., 2000; Cao et al., 2002; Cherrington et al., 2002; Ros et al., 2003b; Kuroda et al., 2004; Lu and Klaassen, 2008). During development, in Sprague-Dawley (SD) rats, Mrp1 mRNA levels decrease gradually from birth to the adult liver (de Zwart et al., 2008). However, in the Wistar rat liver, Mrp1 transcripts expression does not change from the fetal to adult liver (Rosati et al., 2003; Garrovo et al., 2006), while it decreases significantly in aging rats (Rosati et al., 2003). During pregnancy, in SD rats, liver Mrp1 mRNA and protein are not altered at gestational days 20–21 (Cao et al., 2002). In primary rat hepatocytes in culture, Mrp1 mRNA and protein levels increase after 24 hours during a 72-hour culturing period (Rippin et al., 2001). Further, in Wistar-Kyoto (WK) rats, there are no gender differences in Mrp1 mRNA expression (Merrell et al., 2008).

In mouse liver, hepatic Mrp1 mRNA and protein expression levels are also low (Maher et al., 2005a, 2005b; Tanaka et al., 2009), with higher expression in females than males (Maher et al., 2005b). By contrast, canine hepatic Mrp1 expression levels are relatively high, which is different from that of human and rodents (Conrad et al., 2001).

Regulation of hepatic MRP1/Mrp1 expression by xenobiotics

In both human and cynomolgus monkey hepatocytes, MRP1 mRNA levels are shown to increase by rifampicin or omeprazole treatment and tend to decrease with dexamethasone treatment (Nishimura et al., 2008).

In acetaminophen-overdose patients, MRP1 mRNA, but not protein, increases significantly, compared to normal livers (Barnes et al., 2007). In male C57BL/6J mice, Mrp1 mRNA and protein levels are induced significantly in both wild-type and *Nrf2*-knockout mice treated with acetaminophen. This suggests that Mrp1 induction by acetaminophen is independent of Nrf2. Further, Kupffer cell depletion prevents Mrp1 induction by acetaminophen treatment (Aleksunes et al., 2005, 2006, 2007, 2008b; Campion et al., 2008).

In mice and rats, carbon tetrachloride increases liver Mrp1 expression shortly after treatment (Aleksunes et al., 2005, 2006; Okumura et al., 2007). Treatment of C57BL/6J mice with the periportal hepatotoxicant, allyl alcohol, also increases Mrp1 mRNA and protein. The observed changes are independent of Kupffer cell function (Campion et al., 2009). In mouse

liver, Mrp1 mRNA levels increase during the first 3–6 hours after oral administration of 200 mg/kg of the phenolic antioxidant, butylated hydroxyanisole (Hu et al., 2006).

In C57BL/6 mice, hepatic Mrp1 mRNA expression is shown to decrease by ciprofibrate, diethylhexylphthalate, or butylated hydroxyanisole treatment (Maher et al., 2005a), but does not change by trans-stilbene oxide after 4 days of treatment (Slitt et al., 2006a). In male CD-1 mice, Mrp1 mRNA does not change after 10 days of clofibrate dosing (Moffit et al., 2006).

In SD rats, liver Mrp1 mRNA increases significantly by pregnenolone 16a-carbonitrile treatment (Cherrington et al., 2002). However, rat Mrp1 mRNA expression does not change by treatment with oltipraz, phenobarbital, trans-stilbene oxide, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), indole-3-carbinol, β -naphthoflavone, polychlorinated biphenyl 126, polychlorinated biphenyl 99, diallyl sulfide, spironolactone, dexamethasone, clofibric acid, diethylhexylphthalate, perfluorodecanoic acid, ethoxyquin, isoniazid, acetylsalicylic acid, or streptozotocin (Cherrington et al., 2002; Slitt et al., 2006b; Merrell et al., 2008). In Wistar rats, antidepressants or low doses of St. John's wort (*Hypericum perforatum*; SJW) does not change Mrp1 expression. However, higher doses of St. John's wort decrease Mrp1 expression in fetal livers (Shibayama et al., 2004; Garrovo et al., 2006). In C57BL/6 mice, liver Mrp1 mRNA does not change significantly by phenobarbital, oltipraz, or 1,4-bis-[2-(3,5-dichloropyridyloxy)] benzene (TCPOBOP) treatment for 3 days (Beilke et al., 2008).

In 3-week-old male SD rats fed fat-free total parenteral nutrition for 4 days, liver Mrp1 mRNA levels increased significantly (Nishimura et al., 2005). In male SD rats fed docosahexaenoic acid alone or docosahexaenoic acid in combination with low-ingestion vitamin E (docosahexaenoic acid-lowVE) for 14 days, liver Mrp1 mRNA and protein levels tended to be higher than that in linoleic-acid-treated rats (Kubo et al., 2006).

In activated human or rat hepatic stellate cells, Mrp1 expression is higher than in hepatocytes. Mrp1 protein staining is correlated positively with the hepatic stellate cell marker, desmin staining, in livers from carbon-tetrachloride-treated rats. Cytokines and oxidative stress do not change Mrp1 expression in hepatic stellate cells (Hannivoort et al., 2008).

Regulation of hepatic MRP1/Mrp1 expression in diabetes and chronic kidney disease

In SD rats with type 2 diabetes or 5/6 nephrectomy-induced chronic kidney disease, liver Mrp1 expression does not change significantly (Lu and Klaassen, 2008; Nowicki et al., 2008). However, in ob/ob female mice, Mrp1 mRNA and protein levels are decreased, in comparison to wild types. Mrp1 mRNA expression is not different between male ob/ob and wild-type mice, while protein expression is significantly higher in ob/ob males than in wild types (Cheng et al., 2008).

Regulation of hepatic MRP1/Mrp1 expression during hemolysis

In rats with increased bilirubin production due to hemolysis by phenylhydrazine, Mrp1 mRNA and protein levels are increased and reach a peak at 3 days after treatment. Pretreatment with the hemeoxygenase inhibitor, tin mesoporphyrin IX (SnMP), prevents

overexpression of Mrp1 and blocks the increase in serum unconjugated bilirubin. Thus, upregulation of Mrp1 during hemolysis is mediated by serum-unconjugated bilirubin and/or other products of hemeoxygenase metabolism, further supporting a role for Mrp1 in serum-unconjugated bilirubin transport and protection from its cellular toxicity through adaptive induction (Cekic et al., 2003).

Regulation of hepatic MRP1/Mrp1 expression in cancers and tumors

MRP1 mRNA expression in hepatocellular carcinomas has been determined to be 1/10 of that for MRP2 and MRP3, with levels higher than that in the normal liver (Nies et al., 2001). In cancerous human livers, MRP1 mRNA levels are similar between metastatic tumors and hepatocellular carcinomas, and between cancerous and noncancerous portions of livers from patients with hepatocellular carcinomas (Hinoshita et al., 2001; Bonin et al., 2002; Moustafa et al., 2004). In hepatocellular carcinomas, MRP1 mRNA and protein levels increase, in comparison to surrounding nontumor tissue. These changes are well associated with tumor differentiation grade and microvascular invasion (Vander Borgh et al., 2008a). MRP1 is diffusely expressed in the basolateral membrane of tumor cells and localized in intracellular membranes of carcinoma cells. MRP1 staining is negative in tumor cells of all K19-negative hepatocellular carcinoma cases (Nies et al., 2001; Komuta et al., 2008). High MRP1 expression in hepatocellular carcinomas has been associated with poor survival (Vander Borgh et al., 2008a).

In the “hepatoblast subtype” of hepatocellular carcinomas, thought to be derived from hepatic progenitor cells, MRP1 mRNA and protein levels are also significantly increased (Vander Borgh et al., 2008a). In hepatoblastomas, the pattern and intensity of MRP1 expression are very similar to normal livers (Vander Borgh et al., 2008b). In surrounding nonlesional parenchyma, MRP1 protein is expressed in the basolateral membrane of the interlobular bile ducts and hepatic progenitor cells and reactive ductules (Vander Borgh et al., 2008a). MRP1 expression is increased in cholangiolocellular carcinoma. In cholangiolocellular carcinoma areas, tumor cells show diffuse basolateral MRP1 expression. The intensity of staining is stronger, in comparison to non-neoplastic hepatic progenitor cells and ductular reactions (Komuta et al., 2008).

Regulation of hepatic MRP1/Mrp1 expression in cholestasis

In patients with progressive familial intrahepatic cholestasis type 3 (PFIC-3), hepatic MRP1 mRNA levels are significantly increased, but not in PFIC-2 patients (Keitel et al., 2005). In patients with primary biliary cirrhosis, MRP1 mRNA and protein expression levels are increased, in comparison to normal patients (Ros et al., 2003a; Barnes et al., 2007), while in patients with liver cirrhosis or chronic extrahepatic cholestasis, MRP1 expression does not change (Bonin et al., 2002).

In neonatal rats from mothers with obstructive cholestasis during pregnancy, liver Mrp1 mRNA levels are found to be elevated. Liver Mrp1 mRNA expression in neonates is even higher when rats are treated with ursodeoxycholic acid during pregnancy (Macias et al., 2006).

In rats and mice undergoing bile duct ligation, liver Mrp1 mRNA and protein increase at 3–6 days after surgery, compared to sham-operated animals. Mrp1 protein is increased in hepatocyte basolateral membrane and cytoplasm at 4 days after bile duct ligation (Pei et al., 2002; Stedman et al., 2006; Lickteig et al., 2007b; Slitt et al., 2007).

Mrp1 protein expression is increased by treatment of mice with lithocholic acid for 4 days or in mice pretreatment with oltipraz for 3 days prior to cotreatment with lithocholic acid and oltipraz for additional 4 days. Similar cotreatments of mice with phenobarbital/lithocholic acid and TCPOBOP/lithocholic acid do not change significantly Mrp1 expression (Beilke et al., 2008). In CD-1 mice, administration of conjugated and unconjugated bile acids does not alter Mrp1 mRNA expression to any remarkable extent by 24 hours after treatment (Hartmann et al., 2002).

Regulation of hepatic MRP1/Mrp1 expression in response to inflammatory process and infection

In human livers infected with hepatitis viruses, MRP1 expression is reduced, compared to noninfected livers (Hinoshita et al., 2001). In patients with hepatitis C infection, MRP1 mRNA expression is increased, in comparison to noninfected patients (Ros et al., 2003a).

In SD rats treated with lipopolysaccharide (LPS), liver Mrp1 mRNA expression is increased. Pretreatment with dexamethasone prevented LPS-mediated increases in Mrp1, whereas pretreatment with aminoguanidine or gadolinium chloride, an inhibitor of inducible nitric oxide synthetase and Kupffer cell function, respectively, has no effect on LPS-induced Mrp1 changes (Cherrington et al., 2004). In rats, liver Mrp1 mRNA and protein are increased at 6 hours after endotoxin administration. This was confirmed in isolated hepatocytes (Vos et al., 1998). Treatment of CD-1 mice with LPS- and tumor necrosis factor- α (TNF- α) does not change significantly liver Mrp1 mRNA at 6 hours. Treatment with interleukin (IL)-1 β or IL-6 decreases Mrp1 mRNA at 6 hours after treatment. Treatment with all three cytokines in combination does not significantly alter Mrp1 mRNA expression (Hartmann et al., 2002). In C57BL/6J mice treated with LPS for 16 hours, liver Mrp1 mRNA levels are induced (Lickteig et al., 2007b).

Regulation of hepatic MRP1/Mrp1 expression during partial hepatic ischemia

In Male SD rats, partial hepatic ischemia for 60 minutes and reperfusion do not alter basolateral transporter Mrp1 mRNA and protein expression for up to 48 hours after reperfusion (Tanaka et al., 2008).

Regulation of hepatic MRP1/Mrp1 expression in fatty liver

In male SD rats with nonalcoholic steatohepatitis (NASH)-induced by feeding a methionine- and choline-deficient (MCD) diet for 8 weeks, or in simple fatty liver (SFL) induced by feeding a high-fat (HF) diet for 8 weeks, liver Mrp1 mRNA levels do not change (Lickteig et al., 2007a).

Regulation of hepatic MRP1/Mrp1 expression during liver damage and regeneration

In patients with submassive liver necrosis, MRP1 mRNA expression is found to increase, as compared to patients with normal livers. Strong MRP1 reactivity is seen in regenerating human bile ductules at the interface between portal tracts and necrotic areas (Ros et al., 2003a).

In Wistar rats, partial hepatectomy increases hepatic Mrp1 mRNA levels. This induction lasts at least 48 hours (Vos et al., 1999; Ros et al., 2003b). However, Mrp1 mRNA levels return to normal values 9 days after surgery. Administration of 2-acetylaminofluorene along with partial hepatectomy increases liver Mrp1 mRNA levels significantly in Wistar rats even at 9 days after surgery and treatment. In this model, Mrp1 protein expression is strongly localized in periportal progenitor cells (Ros et al., 2003b).

Regulation of hepatic MRP1/Mrp1 expression in genetic disease and gene-knockout animal models

In Eisai hyperbilirubinuria rats, which are *Mrp2*-deficient, hepatic Mrp1 mRNA levels are not significantly different from normal SD rats (Kuroda et al., 2004). In male or female *Mrp2*-knockout mice, Mrp1 mRNA levels are expressed in very low levels (Chu et al., 2006).

In *Mrp6*-knockout mice, Mrp1 mRNA levels are not significantly different from wild types (Li et al., 2007). In *Hnf1 α* -knockout and *Nrf2*-knockout mice, Mrp1 mRNA levels are not significantly different from their respective wild types, either (Maher et al., 2006b; Aleksunes et al., 2008b). In *Tnfr1*-knockout, *Il1r1*-knockout, and *Il6*-knockout mice, hepatic Mrp1 mRNA does not change, in comparison to in wild types, after saline treatment. LPS treatment significantly increases Mrp1 mRNA expression in all these mice (Lickteig et al., 2007b).

In *Fxr*-knockout, *Pxr/Fxr*-double knockout, *Tnfr1*-knockout, *Il1r1*-knockout, and *Il6*-knockout mice, Mrp1 mRNA and protein expression levels are increased at 3–6 days after bile duct ligation, compared to their sham-operated counterparts. Mrp1 protein is increased in both hepatocyte basolateral membrane and cytoplasm (Pei et al., 2002; Stedman et al., 2006; Lickteig et al., 2007b; Slitt et al., 2007).

In liver-specific I κ B kinase β (IKK β)-deficient (*IKK β* hep) mice, hepatic Mrp1 mRNA levels are significantly higher than in *IKK β* -floxed [*IKK β /f/f*] mice after saline treatment or sham operation of bile duct ligation. LPS treatment increases hepatic Mrp1 mRNA levels in both strains (Lickteig et al., 2007b).

Regulation of hepatic MRP1/Mrp1 expression in cell lines by xenobiotics

In HepG2 cells, MRP1 is expressed at low levels, but is higher than in human livers (Pascolo et al., 2000; Lee and Piquette-Miller, 2001; Pascolo et al., 2001; Schrenk et al., 2001; Teng et al., 2003; Hilgendorf et al., 2007). In nonconfluent HepG2 cells lacking cell-cell contact, MRP1 protein is absent in plasma membrane, while in confluent cells, MRP1 is localized to lateral membranes. The presence of a lateral domain seems necessary for MRP1

plasma-membrane localization. In the lateral membrane of proliferating hepatocytes, MRP1 protein levels are greatly increased (Roelofsen et al., 1997). In HepG2 cells, MRP1 mRNA expression levels are increased by IL-6 treatment (Lee and Piquette-Miller, 2001), while MRP1 protein levels are induced by sulforaphane and erucin treatment in a dose-dependent manner (Harris and Jeffery, 2008). However, in HepG2 cells treated with the glutathione-depleting agents, buthionine sulfoximine or t-butylhydroquinone, MRP1 mRNA does not change (Lee et al., 2001).

In the hepatocellular carcinoma cell line, Huh7, cells, MRP1 is nondetectable (Teng et al., 2003). In Huh7 cells that survive toxic cisplatin exposure, MRP1 does not change (Wakamatsu et al., 2007), while in the cisplatin-resistant human liver carcinoma cell line, 7404-CP20, and in another cisplatin-resistant cell line, KB-CP20, MRP1 protein expression is considerably reduced (Shen et al., 2000). Mrp1 mRNA levels are higher in the human hepatocellular carcinoma cell line, BEL-7402/5-FU, cells than in BEL-7402. In BEL-7402/5-FU cells, treatment with a tripeptide tyroservatide (YSV) does not alter MRP1 mRNA expression, but reduces MRP1 protein (Shi et al., 2008). In five human intrahepatic cholangiocarcinoma cell lines (KKU-100, KKU-M055, KKU-M156, KKU-M214, and KKU-OCA17), MRP1 mRNA is highly expressed (Tepsiri et al., 2005).

In Hepa 1–6 cells treated with the bile acids, taurocholate and cholic acid, both mRNA levels for Mrp1 and small heterodimer partner (SHP) are induced. Treatment of Hepa 1–6 cells with IL-1 β increases Mrp1 mRNA expression in a dose-dependent manner, while no changes in Mrp1 mRNA levels are detected with TNF- α or IL-6 treatment (Hartmann et al., 2002).

In unpolarized rat hepatoma Fao cells, Mrp1 is expressed in much higher levels than in normal rat liver. Treatment with 50 μ M of taurocholic acid, chenodeoxycholic acid, or ursodeoxycholic acid for 6 days does not significantly change Mrp1 gene expression in Fao cells or Can 3-1 and Can10 cells, which are derived from the Fao cell line. This concentration of bile acids is similar to the serum total bile-acid concentrations reached in the liver parenchyma in situations of mild cholestasis (Cassio et al., 2007). In the hepatocytoma fusion cell line, HPCT-1E3, derived from primary rat hepatocytes and Fao Reuber hepatoma cells (H35), Mrp1 expression profile is almost identical to that found in normal rat liver (Halwachs et al., 2005). In the rat hepatoma/human fibroblast hybrid cell line, WIF-B9, rat Mrp1 mRNA is highly expressed, in comparison to normal rat liver. Human MRP1 expression is not detected in WIF-B9 cells (Briz et al., 2007).

In rat small hepatocytes, which are hepatocyte progenitor cells of the adult liver, Mrp1 mRNA levels are increased approximately 2-fold during 5–9 weeks in culture (Sidler Pfandler et al., 2004). Similarly, MRP1 mRNA expression is higher in hepatic oval cells than rat hepatocytes (Zhang et al., 2007a). In freshly isolated Thy-1-positive cells, cholangiocytes and cultured RLF phi 13 progenitor cells, Mrp1 mRNA is highly expressed, while it is undetectable in freshly isolated rat hepatocytes (Ros et al., 2003b).

Mechanism of regulation of hepatic MRP1/Mrp1 expression

The Human MRP1 gene 5'-flanking sequence was first isolated from HL60/ADR cells and the organization of the 5'-end region of MRP in the genome has been defined. Transcription activity analysis, based on a 2.2-kilobase (kb) sequence, shows that the promoter activity is contained in a nucleotide sequence located from -91 to +103 bp in a GC-rich region and is modulated by both positive and negative regulatory elements. A number of regulatory elements for AP1, AP2, and SP1 and ERE, GRE, CRE, PRE, and NE1 are found in the promoter-proximal region. Basal activity of promoter is regulated by at least one SP1-type binding site, and SP1 is one of the components that binds directly to the sequence containing the sites (Zhu and Center, 1994, 1996). All three promoters of human, rat, and mice MRP1/Mrp1 genes reside in a CpG island and are GC-rich, TATA-less, and CAAT-less. Sequence conservation between rodent and human promoters is limited to a proximal region of 100 nucleotides, containing binding sites for members of the SP1 family, and a putative activator protein-1 (AP1) element. Transient transfection analysis demonstrates that the conserved GC-boxes of all three genes are the major determinants of basal activity. Each GC-box can be bound by SP1 or SP3, based on electrophoretic mobility-shift assays. The 5'-untranslated region (UTR) of human MRP1 contains an insertion of approximately 160 nucleotides comprising a GCC-triplet repeat and a GC-rich tandem repeat that is absent from the rodent sequences. Unlike the rodent genes, the human MRP1 5'-UTR also binds SP1, but not SP3, and the human promoter retains substantial activity even in the absence of the conserved GC-boxes (Muredda et al., 2003). Besides, positive *cis*-acting elements are located between -91 and -411 bp of the MRP1 promoter, adding more activity to the promoter of MRP1 gene. Negative elements are present between -1,123 and -2,008 bp of the promoter. Both positive and negative elements are located between -411 and -1,123 bp (Wang and Beck, 1998). Using nuclear extracts and a 1-kb region encompassing the 5' flanking region of MRP1 in deoxyribonuclease I footprinting and gel mobility-shift assays, 13 protein-binding sites were identified and six of them are sequence specific (Kurz et al., 2001). Three E-boxes have been identified in the promoter region of human MRP1 gene and they mediate activation of MRP1 by MYCN in neuroblastoma cells (Manohar et al., 2004).

A potential AP1-like antioxidant response element (ARE)/AP1 binding site at -511 to -477 bp has been isolated from the human MRP1 gene. Although this element behaves as a classic enhancer when introduced into the upstream region of a minimal promoter and is able to bind several members of the c-Jun family of transcription factors, it does not mediate an oxidative response in the context of the MRP1 promoter (Kurz et al., 2001).

Studies show that MRP1 gene expression is suppressed by p53 and activated by mutant p53. The inhibitory effect on the promoter activity by wild-type p53 is mainly associated with the region from -91 to +103 bp, where several SP1 transcription-factor-binding sites are localized (Wang and Beck, 1998; Sullivan et al., 2000). However, the promoter does not contain any identifiable potential p53-binding sites, and it has been suggested that suppression may be exerted through an SP1-mediated mechanism (Wang and Beck, 1998). More recently, Muredda et al. showed that the tumor-suppressor protein, p53, can repress the human and rodent MRP1/Mrp1 promoters by a mechanism that was independent of the SP1 elements (Muredda et al., 2003).

MRP1 gene and protein expression in HepG2 cells exhibits hypoxic time-dependent induction and is synchronous with alterations in hypoxia-inducible factor-1- α (HIF-1- α). Overexpression of HIF-1- α results in MRP1 induction, suggesting that HIF-1- α is involved in MRP1 gene expression in response to hypoxia. HIF-1- α might also mediate the induction of MRP1 gene expression by the activation of the ERK/MAPK pathway (Zhu et al., 2005, 2007).

Studies with specific agonists for the nuclear receptors, pregnane X receptor (PXR), constitutive androstane receptor (CAR), peroxisome proliferator-activated receptor alpha (PPAR- α) and farnesoid X receptor (FXR), as well as AhR, along with studies using gene-knockout animals, have shown that only PXR might be involved in the regulation of MRP1/Mrp1 gene expression, as demonstrated by the inducing effects of rifampicin and pregnenolone 16 α -carbonitrile.

Hayashi et al. demonstrated that Nrf2 was required for the constitutive and inducible expression of Mrp1 in mouse embryo fibroblasts (Hayashi et al., 2003). Studies with Nrf2-specific activators and *Nrf2*-knockout mice indicate that Nrf2 is not involved in the regulation of hepatic Mrp1 gene expression in response to oxidative stress resulting from toxic acetaminophen and carbon tetrachloride treatment, and that other factors are responsible for Mrp1 induction by these hepatotoxicants.

MRP1 mRNA and protein levels are increased concomitantly with the disappearance of MRP2 in hepatocytes, mainly in the G1 phase (high density) and hepatocytes that have progressed into the S-phase or beyond (low density) (Roelofsen et al., 1999). MRP1 is highly expressed in hepatic progenitor cells, and its expression is associated with liver regeneration. MRP1 protein levels are greatly increased in the lateral membrane of proliferating hepatocyte-derived cells. These observations suggest that MRP1 might be associated with cell proliferation and differentiation during liver regeneration.

Studies using specific chemical inhibitors or activators, including U0126 and butylated hydroxyanisole, show that the ERK/MAPK and c-Jun N-terminal kinase (JNK) pathways are involved in MRP1 gene regulation in HepG2 cells (Shinoda et al., 2005; Hu et al., 2006; Zhu et al., 2007)

Mrp1 expression is increased in hepatitis C virus-infected liver or endotoxin treatment, but not by exposure to individual cytokines. This suggests that Mrp1 is regulated by pathways directly mediating infection and inflammatory responses.

Less is known about MRP1/Mrp1 regulation by post-translational modifications, although MRP1 has also been shown to be phosphorylated primarily on serine residues (Ma et al., 1995).

Conclusions

Hepatic MRP1/Mrp1 expression is low in normal humans and rodents, but high in the canine liver. MRP1/Mrp1 is expressed in hepatocytes and the basolateral pole of bile ducts, and its basal expression is regulated developmentally. MRP1/Mrp1 is also regulated in a gender-

dependent manner. MRP1/Mrp1 expression is induced by PXR ligands, but not by AhR, CAR, PPAR- α , or FXR ligands. MRP1/Mrp1 expression also increases during cholestasis, hepatitis C virus infection, endotoxin exposure, hemolysis, liver cancers and tumors, and liver regeneration, as well as during oxidative stress resulting from hepatotoxicant treatment. MRP1/Mrp1 expression does not change in fatty liver, diabetes, and chronic kidney disease in rats, during partial hepatic ischemia-reperfusion, or in *Mrp2*⁻, *Mrp6*⁻, *Hnf1 α* ⁻, *Nrf2*⁻, *Tnfr1*⁻, *Il6*⁻, *Il1r1*⁻, *Fxr*⁻, and *Pxr*-knockout mice. Regulation of changes in MRP1/Mrp1 expression is more common in conditions and treatment resulting in cell proliferation and differentiation than in response to xenobiotic exposure.

The MRP1 gene promoter is GC-rich, TATA-less, and CAAT-less. It contains a number of regulatory elements for AP1, AP2, and SP1 and E-box, ARE, ERE, GRE, CRE, PRE, and NE-1. SP1 contributes to basal promoter activity. Transcription factors MYCN, p53, HIF-1- α , PXR, and Nrf2 are involved in MRP/Mrp1 gene expression, while the involvement of CAR, PPAR- α , and FXR is minimal. MRP/Mrp1 gene expression is also regulated by the ERK/MAPK and c-Jun N-terminal kinase (JNK) pathways and during cell cycling. The human, rat, and mouse MRP1/Mrp1 gene promoters have limited homology across species.

The mechanisms for the regulation of MRP1/Mrp1 gene expression in the different disease states and in tissue- and species-specific expression remain to be investigated further.

Regulation of hepatic ABCC2/MRP2 transporters by xenobiotics and in disease states

Function of MRP2/Mrp2

MRP2 localizes exclusively to the apical membrane domain of polarized cells as hepatocytes, renal proximal tubule epithelia, enterocytes, and syncytiotrophoblasts of the placenta. Substrates for MRP2 include a wide range of compounds, such as conjugates of lipophilic substances with glutathione, glucuronate, or sulfate, as exemplified by leukotriene C₄, bilirubin glucuronosides, and some steroid sulfates. In addition, MRP2 can also transport uncharged compounds in cotransport with glutathione and thus can modulate the pharmacokinetics of many drugs. The localization and spectrum of substrates of MRP2 render its function in the terminal excretion and detoxification process for endogenous and xenobiotic organic anions. Several naturally occurring mutations lead to the absence of functional MRP2 protein from the apical membrane of hepatocytes. This is the genetic basis of a human condition known as Dubin-Johnson syndrome, which is associated with conjugated hyperbilirubinaemia due to improper biliary excretion (Taniguchi et al., 1996). MRP2 is present in the canalicular membrane of hepatocytes and is known to be a major transporter of organic anions from the liver into bile. Hepatic MRP2 is an important driving force in bile formation and flow. Deficiency of MRP2 causes an increased concentration of bilirubin glucuronosides in blood due to basolateral egress in the absence of proper biliary elimination. Knowledge on molecular characteristics, functions, and the clinical relevance of MRP2 can be found in several reviews (Borst et al., 2006; Jedlitschky et al., 2006; Nies and Keppler, 2007).

MRP2/Mrp2 expression in normal liver

Human MRP2 is highly expressed in the liver, in comparison to other organs and tissues (Kool et al., 1997; Payen et al., 2000; Hilgendorf et al., 2007). In the liver, MRP2 is predominantly expressed in hepatocytes and localized to their apical membrane domains (Keppler and König, 1997), where it shows its typical canalicular staining (Ros et al., 2003a). This pattern is constant across species. MRP2 expression levels are highly variable interindividually (Meier et al., 2006). MRP2 is also expressed at substantial levels in cultured human hepatocytes (Payen et al., 2000; Jigorel et al., 2006). MRP2/Mrp2 protein levels in freshly isolated hepatocytes are comparable with those in the intact liver (Li et al., 2009).

Among rats, dogs, monkeys, and humans, the relative hepatic MRP2/Mrp2 protein levels differ with rats>monkeys>dogs and humans (Li et al., 2009). Low hepatic canine MRP2 expression levels have been reported (Conrad et al., 2001). In mice, Mrp2 expression does not differ between gallstone-susceptible C57L/J and gallstone-resistant AKR/J strains (Muller et al., 2002).

In rodents, regardless of species and strains, including Wistar, SD, and Fisher 344 rats and C57L/J and AKR/J mice, hepatic Mrp2 mRNA is highly expressed (Cherrington et al., 2002; Muller et al., 2002; Ros et al., 2003b; Cizkova et al., 2005; Maher et al., 2005b; Garrovo et al., 2006). In rats, much more Mrp2 has been imaged in periportal than in perivenous areas within the liver acinus (Micuda et al., 2008). Mrp2 protein tends to a decrease in the rat-liver slices or hepatocytes during incubation after liver perfusion and cell isolation (Rippin et al., 2001; Aoki et al., 2008b). However, Mrp2 mRNA shows a transient upregulation at 24 and 48 hours during incubation of hepatocyte, suggesting that transcriptional and post-translational regulation occurs during the culture of primary cells (Rippin et al., 2001). Cryopreservation of hepatocyte decreases Mrp2 protein expression in membranes (Li et al., 2009).

In SD rats, hepatic Mrp2 expression is higher in females than in males (Rost et al., 2005; Simon et al., 2006; Suzuki et al., 2006; de Zwart et al., 2008), while in WK rats, liver Mrp2 mRNA expression does not differ between genders (Merrell et al., 2008). In C57BL/6 mice, basal hepatic Mrp2 expression in females is about 2-fold higher than in males (Petrick and Klaassen, 2007).

During development, human liver MRP2 is expressed at midgestational age and in the 14-week fetal liver. The mean fetal MRP2 expression is 30–50% of the adult. Human MRP2 protein size is the same between fetal and adult livers. In the fetal liver, MRP2 shows lessclear canalicular staining, compared to the adult, which shows its characteristic sharp-linear canalicular staining (Chen et al., 2005; Cizkova et al., 2005). In SD rats, Mrp2 mRNA and protein are expressed at low levels before birth. Weak Mrp2 immunofluorescence is observed only in livers at embryonic day 16 (E16) (Zinchuk et al., 2002; Gao et al., 2004). After birth, both liver Mrp2 mRNA and protein increase in an age-dependent manner in both genders. However, the temporal and magnitude of these increases are different. Transcriptional and post-transcriptional mechanisms for Mrp2 regulation occur during

development (Johnson et al., 2002a; Zinchuk et al., 2002; Tomer et al., 2003; Cizkova et al., 2005; de Zwart et al., 2008).

In Wistar rats, Mrp2 mRNA expression is increased from the fetal to adult liver (Rosati et al., 2003; Garrovo et al., 2006). In SD rats, liver Mrp2 protein levels are decreased during pregnancy (Cao et al., 2002). In C57BL/6 mice, Mrp2 mRNA expression is increased markedly from 2 days before birth to parturition and remains relatively constant thereafter (Maher et al., 2005b).

Regulation of hepatic MRP2/Mrp2 expression by xenobiotics

Treatment of patients with gallstones and male and female rhesus monkeys with rifampicin increases hepatic MRP2 mRNA and protein expression (Kauffmann et al., 1998; Marschall et al., 2005). MRP2 protein is induced in a gender-dependent manner (Kauffmann et al., 1998). Similarly, treatment of human and cynomolgus monkey hepatocytes, and human liver slices with omeprazole or rifampicin, increases MRP2 expression (Jigorel et al., 2006; Nishimura et al., 2008; Olinga et al., 2008). In SD rats, treatment with pregnenolone 16a-carbonitrile does not markedly change Mrp2 mRNA levels in liver, compared to controls, while it significantly increases Mrp2 protein levels in a time-dependent manner. As early as 10 days of age, pregnenolone 16a-carbonitrile treatment increases Mrp2 protein expression in liver, whereas it does not change mRNA ontogeny. Pregnenolone 16a-carbonitrile increases Mrp2 protein by a gender-independent post-transcriptional mechanism. Analysis of Mrp2 mRNA is not always a good indicator for Mrp2 protein expression *in vivo* (Cherrington et al., 2002; Johnson et al., 2002a, 2002b; Johnson and Klaassen, 2002). In BALB/c mice, treatment with pregnenolone 16a-carbonitrile significantly increases hepatic Mrp2 mRNA levels (Han and Sugiyama, 2006). Treatment of C57/BL-6 mice also with pregnenolone 16a-carbonitrile or RU486 significantly increases liver Mrp2 mRNA (Teng et al., 2003; Teng and Piquette-Miller, 2005).

In SD rats treated with dexamethasone, liver Mrp2 mRNA levels do not change markedly (Cherrington et al., 2002; Johnson and Klaassen, 2002). However, Mrp2 protein is significantly increased (Demeule et al., 1999b; Johnson and Klaassen, 2002; Chandra et al., 2005a; Micuda et al., 2008). The induction of Mrp2 protein by dexamethasone produces spatially disproportional changes, with the most prominent increases in perivenous hepatocytes (Micuda et al., 2008). Rat hepatocyte isolation does not affect Mrp2 mRNA levels significantly (Nishimura et al., 2008). Further, treatment with dexamethasone induces Mrp2 expression in rat hepatocytes in culture (Courtois et al., 1999; Kubitz et al., 1999a; Luttringer et al., 2002; Lee et al., 2008) and tends to decrease MRP2 mRNA levels in both human and cynomolgus monkey hepatocytes. Cynomolgus monkey hepatocytes appear to be more sensitive in response to treatment than human hepatocytes (Nishimura et al., 2008). In mice, dexamethasone treatment does not change liver Mrp2 mRNA (Wang et al., 2008).

In male SD and WK rats of both genders, treatment with phenobarbital does not change liver Mrp2 mRNA levels (Ogawa et al., 2000; Hagenbuch et al., 2001; Cherrington et al., 2002; Courtois et al., 2002; Johnson et al., 2002b; Merrell et al., 2008). However, in SD rats, Mrp2 protein levels are increased by phenobarbital (Johnson et al., 2002b; Chandra et al., 2005a). In contrast, Ogawa et al. and Hagenbuch et al. observed that liver Mrp2 protein levels did

not change in male SD rats treated with phenobarbital (Ogawa et al., 2000; Hagenbuch et al., 2001). In male Wistar rats treated with phenobarbital for 4 days, hepatic Mrp2 mRNA levels are significantly increased, with levels still significantly increased after a 24-hour washout period. Mrp2 mRNA levels return to control values after a 48-hour phenobarbital wash-out period (Patel et al., 2003). Treatment with phenobarbital results in the induction of MRP2 gene expression in human liver slices after 5 and 24 hours of incubation (Olinga et al., 2008). Similarly, phenobarbital markedly increases MRP2/Mrp2 mRNA and protein expression in both primary rat and human hepatocytes (Courtois et al., 2002; Jigorel et al., 2006). In Swiss albino or C57BL/6 mice treated with phenobarbital, hepatic Mrp2 mRNA levels do not change (Wagner et al., 2005; Beilke et al., 2008), while Mrp2 protein levels are significantly induced in Swiss albino mice (Wagner et al., 2005). Therefore, MRP2 appears to be regulated differently in response to phenobarbital *in vivo* and *in vitro* and in humans and rodents.

In SD rats, liver Mrp2 mRNA levels do not markedly change by treatment with perfluorodecanoic acid, diethylhexylphthalate, or clofibrilic acid (Cherrington et al., 2002; Johnson and Klaassen, 2002), while Mrp2 protein is significantly decreased by these treatments (Johnson and Klaassen, 2002). Treatment of male CD-1 mice with clofibrate (500 mg/kg, intraperitoneally; i.p.) for 10 days does not change Mrp2 mRNA in the liver (Moffitt et al., 2006). Treatment of male ICR mice with drug bezafibrate (100 mg/kg; for 6 days) significantly increases Mrp2 mRNA and protein expression (Nishioka et al., 2005).

Exposure of rats to streptozotocin, indole-3-carbinol, polychlorinated biphenyl 99, diallyl sulfide, isoniazid, acetylsalicylic acid, benzo[a]pyrene, 1-chloro-2,4-dinitrobenzene, zinc acetate, or 3-methylcholanthrene for 3–4 days does not significantly increase Mrp2 mRNA or protein levels in livers (Ogawa et al., 2000; Cherrington et al., 2002; Johnson et al., 2002b; Kameyama et al., 2008).

Treatment of BALB/c mice with 3-methylcholanthrene (once-daily for 4 days) significantly increases hepatic Mrp2 mRNA levels (Han and Sugiyama, 2006). Treatment of Swiss albino mice with atorvastatin does not change hepatic Mrp2 mRNA and protein levels (Wagner et al., 2005).

In male C57BL/6 mice treated with β -naphthoflavone, liver Mrp2 mRNA expression is significantly increased (Maher et al., 2005a). Similarly, MRP2 mRNA expression is significantly increased in human liver slices by β -naphthoflavone (Olinga et al., 2008). In another study, SD rats treated with β -naphthoflavone, Mrp2 mRNA or Mrp2 protein were not significantly increased (Johnson et al., 2002b).

In SD rats treated with polychlorinated biphenyl 126, liver Mrp2 mRNA and protein levels do not significantly change (Johnson et al., 2002b), while in male C57BL/6 mice, Mrp2 mRNA expression is significantly increased by polychlorinated biphenyl 126 (Maher et al., 2005a).

Treatment of SD rats with TCDD does not change Mrp2 mRNA or protein (Johnson et al., 2002b). TCDD does not change MRP2 mRNA expression in primary human hepatocytes either (Jigorel et al., 2006). However, TCDD tends to increase hepatic Mrp2 mRNA

expression in mice (Maher et al., 2005a; Petrick and Klaassen, 2007). Pretreatment of C57BL/6 mice with TCDD for 4 days enhances TCPOBOP-induced Mrp2 expression (Petrick and Klaassen, 2007).

Treatment of SD rats with spironolactone, oltipraz, and ethoxyquin does not markedly change Mrp2 mRNA (Cherrington et al., 2002; Johnson and Klaassen, 2002), while Mrp2 protein levels are significantly increased (Johnson and Klaassen, 2002). Treatment with oltipraz does not change hepatic Mrp2 mRNA levels in WK rats (Merrell et al., 2008). In male C57BL/6 mice treated with oltipraz, liver Mrp2 mRNA expression is significantly increased (Maher et al., 2005a). The induction of Mrp2 in mice treated with Nrf2 activators oltipraz and butylated hydroxyanisole is Nrf2-dependent (Maher et al., 2007). However, Beilke et al. observed no significant changes in Mrp2 mRNA expression in C57BL/6 mice by oltipraz treatment for 3 days (Beilke et al., 2008).

Treatment of male SD rat-liver slices with SIN-1 [3-(4-morpholinyl) sydnonimine hydrochloride; an ONOO⁻ donor] and Spermine NONOate (SpNO; a nitric oxide donor) increases Mrp2 mRNA levels. In gadolinium-chloride-pretreated slices, the increase in Mrp2 mRNA by Spermine NONOate is not observed (Aoki et al., 2008b).

In Swiss Webster mice treated with the phenolic antioxidant, butylated hydroxyanisole, by oral administration, MRP2 mRNA expression does not change (Hu et al., 2006), while Maher et al. observed that hepatic Mrp2 mRNA expression was significantly increased in male C57BL/6 mice treated with butylated hydroxyanisole (Maher et al., 2005a).

Treatment of C57BL/6 or Swiss albino mice with TCPOBOP results in the induction of Mrp2 expression (Maher et al., 2005a; Wagner et al., 2005; Petrick and Klaassen, 2007). By contrast, Beilke et al. observed no significant changes in Mrp2 mRNA expression in C57BL/6 mice treated with TCPOBOP for 3 days (Beilke et al., 2008).

Treatment of C57BL/6J mice with allyl alcohol does not change Mrp2 mRNA, but leads to a transient increase in protein expression. The presence of Kupffer cells does not influence the changes in Mrp2 expression produced by allyl alcohol (Campion et al., 2009).

In male C57BL/6J mice, hepatic Mrp2 mRNA and protein expression are not changed significantly by acetaminophen at doses of 200–300 mg/kg, but is induced significantly at a dose of 400 mg/kg or higher (Aleksunes et al., 2005, 2006, 2007, 2008b). Treatment of Kupffer-cell-depleted mice with acetaminophen (500 mg/kg i.p.) increases Mrp2 protein by 4- to 5-fold (Campion et al., 2008). Similarly, treatment of male Wistar rats with acetaminophen (1 g/kg body-weight dose) induces liver Mrp2 protein (Ghanem et al., 2004).

In male C57BL/6J mice, Mrp2 mRNA levels do not change significantly by treatment with carbon tetrachloride at a dose of 10 μ L/kg. However, Mrp2 mRNA and protein induction is seen at a higher dose of 25 μ L/kg (Aleksunes et al., 2005, 2006). In male SD rats, exposure to carbon tetrachloride (640 mg/kg per 2 days for 45 days) in a long-term treatment decreases hepatic Mrp2 mRNA expression (Okumura et al., 2007). In rats with chronic hepatic failure induced by carbon tetrachloride (1.0 mg/kg, subcutaneously; s.c., 3 times per

week) for 2 or 3 months, Mrp2 mRNA expression was significantly lower than in the control group (Khemawoot et al., 2007).

Male SD rats receiving docosahexaenoic acid or docosahexaenoic acid in combination with low vitamin E (docosahexaenoic acid-lowVE) supplementation for 14 days tended to have lower levels of Mrp2 mRNA and protein, in comparison to rats fed linoleic acid (LA). However, this difference is not statistically significant (Kubo et al., 2006).

In Long-Evans rats, Mrp2 mRNA gene expression is increased in males 4–21 days after birth and decreased 60 days after birth by oral treatment of pregnant rats with DE-71, which is predominately composed of polybrominated diphenyl ether congeners 47, 99, 100, 153, and 154, with low levels of brominated dioxin and dibenzofuran contaminants (Szabo et al., 2009).

In rats treated with dichloroethylene for 1 hour by oral administration, Mrp2 staining in the canalicular membranes of zone 3 hepatocytes is decreased (Marumo et al., 2004). In male Wistar rats, long-term (i.e., 3 months) exposure to aluminum hydroxide decreases liver Mrp2 expression (Gonzalez et al., 2004). In Wistar rats, exposure to arsenic increases Mrp2 protein. This induction correlates with total arsenic content in bile (Li et al., 2004). Exposure of male SD rats to inorganic arsenate (iAsV) and arsenite (iAsIII) increases Mrp2 expression in liver (Cui et al., 2004). In mice fed a diet supplemented with 0.125% of the herbicide, 2,4,5-trichlorophenoxyacetic acid, hepatic Mrp2 mRNA and protein levels are increased (Wielandt et al., 1999).

In patients with biliary obstruction due to bile duct carcinoma, liver MRP2 mRNA levels do not change, regardless of whether or not patients received inchinkoto, an herbal medicine recognized in China and Japan as a “magic bullet” for treating jaundice. However, MRP2 protein levels are significantly higher in inchinkoto-treated patients than in -untreated patients (Watanabe et al., 2009). In SD rats treated with genipin, an intestinal bacterial metabolite of geniposide (a major ingredient of inchinkoto), Mrp2 protein function in canalicular membrane vesicles, measured by the adenosine triphosphate (ATP)-dependent uptake of Mrp2-specific substrates, is increased, but not mRNA levels. In immunoelectron microscopic studies, canalicular membrane and microvilli Mrp2 protein staining increases markedly (Shoda et al., 2004).

In male Wistar rats, liver Mrp2 mRNA expression tends to increase after 6 hours of anesthesia with sevoflurane or propofol and decreases with dexmedetomidine treatment, although the changes are not statistically significant. Mrp2 mRNA expression returns to the control values at 24 hours after awakening from anesthesia by sevoflurane, propofol, or dexmedetomidine. By contrast, liver Mrp2 increases significantly in rats receiving isoflurane and does not return to normal levels 24 hours after awakening from anesthesia (Nakazato et al., 2009).

In male Wistar rats, Mrp2 protein expression is significantly decreased by treatment with acetylcholinesterase reactivators K027 [1-(4-carbamoyl pyridinium)-3-(4-hydroxyiminomethyl pyridinium) propane dibromide] at a dose of 50% LD₅₀, HI-6 [1-(4-carbamoylpyridinium)-3-(2-hydroxyimino methylpyridinium) oxapropane dichloride], or

obidoxime [1,3-bis(4-hydroxyiminomethyl pyridinium) oxapropane dichloride] at doses of 5% and 50% LD₅₀. No statistical differences in Mrp2 expression are seen when animals receive K027 at a dose of 5% LD₅₀, in comparison with control animals (Pejchal et al., 2008).

In rats on 12-week chronic ethanol consumption, liver Mrp2 mRNA and protein are downregulated over time. Confocal immunofluorescence microscopy shows disruption in Mrp2 localization from the canalicular membrane and intracellular relocation. The pattern of immunostaining starts to change after 1 week of ethanol feeding. Eight weeks appears to be the critical time point for this decrease in expression and protein relocation (Zinchuk et al., 2007).

Treatment of male SD rats with a single dose of cisplatin (5 mg/kg) does not change liver Mrp2 expression up to 15 days after treatment (Demeule et al., 1999a). However, treatment of primary rat hepatocytes with cisplatin results in Mrp2 induction (Kauffmann et al., 1997; Young et al., 2006).

In male Wistar rats treated with the folate antagonist, methotrexate, liver Mrp2 expression is downregulated. This change is reversed by leucovorin treatment (Shibayama et al., 2006).

In 2 epileptic patients, treatment with the antiepileptic drug, carbamazepine, significantly induced MRP2 mRNA levels (Oscarson et al., 2006).

Treatment of male Wistar rats with St John's wort (*Hypericum perforatum*) (400 mg/kg/day) for 10 days increases Mrp2 protein expression over time, to a maximum level at 10 days after treatment. Then, Mrp2 protein decreases gradually and returns to control levels at 20 days after the end of treatment (Shibayama et al., 2004). Mrp2 levels are increased in the livers of Wistar rats receiving high doses of St John's wort extract (100 and 1,000 mg/kg/day) during pregnancy, whereas Mrp2 levels decrease in fetal liver (Garrovo et al., 2006).

In male Wistar rats treated with taurine, the most abundant free amino acid in mammals, hepatic Mrp2 protein expression increases. This treatment prevents Mrp2 retrieval from the canalicular membrane induced by LPS (Muhlfeld et al., 2003).

In male SD rats treated with immunosuppressants cyclosporine A (15 mg/kg/day) or sirolimus (0.4 mg/kg/day) for 2 weeks, Mrp2 mRNA decreases. Treatment with both immunosuppressants in combination results in an even greater reduction in Mrp2 mRNA levels (Bramow et al., 2001).

Treatment of C57BL/6 mice with 2-acetylaminofluorene results in a dose-dependent induction of Mrp2 mRNA, but no induction is observed in *Pxr*-knockout mice by the same treatment (Anapolsky et al., 2006). Treatment of male Wistar rats with oral 2-acetylaminofluorene does not alter Mrp2 protein or mRNA levels (Tang et al., 2000). Treatment of primary male Wistar rat hepatocytes with cycloheximide (5 µmol/L; a protein-synthesis inhibitor) and 2-acetylaminofluorene (40 µmol/L) increases Mrp2 gene expression in a dose- and time-dependent manner (Kauffmann et al., 1997).

In male SD rats, liver Mrp2 mRNA and protein levels are increased after estrogen and decrease after testosterone treatment, whereas no significant effects by these hormonal treatments are seen in females. Hypophysectomy and hormone-replacement studies showed that liver Mrp2 levels are regulated by the combination of thyroxine and different growth-hormone secretory patterns (Simon et al., 2006).

Treatment of male and female rhesus monkeys with tamoxifen (25 mg/kg per day; over 7 days) resulted in a strong increase in liver MRP2 mRNA and protein (Kauffmann et al., 1998). Treatment of female SD rats with the steroid, dehydroepiandrosterone, does not influence liver Mrp2 expression. In male rats, dehydroepiandrosterone treatment leads to a slight, but significant, decrease in Mrp2 expression (Rost et al., 2005). Mrp2 protein levels are lowered in female rats after testosterone treatment (10 mg/day for 7 days), closer to those seen in males (Suzuki et al., 2006). In male SD rats treated with trans-stilbene oxide (a synthetic proestrogen), liver Mrp2 mRNA levels do not change at 3 or 12 hours, but are twice as high 4 days after treatment. However, treatment with trans-stilbene oxide does not change liver Mrp2 expression in WK rats or C57BL/6 mice (Slitt et al., 2006a, 2006b). These results suggest that gender-related differences exist in Mrp2-mediated hepatobiliary transport, and that Mrp2 is differentially regulated by sex hormones.

SD rats receiving either fat-free total parenteral nutrition or the total parenteral nutrition with 20% of the calories derived from fat (soybean-oil emulsion) have liver Mrp2 mRNA levels significantly lower than those in rats on a regular diet (Nishimura et al., 2005). Liver Mrp2 protein levels and its canalicular localization are suppressed in rats fed a choline-deficient l-amino-acid-defined (CDAA) diet (Makino et al., 2008).

Regulation of hepatic MRP2/Mrp2 expression in extrahepatic diseases

In SD rats with chronic renal failure induced by partial nephrectomy, Mrp2 mRNA levels are increased (Laouari et al., 2001; Lu and Klaassen, 2008; Naud et al., 2008), while changes in Mrp2 protein expression are not consistent (Laouari et al., 2001; Naud et al., 2008). In rats with chronic kidney disease, liver Mrp2 mRNA levels are higher in females than in males. Also, hepatic Mrp2 mRNA expression does not correlate with severity of chronic kidney disease in nephrectomy of rat (Lu and Klaassen, 2008). Treatment of hepatocytes with uremic serum increases Mrp2 mRNA levels, but does not change Mrp2 protein expression (Naud et al., 2008).

In male Wistar rats undergoing intestinal ischemia-reperfusion, liver Mrp2 mRNA and protein expression is decreased at 6 hours. This decrease is associated with an increase in serum levels of IL-6 (Ogura et al., 2008). In male Wistar rats with colitis induced by treatment with trinitrobenzene sulfonic acid for 24 hours, Mrp2 immunostaining diminishes. This change is prevented by polymyxin B treatment, but not by the other antibiotics, such as penicillin G or metronidazole (Kawaguchi et al., 2000). In male SD rats with bowel injury induced by indomethacin (8.5 mg/kg, i.p.; 3 days), liver Mrp2 mRNA and protein expression is significantly reduced. This is caused by nitric oxide arising from bowel injury (Fujiyama et al., 2007).

In female Lewis or SD rats with arthritis induced by adjuvant *Mycobacterium butyricum*, liver Mrp2 expression decreases (Achira et al., 2002; Uno et al., 2009).

Regulation of hepatic MRP2/Mrp2 expression in diabetes

In type 2 diabetic male SD rats induced by feeding a high-fat diet, followed by a single dose of streptozotocin, Mrp2 protein levels do not change 14 days after treatment (Nowicki et al., 2008).

In ob/ob mice, liver Mrp2 mRNA is not significantly different from wild-type females or males, while Mrp2 protein levels are 3.4- and 2.9-fold higher in females and males, respectively, compared to wild types. No significant gender-related differences in Mrp2 expression were observed (Cheng et al., 2008).

Regulation of hepatic MRP2/Mrp2 expression in cancers and tumors

In patients with metastatic tumor or hepatocellular carcinomas, MRP2 mRNA levels are similar in liver samples from these two groups (Hinoshita et al., 2001). In patients with hepatocellular carcinomas, MRP2 mRNA levels are similar between cancerous and noncancerous portions of the liver (Moustafa et al., 2004). In hepatocellular carcinomas and peritumorous non-neoplastic tissue, MRP2 mRNA and protein expression levels do not differ significantly (Zollner et al., 2005). In human hepatocellular carcinomas, MRP2 and MRP3 mRNA expression levels are at least 10-fold higher than those for MRP1. MRP2 is localized in the plasma membrane in a polarized fashion, either in trabecular structures resembling the canalicular membrane or in the luminal membrane, when cells have a pseudoglandular arrangement (Nies et al., 2001). The MRP2 mRNA expression is significantly increased in neoplastic, and even higher in perineoplastic, lesions (Bonin et al., 2002).

In patients with hepatoblastomas, the expression pattern and intensity of MRP2 staining is similar to that in the normal livers, and the expression pattern does not change by chemotherapy (Vander Borcht et al., 2008b).

In male Wag/Rij rats with metastatic liver tumors developed by intraportal injection of CC531 colon adenocarcinoma cells, Mrp2 protein levels are increased at week 2 in the peritumor area, compared to livers from sham-operated rats (Liu et al., 2007).

During chemically induced hepatocarcinogenesis at two different stages corresponding to 20 and 32 weeks after carcinogen administration in rats, a marked progressive loss of Mrp2 RNA is found. The change is due to mechanisms other than those controlled by FXR/SHP/FTF (Monte et al., 2005).

In isolated hepatocytes from preneoplastic rat livers, Mrp2 expression decreases markedly. After incubation with ethacrynic acid, Mrp2 levels are increased dose dependently and progressively in the intracellular microsomal membrane fraction at all the times studied. The rate of this increase is notably higher in preneoplastic rat hepatocytes (Parody et al., 2007).

Regulation of hepatic MRP2/Mrp2 expression in primary and induced cholestasis

In patients with obstructive jaundice caused by peri-ampullary tumor growth, liver MRP2 mRNA levels are similar to nonjaundiced patients (Schaap et al., 2009). In obstructive cholestatic patients with adequate percutaneous transhepatic biliary drainage (PTBD), liver MRP2 mRNA levels do not change, while in poorly drained patients, MRP2 mRNA expression is reduced. Immunostainings of MRP2 outline the canalicular membrane domains, but the outlines seem fuzzy to varying degrees in specimens obtained from cholestatic liver, especially in livers that have been poorly drained, in contrast to the linear and intense localization in livers of control subjects. The staining correlated with impaired bilirubin conjugate and bile-acid excretion (Shoda et al., 2001). Thus, MRP2 mRNA and protein expression may be altered in the cholestatic liver of patients undergoing percutaneous transhepatic biliary drainage (Shoda et al., 2001).

In patients with inflammation-induced icteric cholestasis (mainly cholestatic alcoholic hepatitis), MRP2 mRNA levels remain unchanged, in comparison to controls, but canalicular immunolabeling for MRP2 is decreased (Zollner et al., 2001).

In patients with cirrhosis or chronic extrahepatic cholestasis, no difference of MRP2 expression was observed, in comparison to controls (Bonin et al., 2002).

In infants with biliary atresia, liver MRP2 shows higher mean expression than adults, but this difference is not statistically significant (Chen et al., 2005).

Primary sclerosing cholangitis is a cholestatic liver disease of unknown etiology. In patients with this condition, MRP2 mRNA levels are decreased markedly (Oswald et al., 2001). In rats with 2,4,6-trinitrobenzenesulfonic-acid-induced primary sclerosing cholangitis, Mrp2 protein is downregulated during the acute phase of inflammation. In chronic cholangitis induced by 2,4,6-trinitrobenzenesulfonic acid treatment for 12 weeks, Mrp2 protein and mRNA is also downregulated (Geier et al., 2002a).

In rats with 17 α -ethynylestradiol-induced cholestasis, liver Mrp2 mRNA does not change, but protein expression in the canalicular membrane is significantly reduced (Trauner et al., 1997; Kamisako and Ogawa, 2005; Ruiz et al., 2006, 2007). Male Wistar rats fed a diet containing 1% (wt/wt) diosgenin or diosgenin-ethinyl estradiol for 7 days do not show any changes in hepatic Mrp2 mRNA (Kamisako and Ogawa, 2005). Treatment of adult male Wistar rats with spironolactone alone or in combination with 17 α -ethynylestradiol for 6 and 12 hours increases Mrp2 mRNA expression at 6 and 12 hours after treatment (Ruiz et al., 2007).

Alpha-naphthyl isothiocyanate produces acute intrahepatic cholestasis in rodents. In α -naphthyl-isothiocyanate-treated mice, Mrp2 mRNA expression is induced over time. Canalicular Mrp2 staining is enhanced 48 hours after treatment throughout the liver lobule (Cui et al., 2009). In contrast, Tanaka et al. observed unaltered liver Mrp2 mRNA levels after α -naphthyl isothiocyanate treatment of mice, while liver Mrp2 protein expression was increased (Tanaka et al., 2009). Treatment of male SD rats with a single dose of α -naphthyl isothiocyanate does not change liver Mrp2 mRNA or protein levels (Ogawa et al., 2000).

Regulation of hepatic MRP2/Mrp2 expression during pregnancy and cholestasis

In neonatal rats whose mothers had obstructive cholestasis during pregnancy, liver Mrp2 mRNA levels do not change, regardless of treatment with or without ursodeoxycholic acid (Macias et al., 2006). In SD rats during pregnancy and postpartum, Mrp2 mRNA levels do not change, whereas Mrp2 protein expression significantly decreases in pregnancy and return to control levels post-partum (Cao et al., 2001).

Regulation of hepatic MRP2/Mrp2 expression in primary biliary cirrhosis

In patients with primary biliary cirrhosis, liver MRP2 mRNA and protein expression and localization do not change significantly (Zollner et al., 2001; Kojima et al., 2003; Ros et al., 2003a; Zollner et al., 2003b; Barnes et al., 2007). Kojima et al. observed irregular MRP2 immunostaining, suggesting redistribution into intracellular structures in primary biliary cirrhosis III (Kojima et al., 2003). Kullak-Ublick et al. observed decreased MRP2 expression with progressive cholestasis in primary biliary cirrhosis patients with stage IV disease (Kullak-Ublick et al., 2002).

Regulation of hepatic MRP2/Mrp2 expression by bile acid treatment

Human liver MRP2 expression is upregulated by exposure to lithocholic acid (Elias and Mills, 2007). In healthy gallstone patients, treatment with ursodeoxycholic acid (1 g/day for 3 weeks) does not change significantly MRP2 mRNA or protein expression (Marschall et al., 2005). In mice, treatment with ursodeoxycholic or cholic acid upregulates Mrp2 expression (Fickert et al., 2001; Guo et al., 2003; Zollner et al., 2003a; Teng and Piquette-Miller, 2007). Pretreatment of mice with phenobarbital or TCPOBOP enhances MRP2 expression induced by a 1% cholic-acid-containing diet, but there is no significant change by cholic acid in combination with pregnenolone 16 α -carbonitrile (Guo et al., 2003; Teng and Piquette-Miller, 2007). Cotreatment of C57BL/6 mice with oltipraz/lithocholic acid significantly increases liver Mrp2 protein, in comparison to corn-oil vehicle (Beilke et al., 2008).

In rats, treatment with hydrophilic bile salts or treatment with bile salts of more hydrophilicity increases liver Mrp2 expression (Asamoto et al., 2001). Incubation of rat primary hepatocytes with a medium containing 5% ascites fluid or 5% bile also increases Mrp2 mRNA expression (Tamai et al., 2003).

Regulation of hepatic MRP2/Mrp2 expression by bile duct ligation

In male Wistar or SD rats that underwent bile duct ligation, hepatic Mrp2 and protein levels are significantly decreased at 24 and 72 hours. These decreases persist for 7–14 days after surgery (Trauner et al., 1997; Ogawa et al., 2000; Donner and Keppler, 2001; Denson et al., 2002; Denk et al., 2004; Kamisako and Ogawa, 2005; Villanueva et al., 2008). In Wistar rats after bile duct ligation, liver Mrp2 protein showed maximal reduction between 7 and 14 days after surgery (Villanueva et al., 2006). In male SD rats with bile duct ligation, decreases in hepatic Mrp2 protein expression are detected as early as at 6 hours after surgery (Hyogo et al., 2001). In SD rats treated by selective bile duct ligation for 3 days, liver Mrp2 protein expression levels are significantly lower in the obstructed lobe, in comparison to the

nonobstructed lobe (Kanno et al., 2003). In a rat model of intrahepatic and obstructive cholestasis, Mrp2 expression is also downregulated.

Paulusma et al. observed that bile duct–ligated male Wistar rats had decreased liver Mrp2 protein expression and transporter function at 16–72 hours after surgery without any significant changes in Mrp2 RNA levels. A strong reduction in Mrp2 staining occurred at 48 hours after surgery, which was initially detected in periportal areas of the liver lobule and progressed toward pericentral areas after 96 hours. Decreased Mrp2 staining was accompanied by intracellular localization of the protein in pericanalicular vesicular structures. Restoration of bile flow after a 48-hour bile duct ligation period resulted in a slow recovery of Mrp2-mediated transport and protein levels. These observations suggest that downregulation of Mrp2 during bile duct ligation-induced obstructive cholestasis is mainly post-transcriptionally regulated. This downregulation is caused by endocytosis of apical transporters and increased breakdown of Mrp2, probably in lysosomes. This breakdown of Mrp2 is more pronounced in periportal areas of the liver lobule (Paulusma et al., 2000).

In C57BL/6 bile duct–ligated mice, Mrp2 mRNA expression does not change at 1 and 3 days after surgery, in comparison to sham-operated controls. By 7 and 14 days after surgery, Mrp2 mRNA expression is 1.5-fold higher than in controls (Slitt et al., 2007). By contrast, Bohan et al. showed decreases in Mrp2 mRNA and protein expression at 14 days following common bile duct ligation in C57BL/J mice (Bohan et al., 2003). In other studies, Mrp2 mRNA or protein levels do not change significantly in mice that underwent common bile duct ligation analyzed 7 days after surgery (Wagner et al., 2003; Mennone et al., 2006; Wagner et al., 2007).

Regulation of hepatic MRP2/Mrp2 expression during inflammatory responses

Treatment of Wistar or SD rats with lipopolysaccharide (from *Salmonella typhimurium*, *Escherichia coli* 0127:B8, *E. coli* 055:B5, and *E. coli* 0111:B4) significantly decreases liver Mrp2 mRNA and protein levels as early as 6–12 hours. This decrease lasts for several days after LPS treatment. The effect of LPS is time- and dose dependent (Trauner et al., 1997; Vos et al., 1998; Kubitz et al., 1999b; Nakamura et al., 1999; Dombrowski et al., 2000; Tang et al., 2000; Cherrington et al., 2004; Donner et al., 2004; Shibayama et al., 2006; Aoki et al., 2008a). Similar to LPS treatment, Shiga-like toxin II, derived from *E. coli* O157:H7, downregulates liver Mrp2 protein in male Wistar rats. Pentoxifylline (a TNF- α production inhibitor) could not protect Shiga-like toxin II–induced decrease in biliary clearance of doxorubicin and downregulation of Mrp2 (Hidemura et al., 2003). In male SD rats, liver Mrp2 transcription is downregulated transiently after induction of mild, nonlethal sepsis by cecal ligation and single puncture (CLP) and is downregulated persistently after fulminant sepsis by cecal ligation and double puncture (2CLP) (Kim et al., 2000). LPS treatment results in a selective early retrieval of rat Mrp2 from the canalicular membrane, whereas the canalicular morphology remains unchanged. Downregulation of Mrp2 mRNA is a later event (Kubitz et al., 1999b; Dombrowski et al., 2000). With LPS treatment, Mrp2 protein decreases more prominently in pericentral hepatocytes, with only minor reductions in periportal hepatocytes (Donner et al., 2004). *Klebsiella pneumoniae* (0.5 mg/kg) and

Pseudomonas aeruginosa (0.5 mg/kg) endotoxins do not downregulate Mrp2. Treatment of male Wistar rats with *E. coli* endotoxin (0.5 mg/kg; *E. coli* O55:B5) by intravenous (i.v.) administration has no effect on liver Mrp2 expression, probably due to the low dose used (Ueyama et al., 2005).

Treatment of mice with lipoteichoic acid, which is a TLR2 ligand, transiently suppresses Mrp2 RNA levels (Ghose et al., 2009).

In C57BL/6 or CD-1 mice, LPS treatment significantly decreases hepatic Mrp2 mRNA levels as early as 6 hours after treatment. Mrp2 mRNA levels return to approximately 70% of controls (Hartmann et al., 2002; Siewert et al., 2004; Teng and Piquette-Miller, 2005). Treatment of mice with IL-6, IL-1 β , and TNF- α alone or in combination decreases liver Mrp2 mRNA and protein (Hinoshita et al., 2001; Hartmann et al., 2002).

Treatment of mice with turpentine also downregulates liver Mrp2 mRNA and protein levels (Hartmann et al., 2002; Siewert et al., 2004). Treatment of C57BL/6 mice with IL-6 has similar effects (Siewert et al., 2004).

In human liver slices treated with LPS from *E. coli* 055:B5, MRP2 mRNA levels do not change. However, MRP2 protein is absent in human liver slices at 24 hours post-LPS challenge, suggesting that a post-transcriptional mechanism plays a more prominent role in regulating human MRP2 than rat Mrp2 by LPS (Elferink et al., 2004). LPS treatment also decreases Mrp2 mRNA levels in isolated rat hepatocytes or precision-cut rat-liver slices (Vos et al., 1998; Elferink et al., 2004; Aoki et al., 2008b). Similar observations were obtained in isolated rat hepatocytes treated with IL-6 or IL-1 β (Kim et al., 2000; Denson et al., 2002).

Treatment of human hepatocytes with IL-1 β or TNF- α decreases MRP2 mRNA and protein expression (Hisaeda et al., 2004), while treatment of human hepatocytes with IL-6 reduces MRP2 mRNA and protein levels over time (Vee et al., 2009). However, Vee et al. observed that human hepatocytes exposed to TNF- α had no changes in MRP2 mRNA or protein (Vee et al., 2009). In cultured human hepatocytes, MRP2-related fluorescence is restricted to canalicular networks. TNF- α or IL-6 treatment does not change this staining pattern (Konig et al., 1999; Vee et al., 2009).

In rats, aminoguanidine does not change the effect of LPS on Mrp2 mRNA levels (Cherrington et al., 2004; Aoki et al., 2008a). Treatment with dexamethasone counteracts the LPS effect on rat-liver Mrp2 mRNA, Mrp2 protein subcellular distribution, and bromosulfophthalein excretion (Kubitz et al., 1999b). However, Cherrington et al. observed that dexamethasone treatment did not change the effect of LPS on mRNA levels in rats (Cherrington et al., 2004). In rats, pretreatment with Kupffer cells inactivator, gadolinium chloride inhibits the decrease in Mrp2 mRNA expression by LPS (Nakamura et al., 1999). However, this was not observed in the studies by Cherrington (Cherrington et al., 2004).

Regulation of hepatic MRP2/Mrp2 expression by virus infection

In infections with hepatitis viruses, liver MRP2 expression decreases, in comparison to livers of noninfected patients. MRP2 expression is much lower in hepatitis C virus-infected, noncancerous regions of the liver, in comparison to noninfected cases. MRP2 protein staining is significantly reduced in noncancerous hepatic cells in most hepatitis C virus-infected than noninfected cases (Hinoshita et al., 2001). By contrast, in a study by Ros et al., MRP2 mRNA expression levels were relatively stable in patients with hepatitis C virus infection, in comparison to patients with noninfected livers (Ros et al., 2003a).

Regulation of hepatic MRP2/Mrp2 expression during liver ischemia-reperfusion

During liver ischemia-reperfusion, hepatic Mrp2 mRNA and protein expression decreases significantly in rats (Shu et al., 2007; Yu et al., 2007a; Tanaka et al., 2008). In rat hepatocytes under hypoxic conditions, Mrp2 mRNA and protein levels are significantly decreased. These changes are accompanied by decreases in mRNA levels for HNF-4- α , RXR- α , and FXR (Fouassier et al., 2007).

Regulation of hepatic MRP2/Mrp2 expression in fatty liver and steatosis

In obese Zucker rats, an established model for nonalcoholic fatty liver disease, liver Mrp2 protein levels are significantly reduced, while Mrp2 mRNA levels tend to reduce. Downregulation of Mrp2 seems to involve both transcriptional and post-transcriptional mechanisms. This probably relates to insulin and leptin resistance (Kim et al., 2004; Pizarro et al., 2004; Geier et al., 2005a).

In rats with simple fatty liver (SFL) induced by feeding a high-fat diet for 8 weeks, liver Mrp2 mRNA levels do not change significantly. In rats with nonalcoholic steatohepatitis (NASH) induced by feeding a methionine- and choline-deficient (MCD) diet, liver Mrp2 protein levels are increased strikingly (Lickteig et al., 2007a). In rats fed the standard or the high-fat and high-sucrose (HF1) diet for 4 weeks, there is no gender difference of liver Mrp2 protein expression (Osabe et al., 2008).

Regulation of hepatic MRP2/Mrp2 expression during liver regeneration

Following 70% partial hepatectomy, rat Mrp2 mRNA and protein expression does not change, when compared to sham-operated animals (Gerloff et al., 1999; Vos et al., 1999; Ros et al., 2003b; Chang et al., 2004; Dransfeld et al., 2005). However, MRP2 expression is decreased at 36 and 72 hours postoperatively in the 90% hepatectomy group (Chang et al., 2004).

In patients with submassive liver necrosis, liver MRP2 mRNA expression is relatively stable, as compared to patients with normal liver function (Ros et al., 2003a). In rats with acute liver injury induced by carbon tetrachloride, Mrp2 expression does not change (Geier et al., 2002b).

Regulation of hepatic MRP2/Mrp2 expression in genetic diseases and gene knockout animal models

In saline-treated *Tnfr1*-knockout, *Il1r1*-knockout, and *Il6*-knockout mice, liver Mrp2 mRNA levels are not different from those in wild types with the same treatment. Treatment of these strains of null mice with LPS decreases Mrp2 mRNA levels relative to their untreated counterparts (Lickteig et al., 2007b). In *Tnfr1*-knockout, *Il1r1*-knockout, and *Il6*-knockout mice subjected to bile duct ligation, liver Mrp2 mRNA levels do not change, in comparison to wild types or sham-operated knockout animals. However, In sham-operated *Tnfr1*-knockout mice, there are significant elevations in Mrp2 mRNA levels, in comparison to sham-operated wild-type mice (Lickteig et al., 2007b). Bohan et al. observed that bile duct ligation decreases liver Mrp2 expression in *Tnfr1*-knockout and wild-type mice, in comparison to the sham-operated counterparts. There was no difference in Mrp2 expression between *Tnfr1*-knockout and wild-type mice, regardless of whether or not they were bile duct ligated (Bohan et al., 2003).

There is no difference in hepatic Mrp2 mRNA levels in liver-specific I κ B kinase β (IKK β)-deficient (*IKK β hep*) and *IKK β* -floxed [*IKK β /f/f*] mice after saline treatment. LPS treatment decreases liver Mrp2 mRNA levels in *IKK β /f/f* mice (Lickteig et al., 2007b).

Among three strains of C57BL6/6J mice (i.e., endotoxin-sensitive wild-type, *Icam*-knockout, and Fas-receptor-deficient *lpr* mice), Mrp2 mRNA levels are not different after sham operation, but are decreased in all these strains upon common bile duct ligation. Mrp2 protein levels also reduce in wild-type mice and *lpr* mice with more decreases in *lpr* mice following common bile duct ligation (Wagner et al., 2007). Between endotoxin-resistant C3H/HeJ wild-type and C3H/HeJ *lpr* mice, there is no difference in Mrp2 expression by common bile duct ligation or sham operation. Common bile duct ligation does not change liver Mrp2 expression in these C3H/HeJ mice (Wagner et al., 2007).

In *Abcg2*-knockout mice, liver Mrp2 mRNA levels are not different from wild types (Nezasa et al., 2006). In *Spgp*-knockout mice, liver Mrp2 increases moderately (Lam et al., 2005). In *Mrp6*-knockout mice, liver Mrp2 mRNA levels are not significantly different from wild types (Li et al., 2007). In *Mrp4*-knockout mice, liver Mrp2 expression tends to be lower than in wild types. Common bile duct ligation does not change Mrp2 expression 7 days after surgery in the *Mrp4*-knockouts (Mennone et al., 2006). In mice deficient in the *Osta* subunit of the heteromeric organic solute and steroid transporter (*Osta*-*Ost β*), liver Mrp2 mRNA is increased (Ballatori et al., 2008).

In gamma-glutamyl transpeptidase (GGT)-deficient mice, liver Mrp2 expression levels are reduced to half of wild-type values (Habib et al., 2000). In hepatocyte-specific glutamate-cysteine ligase catalytic subunit-knockout mice, a marked induction of Mrp2 expression is observed in the liver (Maher et al., 2007).

In *Nrf2*-knockout mice, constitutive hepatic Mrp2 mRNA expression is similar to wild types (Aleksunes et al., 2008b; Tanaka et al., 2009). Treatment with acetaminophen does not alter Mrp2 mRNA expression in either genotype, but decreases slightly Mrp2 protein expression

in the *Nrf2*-knockouts (Aleksunes et al., 2008b). Treatment of *Nrf2*-knockout mice with α -naphthyl isothiocyanate does not change Mrp2 expression in the liver (Tanaka et al., 2009).

In *Fxr*-knockout mice, Mrp2 expression does not change, in comparison to wild types. Treatment with 1% cholic acid does not alter Mrp2 expression (Schuetz et al., 2001; Guo et al., 2003). Common bile duct ligation does not change Mrp2 protein expression 7 days after surgery in these knockouts (Wagner et al., 2003). Treatment with α -naphthyl isothiocyanate does not change Mrp2 expression at 48 hours (Cui et al., 2009). In *Pxr*-knockout mice, basal hepatic Mrp2 mRNA levels are 2-fold higher than in wild types (Teng and Piquette-Miller, 2007). Treatment with RU486, α -naphthyl isothiocyanate, or 2-acetylaminofluorene does not change liver Mrp2 mRNA levels in these knockouts (Teng et al., 2003; Anapolsky et al., 2006; Cui et al., 2009). Treatment with cholic acid or cholic acid in combination with pregnenolone 16 α -carbonitrile induces Mrp2 expression (Teng and Piquette-Miller, 2007). In *Hnf1 α* -knockout mice, liver Mrp2 mRNA levels are not different from those in wild types (Maher et al., 2006b). In *Car*-knockout mice, the basal liver Mrp2 mRNA expression is slightly lower than in wild types (Slitt et al., 2006a).

In Eisai hyperbilirubinuria rats, which are animal models of Dubin-Johnson syndrome, basal Mrp2 mRNA is about 20-fold lower than normal SD rats. Treatment of Eisai hyperbilirubinuria rats with tienilic acid at a dose of 300 mg/kg decreases Mrp2 mRNA expression (Nishiya et al., 2006).

In ATP8B1/Atp8b1 (a putative aminophospholipid flippase)-knockdown human or rat hepatocytes, Mrp2 basal mRNA or protein are not altered, in comparison to control wild-type hepatocytes (Cai et al., 2009).

Regulation of hepatic MRP2/Mrp2 expression in other diseases

In patients with Rotor syndrome (RS), a rare-familial conjugated hyperbilirubinaemia with normal liver histology, MRP2 expression does not change, and no sequence alterations are found in 32 exons, adjacent intronic regions, and the promoter region of MRP2. Rotor-type hyperbilirubinaemia is not an allelic variant of MRP2 deficiency (Hrebicek et al., 2007).

In patients with cholesterol calculus, liver MRP2 mRNA and protein expression levels are significantly downregulated, in comparison to the normal liver (Kong et al., 2006).

The Gunn rat is an animal model of Crigler-Najjar syndrome (CNS) type I that develops jaundice due to defects of bilirubin conjugation. In Gunn rats, hepatic Mrp2 protein expression is not significantly different from that of SD rats (Higuchi et al., 2004).

In Long-Evans Cinnamon rats, which provide a pertinent model for basic and clinical studies on hepatitis, Mrp2 expression is not significantly different from that in normal Wistar rats (Chiba et al., 2007).

In a novel mouse model of hepatobiliary injury and biliary fibrosis of cholangiopathy induced by the feeding of 3,5-diethoxycarbonyl-1,4-dihydrocollidine for 1 week, Mrp2 mRNA does not change, while protein expression is significantly reduced over time (Fickert et al., 2007).

Regulation of hepatic MRP2/Mrp2 expression in cell lines by xenobiotics

In HepG2 cells, MRP2 expression is almost the same as that in cultured primary hepatocytes and also correlates with levels in the intact human liver (Lee and Piquette-Miller, 2001; Lee et al., 2001; Teng et al., 2003; Le Vee et al., 2006; Hilgendorf et al., 2007). Treatment of HepG2 cells with clotrimazole, rifampicin, 2-acetylaminofluorene, phenobarbital, or cisplatin induces MRP2 mRNA expression (Courtois et al., 2002; Teng et al., 2003; Harris and Jeffery, 2008). Treatment of HepG2 cells with both sulforaphane or erucin increases MRP2 protein levels in a dose-dependent manner (Harris and Jeffery, 2008). Piper betel leaves (PBLs) and their major constituent, eugenol, decrease MRP2 expression. The addition of eugenol clearly attenuates the inductive effect of cisplatin in MRP2 (Young et al., 2006). IL-1 β and TNF- α reduce MRP2 protein and mRNA expression in HepG2 cells (Hisaeda et al., 2004). On the other hand, treatment of HepG2 cells with 250 mM of buthionine sulfoximine or 80 mM of t-butylhydroquinone for 18 hours, to reduce intracellular glutathione levels, does not alter MRP2 expression (Lee et al., 2001). Treatment of HepG2 cells with doxorubicine, vinblastine, dexamethasone, tamoxifen, or TCDD does not increase MRP2 mRNA or protein significantly, either (Schrenk et al., 2001).

In highly differentiated human hepatoma HepaRG cells, MRP2 mRNA expression is lower than in primary human hepatocytes. MRP2 activity is comparable to that in 1-day-old primary human hepatocytes in culture (Le Vee et al., 2006). Treatment of HepaRG cells with phenobarbital upregulates MRP2 mRNA levels (Le Vee et al., 2006; Lambert et al., 2009). Treatment of primary human hepatocytes and HepaRG cells with IL-1 β results in significant downregulation of MRP2 mRNA in a time-dependent manner (Le Vee et al., 2008).

Hepatocellular carcinoma Huh7 cells express MRP2 at low levels (Teng et al., 2003). In Huh7 cells resistant to cisplatin, MRP2 mRNA expression increases, in comparison to the parental Huh7 cells (Wakamatsu et al., 2007). In the cisplatin-resistant human liver carcinoma cell line, 7404-CP20, and another cisplatin-resistant cell line, KB-CP20, MRP2 protein expression is considerably reduced by cisplatin selection (Shen et al., 2000). In human intrahepatic cholangiocarcinoma cells KKKU-100, KKKU-M055, KKKU-M156, KKKU-M214, and KKKU-OCA17, MRP2 mRNA is moderately expressed (Tepsiri et al., 2005). In adriamycin-resistant subclone BEL/ADM of human hepatoma cell line BEL, MRP2 mRNA expression is significantly higher than in human normal liver cells (L-02) and BEL cells (Zhang et al., 2008).

Treatment of primary rat hepatocytes, as well as rat hepatoma H4IIE cells with 2-acetylaminofluorene, cisplatin, vinblastine, dexamethasone, or phenobarbital, induces Mrp2 gene expression. The cholestatic drug, ethinyl estradiol, increases Mrp2 mRNA, but decreases Mrp2 protein in primary rat hepatocytes, most likely via a post-transcriptional mechanism (Kauffmann and Schrenk, 1998; Schrenk et al., 2001). Treatment of rat primary hepatocyte cultures and H4IIE rat hepatoma cells with the peroxisome proliferators, clofibrate and diethylhexylphthalate, does not change Mrp2 mRNA or protein expression (Schrenk et al., 2001). The Fao cell line is a subclone of rat hepatoma H4IIEC3 cells. Mrp2 is expressed at higher levels in unpolarized parental Fao cells than in the normal liver. In polarized subclone Can 3-1 cells, Mrp2 levels are significantly lower than in Fao cells. In

Fao, Can 3-1, or Can10 cells treated with taurocholic, chenodeoxycholic, or ursodeoxycholic acid at 50 μ M, which is similar to the serum total bile-acid concentrations reached in the liver parenchyma during mild cholestasis or transiently during postprandial periods, no significant differences in Mrp2 gene expression are seen (Cassio et al., 2007). In rat ascites hepatoma AH66 cells cultured with medium containing 5% ascites fluid or 5% bile for 24 hours, Mrp2 mRNA expression increases (Tamai et al., 2003). In F258 rat-liver epithelial cells and 3-methylcholanthrene-resistant F258 cells, Mrp2 mRNA levels are absent or barely detected (Payen et al., 2001).

The rat hepatoma/human fibroblast hybrid cell lines, WIF-B and WIF-B9, were developed for studies requiring the maintenance of hepatocyte-like polarity. In WIF-B cell homogenates, rat Mrp2 protein is detected as a 190-kD glycoprotein. Immunofluorescence microscopy localizes Mrp2 to the apical membrane domain of these cells (Nies et al., 1998). In WIF-B9 cells, rat Mrp2 mRNA is highly expressed, in comparison to the normal rat liver. In WIF-B9, cisplatin-resistant subclone WIF-B9/R and cisplatin-resistant recovery subclone WIF-B9/Rev cells, very low human MRP2 mRNA levels are detected, in contrast to the normal human liver. In WIFB9/R cells, rat Mrp2 mRNA and protein expression are markedly increased, in comparison to WIF-B9 cells and rat hepatocytes. Rat Mrp2 mRNA levels are higher than in WIF-B9/Rev cells (Briz et al., 2007). In HPCT-1E3, a fusion cell line between primary rat hepatocytes and Fao Reuber hepatoma cells (H35), Mrp2 is detected. This expression profile is almost identical to that found in the rat liver (Halwachs et al., 2005).

In mouse hepatoma cell line Hepa-1 cells, treatment with oltipraz, ethoxyquin, or tert-butylhydroquinone induces Mrp2 expression dose dependently (Maher et al., 2007). In Hepa1-6 cells, treatment with IL-1 β or IL-6 significantly decreases Mrp2 mRNA levels and cellular efflux of 5-carboxyfluorescein, whereas TNF- α treatment results in less pronounced, statistically insignificant reductions. Incubation of Hepa 1–6 cells with the bile acids, taurocholate and cholic acid, does not change Mrp2 mRNA levels (Hartmann et al., 2002). Cytokines are key mediators in regulating hepatic anion-transporter expression in inflammatory cholestasis, whereas bile acids likely play a minor role (Hartmann et al., 2002).

In hepatic progenitor cell-like Thy-1 positive cells (oval cells), freshly isolated from rats treated with 2-acetylaminofluorene in combination with carbon tetrachloride, Mrp2 mRNA is only minimally expressed. In progenitor cell-like RLF phi 13 cells or cholangiocytes freshly isolated from normal male Fisher 344 rats, Mrp2 mRNA is undetectable (Ros et al., 2003b). In cultures of small hepatocytes, which are hepatic progenitor cells with hepatic characteristics, Mrp2 mRNA is expressed at day 13 and gradually increased to about 80% of that in mature hepatocytes at day 28 after isolation and plating. Mrp2 protein correlates with mRNA expression. In small hepatocytes in a flat colony, Mrp2 protein is not expressed, but in rising or piled-up cells, Mrp2 protein is well expressed, restricted to the bile canalicular membrane (Oshima et al., 2008). During development into hepatic “organoid-like” clusters of mature hepatocytes, rat small hepatocytes in culture acquire a fully differentiated transporter expression phenotype, and Mrp2 is included (Sidler Pfandler et al., 2004).

In activated hepatic stellate cells from rats, there is no Mrp2 expression (Hannivoort et al., 2008).

In the human fetal hepatocytes cell line, WRL 68, which can undergo differentiation *in vitro*, MRP2 protein is detected (Cizkova et al., 2005).

The isolation of hepatocytes from the intact liver involves collagenase digestion of the tissue, resulting in loss of cell polarization and functional vectorial excretion. Sandwich-cultured hepatocytes repolarize and reestablish vectorial transport. In sandwich-cultured human hepatocytes, MRP2 expression increases slightly. Colocalization of MRP2 and P-glycoprotein to the canalicular domain is clearly observed on day 6 in sandwich cultures of rat and human primary hepatocytes (Hoffmaster et al., 2004).

Mechanisms of hepatic MRP2/Mrp2 regulation

Transcriptional regulation—The human MRP2 gene promoter region has been isolated, and three transcription start sites are known to exist. In a search for transcription-factor binding sites in the MRP2 promoter by a computer-aided sequence analysis, transcription-factor binding sites for the ubiquitous factors, AP1 and SP1, and several liver-abundant transcriptional factor binding sites for hepatocyte nuclear factors HNF1 (hepatic nuclear factor 1), HNF3, and C/EBP have been identified. HNF1 and upstream stimulatory factor (USF)-like elements are also found in the 5′ untranslated region of human MRP2. Experimental analysis showed that a putative silencer element is present in the −1,659/−491 bp region and a liver-specific positive regulatory element is localized in the −491/−258 bp region, which contains a binding site for the liver-abundant transcription factor CCAAT-enhancer binding protein beta-C/EBPβ (−356 to −343 bp). C/EBPβ may activate MRP2 gene expression, and the C/EBPβ site at −356 to −343 bp has a critical role. The MRP2 promoter activity also requires the sequence between +81 and +248 in HepG2 cells (Tanaka et al., 1999). The sequence between nucleotides −517 and −197 is decisive for basal MRP2 expression (Stockel et al., 2000). A Y-box-like element binding for YB-1, but not for NF-Y, is found to be located at bp −290 (referring to the translation start site) of the human MRP2 promoter. This element does not contribute to basal expression. No SP1 site is found in this region (Kauffmann et al., 2001). In the promoter region of the human MRP2 gene, potential binding sites for hepatocyte nuclear factor 4 (HNF-4) is also found, in addition to HNF-1 and HNF-3, besides the CAATT box enhancer binding protein (C/EBP). Studies show that only HNF-1 and −4 increased MRP2 gene expression. HNF-1-α and -1-β transactivate the human MRP2 gene. HNF-1-β has a slightly repressive effect on HNF-1-α transcriptional activity (Kitanaka et al., 2007; Qadri et al., 2009). Induction of MRP2 by HNF4α is independent of the HNF-1 binding site. C/EBP, HNF-3, and HNF-6 inhibit HNF-1-α activity (Qadri et al., 2009). An interferon stimulatory response element (ISRE) has been found at −179/−146 bp of the promoter region of the human MRP2 gene. Interferon regulatory factor 3 (IRF3) has been shown to be involved to bind to ISRE and regulate positively MRP2 gene expression. IL-1β reduces the translocation of IRF3 to nuclei and reduces the binding of IRF3 to ISRE in the MRP2 promoter in human hepatic cells. This inactivation is accomplished via interference with the ERK-signaling pathway (Hisaeda et al., 2004).

The 5'-flanking region of rat Mrp2 gene is the only sequence that shows good homology to the 5'-flanking sequence of the human MRP2 gene, exhibiting 51% nucleotide identity. Both sequences have HNF-1- and USF-like elements in the 5' untranslated region (Tanaka et al., 1999). No TATA-box is present in the rat Mrp2 gene-promoter region. However, several glucocorticoid-responsive elements (GREs), peroxisome proliferators-responsive elements (PPREs), and binding sites for AP1, CBF, CDP2, C/EBP α , EF1A, HNF1, c-Myb, PEA3, and SP1 have been identified. Studies have shown that basal Mrp2 gene expression is regulated by two different fragments in the flanking region comprising bases -307 to -290, which contains an inverted CCAAT element (Y-box) for the enhancer factor 1A (EF1A) and the CCAAT binding factor (CBF), and bases -250 to -214, which contain a GC-box as the binding site for the transcription factor, SP1. Sequences mediating 2-acetylaminofluorene induction are located within a region 250 bases upstream of the translation start site, while the inducing effect of phenobarbital seems to be mediated by another domain located further upstream (Kauffmann and Schrenk, 1998). NF-Y- and SP1-binding sites play a decisive role in the basal expression of the rat Mrp2 gene, while the human MRP2 gene is regulated differently. Both Y- and GC-box in the rat Mrp2 promoter are essential for full basal gene expression, but have no significant relevance for its inducibility by the chemical carcinogen, 2-acetylaminofluorene. The transcription factor, CBF/NF-Y, but not EF1A/YB-1, binds to the Y-box and contributes substantially to basal gene expression. SP1 binds to a GC-box located at bp -242 relative to the translation start site and also contributes to basal expression (Kauffmann et al., 2001). The minimal promoter sequence of the rat Mrp2 gene is confined to the proximal 186 bp. A YB-1-responsive element (YRE-1), mapped at -186/-157 bp, exhibits specific YB-1-binding capacity. YB-1 acts as a potent repressor of Mrp2 promoter activity *in vitro* (Geier et al., 2003b).

Mrp2 is regulated by three distinct nuclear-receptor-signaling pathways that converge on a common response element in the 5'-flanking region of this gene. Studies with specific agonists and gene-knockout animals have shown that PXR, CAR, and FXR are regulators of Mrp2 gene expression. Further studies show that PXR, CAR, and FXR can bind as heterodimers with the retinoid X receptor alpha (RXR α , NR2B1) to a 26-bp sequence, which is identified at 440 bp upstream of the Mrp2 transcription initiation site that contains an ER-8 element (everted repeats of the AGTTCA hexad separated by eight nucleotides), and induce rat Mrp2 reporter gene (1-kb promoter region) expression in a PXR-, CAR-, or FXR-dependent manner. Further, the isolated ER-8 element confers PXR, CAR, and FXR responsiveness when fused to a heterologous thymidine kinase promoter. Mutation of the ER-8 element abolishes the nuclear-receptor response (Kast et al., 2002).

A DR-5 element (direct repeats of hexad separated by five nucleotides) has been identified in the mouse Mrp2 promoter region. RXR α :RAR α -heterodimer binds to the DR-5 element. DR-5 and ER-8 elements are highly conserved sequences between the rat and mouse. ER-8 repeats can also be found in the 5'-flanking region of the mouse Mrp2 gene (Geier et al., 2005b).

Nrf2 appears to regulate Mrp2 gene expression in response to exposure to xenobiotics via an ARE element located at the proximal region of its promoter. Two ARE-like sequences, ARE-2 (-1,391 to -1,381 bp) and ARE-1 (-95 to -85 bp), are found in the 5'-flanking

region of the mouse Mrp2 gene. The proximal region (–185 to +99 bp) contains the elements for basal expression and xenobiotic-mediated induction of the Mrp2 gene (Vollrath et al., 2006).

Role of nuclear receptors and liver-enriched transcription factors in MRP2/Mrp2 gene regulation by xenobiotic exposure, proinflammatory factors, and in disease states—Studies have shown that nuclear receptors PXR, CAR, FXR, PPAR α , RAR, RXR, and HNF family are involved in the regulation of MRP2/Mrp2 gene expression. These transcription factors mediated the regulation of MRP2/Mrp2 gene expression in response to xenobiotic exposure, proinflammatory factors, and in disease states with altered transcriptional activity, which is affected by the alteration of expression levels, translocations, and modifications of these transcription factors. For example, hypoxia induces a marked decrease in mRNA levels for transcription factors HNF-4 α , RXR α , and FXR (Fouassier et al., 2007). Methotrexate treatment also decreases mRNA levels of constitutive androstane receptor (CAR) and pregnane X receptor (PXR) in liver (Shibayama et al., 2006). PXR activation is responsible for 2-acetylaminofluorene–induced liver Mrp2 gene expression in mice (Anapolsky et al., 2006). Induction of drug-metabolizing enzymes and transporter gene expression by the antiepileptic drug, carbamazepine, in the human liver is mediated, at least in part, by the activation of PXR (Oscarson et al., 2006). Mrp2 expression does not change during acute liver injury in rats induced by treatment with carbon tetrachloride, possibly due to the unchanged binding activity of RXR α :RAR α in the Mrp2 gene (Geier et al., 2002b). Time-dependent alterations in PXR mRNA levels are similar to those for Mrp2 in rats with adjuvant-induced arthritis (Uno et al., 2009). Using partial regression analysis, CAR, HNF-4 α , and PXR are associated with human liver MRP2 gene expression (Wortham et al., 2007).

Hepatitis C virus induces MRP2 gene expression through HNF-1 and –4. Hepatitis C virus infection induces HNF-4 and –1 mRNA and protein levels through increasing oxidative stress. Protein-protein interactions between hepatitis C virus nonstructural component 5A and HNF-1 leads to enhanced HNF-1 DNA binding, and HNF-1 is transcriptionally controlled by HNF-4 (Qadri et al., 2006).

Proinflammatory factors downregulate MRP2/Mrp2. This response is transcriptionally mediated by nuclear hormone receptors and liver-specific transcription factors.

Aoki et al. observed that LPS does not change HNF-4 α or –1 α mRNA levels, HNF-1 α protein levels, or its DNA-binding activity of HNF-1 α , whereas LPS increases nuclear HNF-1 α levels and reduces HNF-4 α protein levels and its DNA-binding activity. Aminoguanidine treatment alone increases mRNA levels for HNF-4 α and –1 α , as well as nuclear HNF-1 α accumulation, but does not enhance the LPS-induced increase in nuclear HNF-1 α localization, whereas it attenuates the LPS-induced decrease in nuclear HNF-4 α localization, but has no effect on the LPS-induced decrease of HNF-4 α DNA-binding activities (Aoki et al., 2008a).

RXR α is the heterodimer partner for type II nuclear receptors, including PXR, FXR, CAR, and RAR α . LPS decreases mRNA and protein expression of RXR α , RAR α , PXR, CAR, and

FXR. Aminoguanidine attenuates this LPS-induced decrease in RXR α protein (Aoki et al., 2008a). Inflammatory cytokines suppress mRNA and protein levels for FXR, PXR, CAR, RXR α , and RAR α and thus reduce the binding of these nuclear receptors to their target genes (Geier et al., 2005b).

It is well known that nuclear factor kappa-beta (NF- κ B) plays a key role in inflammatory responses. NF- κ B p65 interacts with the DNA-binding domain of RXR α and disrupts its DNA binding capacity. This may provide a possible mechanism for suppression of MRP2 gene expression under inflammation conditions (Gu et al., 2006).

Treatment with lipoteichoic acid, a TLR2 ligand, results in the transient suppression of Mrp2 RNA expression. Concomitantly, PXR and CAR mRNA expression levels are transiently reduced. Nuclear RXR α protein and TLR2-receptor mRNA and protein are also reduced. Kupffer cells contribute to lipoteichoic-acid-mediated downregulation of hepatic genes, such as Mrp2 (Ghose et al., 2009).

As previously stated, treatment of mice with endotoxin and IL-6 significantly reduces Mrp2 and PXR mRNA and protein levels. The magnitude of endotoxin- and IL-6-induced suppression of Mrp2 is significantly diminished in *Pxr*-knockout mice, suggesting that PXR is involved in the regulation of mouse Mrp2 gene expression, and that it plays a role in the downregulation of Mrp2 during inflammation (Teng and Piquette-Miller, 2005).

Toxic and cholestatic liver injury results in the downregulation of MRP2 gene expression. Cytokines, such as TNF- α , IL-1 β , and IL-6, are attributed to mediate this regulation. In cholestatic liver injury, IL-1 β plays a predominant role in the regulation of Mrp2 expression. HNF-1- and RXR/RAR-independent mechanisms appear to be more important in the regulation of Mrp2 gene expression under endotoxemia (Geier et al., 2003a). In male SD rats, Mrp2 downregulation and IL-1 β upregulation are observed in liver by bile duct ligation. This is temporally associated with downregulation of liver RAR α :RXR α nuclear protein contents and their binding to the *cis* element in the Mrp2 promoter. Retinoid trans-activation and IL-1 β -induced downregulation of Mrp2 promoter activity were mapped to RXR α :RAR α -response elements, providing a mechanism that likely contributes to the downregulation of MRP2 during inflammation (Denson et al., 2000).

PXR levels are reduced in early- and late-stage cholestasis. CAR and SHP do not change significantly, although there was a trend for CAR decrease at both early- and late-stage cholestasis (Kocher et al., 2008). PXR mRNA levels are significantly increased in mice fed cholic acid or cholic acid in combination with pregnenolone 16 α -carbonitrile, in comparison to mice receiving a standard diet. CAR mRNA levels tended to be decreased in wild-type mice by the same treatments and were significantly induced in *Pxr*-knockout mice fed cholic acid, but not cholic acid in combination with pregnenolone 16 α -carbonitrile. Of note, basal CAR mRNA levels do not differ between wild-type and *Pxr*-knockout mice (Teng and Piquette-Miller, 2007).

FXR mRNA levels are significantly induced in wild-type and *Pxr*-knockout mice fed a cholic-acid-containing diet, but not by cholic acid in combination with pregnenolone 16 α -carbonitrile. Basal FXR mRNA levels do not differ between wild-type and *Pxr*-knockout

mice. Indeed, FXR mRNA levels are approximately 50% lower in pregnenolone 16 α -carbonitrile-treated mice, although this did not reach statistical significance (Teng and Piquette-Miller, 2007). Hepatic Mrp2 induction by cholic and ursodeoxycholic acids is independent of FXR function (Zollner et al., 2003a). Basal Mrp2 mRNA expression had no obvious changes between *Fxr*-knockout and wild-type mice and was similar to that in mice receiving a diet containing a 1% lithocholic acid supplement (Kitada et al., 2003). A 1% cholic acid diet slightly induces Mrp2 expression in *Fxr*-knockout mice (F^{-}/P^{+} , F^{-}/P^{-}) (Guo et al., 2003). There is no difference in Mrp2 mRNA levels between *Fxr* knockout and wild-type mice, and 1% cholic acid treatment does not change this (Schuetz et al., 2001).

LPS activates Kupffer cells to secrete IL-1 and TNF- α , which, in turn, activate MAP kinases and decrease Mrp2 expression (Nakamura et al., 1999).

Mrp2 gene expression is regulated through Nrf2 pathway in response to oxidative stress—Oxidative stress is known to be a common feature of many liver diseases, such as cholestatic syndrome. In response to oxidative and/or electrophilic stress, Nrf2 plays a critical role in the transcriptional upregulation of many target genes, including drug-metabolizing enzymes and transporters that are essential for cellular defense and detoxification. Studies with Nrf2 activators and knockout mice suggest that Mrp2 gene expression is dependent on the Nrf2 pathway.

Chromatin immunoprecipitation assays showed the binding of Nrf2 to the Mrp2 gene in response to ursodeoxycholic-acid treatment in mouse hepatocytes (Okada et al., 2008). Treatment of Hepa1c1c7 cells with the Nrf2 activator, tert-butylhydroquinone, also results in the binding of Nrf2 to antioxidant response elements in the promoter region of mouse Mrp2 (−185 bp) (Maher et al., 2007). In HepG2 cells treated with tert-butylhydroquinone, Nrf2 translocates into the nuclei, and MRP2 mRNA expression is significantly increased in a dose- and time-dependent manner. Treatment with siRNA targeting Nrf2 or Keap1 results in decreases or increases in MRP2 mRNA levels, respectively, suggesting that induction of MRP2 by tert-butylhydroquinone is mediated by the Nrf2/Keap1 system (Adachi et al., 2007).

Acute oxidative stress also results in the internalization of Mrp2 from the canalicular membrane through a sequential mechanism that involves reduction in glutathione, Ca^{2+} elevation, nitric oxide production, and nPKC activation (Sekine et al., 2006). Internalized Mrp2 can be reinserted to the canalicular membrane when the intracellular redox status is restored. The redox-sensitive balance of PKA/PKC activation regulates the reversible Mrp2 localization by two different pathways: the microtubule-independent internalization pathway and the microtubule-dependent recycling pathway of Mrp2 (Sekine et al., 2008). In addition, the dynamic changes in actin spatial organization and the activation status of Ca^{2+} -dependent PKC also play roles in the dynamic localization of canalicular Mrp2 in response to changes in redox status (Sanchez Pozzi and Roma, 2009).

Mrp2 activity and canalicular membrane fluidity—Mrp2 activity may be partially enhanced by increases in canalicular membrane fluidity (Nishioka et al., 2005), which is regulated by many factors. Canalicular membrane fluidity is increased by hydrophilic bile

salts (Asamoto et al., 2001). In bile duct–ligated rats, canalicular membrane fluidity decreases in a time-dependent manner, whereas sinusoidal membrane fluidity shows biphasic responses: an initial transient increase in fluidity that is followed by a more prolonged decrease. These changes are closely related to changes in membrane lipid composition (i.e., saturated:unsaturated fatty-acid ratio) (Hyogo et al., 2001). On the other hand, canalicular and sinusoidal membrane fluidity is decreased in obstructed lobes, in comparison to the nonobstructed lobes, in response to localized cholestasis (Kanno et al., 2003). Membrane cholesterol content is a critical determinant of MRP2 transport activity. Reduced cholesterol content impairs the activity of MRP2, which, in turn, causes cholestasis (Paulusma et al., 2009).

Mrp2 and Mrp1 coregulation during cell cycle—Mrp1 mRNA and protein levels are increased concomitantly with the disappearance of Mrp2 in rat hepatocytes mainly in the G1 phase (high density) and hepatocytes that have progressed into the S-phase or beyond (low density) (Roelofsen et al., 1999). A switch from Mrp2 to Mrp1 occurs in the G1 phase of the cell cycle, which is associated with decreased cell polarity. During replication, transporter expression switches from the apically located Mrp2 to the basolaterally located Mrp1 (Roelofsen et al., 1999).

MRP2 gene expression and single-nucleotide polymorphisms (SNPs)—High MRP2 expression is significantly correlated with the 3600A and 4581A MRP2 variants (Meier et al., 2006). MRP2 1446C>G is associated with reduced systemic exposure to pravastatin as a consequence of increased MRP2 expression. The underlying mechanism may involve either a modulating effect of the SNPs on mRNA stability or linkage to other polymorphism(s) acting at the transcriptional level (Niemi et al., 2006). In a 39-year-old Japanese woman, both mutations, C298T and C3928T, led to immature stop codons, which resulted in the instability of MRP2 mRNA and/or defective synthesis of the truncated protein (Shoda et al., 2003).

Translational and post-translational regulation of Mrp2—Mrp2 expression is regulated at the translation level. For example, treatment of rats with pregnenolone 16 α -carbonitrile for 2 days results in significant increases in liver Mrp2 protein. By contrast, a significant decrease in Mrp2 expression is seen in 19-day pregnant rats with no significant differences in Mrp2 mRNA levels. Differences in the degradation of Mrp2 protein cannot explain the posttranscriptional regulation of Mrp2 in control, pregnant, and pregnenolone 16 α -carbonitrile–treated rats. Rather, the differences in protein synthesis showed by the rate of ³⁵S incorporation suggest an intrinsic role for the translational regulation of rat Mrp2 protein. Further, the major transcription-initiation site determined by RNA protection assay is –98 nucleotides in the rat liver, with other minor start sites observed at –213, –163, –132, and –71 nt, and use of transcription sites does not differ among the groups (Jones et al., 2005). The 5′-UTR plays a role in the regulation of Mrp2 protein synthesis. 5′-UTR without any upstream open reading frames (uORFs) (–98 bp) expresses maximal luciferase activity, in comparison to those with one (–132 nucleotides), two (–163 bp), or three (–213 bp) uORFs. Among the uORFs in the Mrp2 5′-UTR, the uORF starting at –109 bp probably plays an important role in the regulation of Mrp2 protein expression (Zhang et al., 2007b).

Mrp2 is regulated at the post-translational level. A series of small ubiquitin-like modifier (SUMO)-related enzymes and their substrates are shown to interact with the linker region of Mrp2 located between the NH₂-terminal nucleotide-binding domain and the last membrane-spanning domain. Mrp2 linker is a substrate of Ubc9-mediated SUMOylation. The IKKE sequence in the linker of rat Mrp2 is the target of SUMOylation. Ubc9 may be involved in regulating the expression level of Mrp2 (Minami et al., 2009).

Role of radixin in MRP2/Mrp2 membrane localization—Membrane MRP2/Mrp2 localization needs radixin. The proteins from the ezrin-radixin-moesin (ERM) family cross-link actin filaments and integral membrane proteins. Radixin is the dominant ERM protein in the liver and concentrates in bile canalicular membranes. In *Rdx*-knockout mice, Mrp2 is decreased, in comparison to other bile canalicular membrane proteins, such as dipeptidyl peptidase IV (CD26) and P-glycoproteins. *In vitro* binding studies show that radixin associates directly with the carboxy-terminal cytoplasmic domain of human MRP2, suggesting that radixin is required for the secretion of conjugated bilirubin by supporting localization of Mrp2 at the canalicular membrane (Kikuchi et al., 2002). MRP2 immunostaining suggests the redistribution of MRP2 into intracellular structures in primary biliary cirrhosis III. Areas of irregular MRP2 immunostaining are associated with largely reduced radixin immunostaining, whereas normal hepatocytes have MRP2 and radixin confined to the canalicular membrane. Colocalization of radixin and MRP2 supports the concept that radixin contributes to the canalicular localization of MRP2 (Kojima et al., 2003). Localization of MRP2 in the canalicular membrane is absent when radixin expression is missing (Shu et al., 2007).

Intracellular trafficking—Studies suggest that many intracellular components, including ATP, Ca²⁺, numerous GTPases, microtubules, cytoplasmic motors, and other unknown factors, are required for physiological regulation of ABC transporter trafficking from the Golgi apparatus to the canalicular membrane. Defects in this complex system are postulated to produce an “intrahepatic traffic jam” that results in defective ABC transporter function in the canalicular membrane and, consequently, in cholestasis (Kipp and Arias, 2000).

Conclusions

MRP2/Mrp2 is highly expressed in livers across species. It is predominantly expressed in hepatocytes and located in their apical membrane domains. Its basal expression is regulated developmentally and also in a gender-dependent manner.

Hepatic MRP2/Mrp2 expression is induced by PXR, GR, and CAR agonists at the transcriptional and post-transcriptional levels. Induction by PXR agonists is gender dependent. Hepatic MRP2/Mrp2 protein levels are repressed by PPAR α agonists post-transcriptionally. Hepatic MRP2/Mrp2 gene expression is induced in mice by AhR agonists at the transcriptional level, but not in rats or humans. Hepatic Mrp2 expression is induced by Nrf2 activators in rats post-transcriptionally and in mice at the transcriptional level. Hepatic MRP2/Mrp2 expression is induced by acute, toxic acetaminophen and carbon tetrachloride treatment in an Nrf2-independent manner, suggesting that other factors are involved in this regulation. Long-term carbon-tetrachloride-induced liver injury results in decreased Mrp2

expression. The mechanisms of the acute and chronic effects of carbon tetrachloride on hepatic MRP2/Mrp2 expression are not well defined. Hepatic MRP2/Mrp2 expression levels also change in response to environmental chemicals and by treatment with therapeutic drugs. Inchinkoto increases MRP2 protein by a post-transcriptional mechanism. Isoflurane, carbamazepine, St. John's wort, taurine, and tamoxifen increase MRP2 expression, while KO27, HI-6, chronic ethanol consumption, methotrexate, cyclosporine A, or sirolimus decrease it.

Hepatic MRP2 expression is induced in chronic renal diseases, by bile-acid treatment, and in *Gclc*- and *Osta*-knockout mice. Hepatic MRP2 expression is decreased during intestinal ischemia-reperfusion, in colitis, bowel injury, adjuvant-induced arthritis, during liver ischemia-reperfusion, 90% hepatectomy-induced liver regeneration, in cholestasis, including poorly drained obstructive cholestasis, endotoxin exposure, and inflammation, virus infection, cholesterol calculus, and in ggt-knockout mouse. Notably, hepatic MRP2 expression is more readily induced in rats than mice after bile duct ligation. Hepatic MRP2 expression does not change during 70% hepatectomy-induced liver regeneration, well-drained obstructive jaundice, cirrhosis or chronic extrahepatic cholestasis, primary biliary cirrhosis, hepatocellular carcinoma, hepatoblastoma, Crigler-Najjar syndrome type I, diabetes type II, Long-Evans Cinnamon rats, hepatobiliary injuries, and biliary fibrosis. *Nrf2*-, *Fxr*-, *Car*-, *Pxr*-, *Mrp4*-, *Mrp6*-, and *Abcg2*-knockout mice do not have altered Mrp2 expression either.

The MRP2 gene promoter is TATA-less and GC-rich. Many regulatory elements are found in this region for binding AP1, SP1, CBF, CDP2, C/EBP, EF1A, HNFs, c-Myb, and PEA3. ER-8 element, DR-5 element, Y-box, ISRE, GREs, as well as PPREs have also been found. Transcription factors SP1, interferon regulatory factor 3 (IRF3), Nrf2, NF-Y, SP1, YB-1, CBF/NF-Y, PXR, CAR, and FXR are known regulators of Mrp2 gene expression. Certain SNPs in the MRP2/Mrp2 gene have been found to affect the MRP2/Mrp2 gene expression. Nuclear receptors and liver-enriched factors mediate the regulation of MRP2/Mrp2 gene expression in response to xenobiotic exposure, proinflammatory factors, and in disease states. The Keap1-Nrf2 pathway mediates the regulation of MRP2/Mrp2 gene expression in response to oxidative stress.

MRP2/Mrp2 gene expression is also regulated by PKA/PKC activation, dynamic changes in actin spatial organization, and the activation status of Ca²⁺-dependent PKC. MRP2/Mrp2 gene expression is also regulated translationally and post-translationally and also during cell cycling. MRP2/Mrp2 activity may be partially enhanced by increases in canalicular membrane fluidity. MRP2/Mrp2 membrane localization needs radixin and is also regulated by intracellular trafficking.

Regulation of hepatic ABCC3/MRP3 transporters by xenobiotics and in disease states

Function of MRP3/Mrp3

MRP3 is found to be expressed in liver, kidney, small intestine, colon, adrenals, pancreas, gallbladder, spleen, urinary bladder, lung, stomach, and tonsils. In normal livers, MRP3

localizes to the basolateral membrane in polarized epithelial cells, such as pericentrally localized hepatocytes and cholangiocytes. MRP3 can transport organic compounds conjugated to glutathione, sulfate or glucuronate, and bile salts and methotrexate. Thus, MRP3 plays a role in bile-salt physiology and defenses against toxic organic anions (Borst et al., 2006, 2007).

MRP3/Mrp3 expression in normal liver

The human MRP3 gene chromosomal locus is demonstrated to be on chromosome 17q22 by fluorescence *in situ* hybridization (Uchiumi et al., 1998). Human MRP3 mRNA is expressed predominantly in the liver at about the same level as MRP2, but 27 times higher than that of MRP1 (Kool et al., 1997; Kiuchi et al., 1998; Uchiumi et al., 1998; Konig et al., 1999; Hinoshita et al., 2001). However, in normal livers, human MRP3 protein is detected at lower levels than anticipated from the mRNA data (Scheffer et al., 2002b). Human MRP3 is localized to the basolateral membrane domain of hepatocytes and is found to be present in bile duct epithelial cells and centrilobular hepatocytes at low levels (Konig et al., 1999; Scheffer et al., 2002b; Ros et al., 2003a; Vee et al., 2009). In cultured primary hepatocytes, 3 days after isolation, basal MRP3 expression is detected (Jigorel et al., 2006).

In C57BL/6 mice, liver Mrp3 mRNA is moderately expressed (Maher et al., 2005b). In mice, Mrp3 protein is typically localized to the basolateral membrane of hepatocytes through the liver from periportal to centrilobular regions. Its staining tends to be higher in centrilobular hepatocytes, with expression decreasing in intensity from the central vein outward to the portal regions (Maher et al., 2008; Tanaka et al., 2009). Basal Mrp3 expression in the female liver is twice as high as in males (Petrick and Klaassen, 2007; Cheng et al., 2008). Postnatally, Mrp3 expression is low until 3 weeks of age and reaches adult levels by 1 month of age in mice (Maher et al., 2005b).

In contrast to humans, rat hepatic Mrp3 mRNA is expressed at low levels. Low Mrp3 levels are specifically found in the basolateral membrane of hepatocytes in the centrilobular zone in normal rat liver and in bile ducts (Kiuchi et al., 1998; Ortiz et al., 1999; Donner and Keppler, 2001; Soroka et al., 2001; Cao et al., 2002; Cherrington et al., 2002; Ros et al., 2003b; Donner et al., 2004; Rost et al., 2005; Lu and Klaassen, 2008). Mrp3 expression is higher in female than in male rats (Xiong et al., 2002; Cherrington et al., 2003; Rost et al., 2005; de Zwart et al., 2008). Rat Mrp3 expression is also transiently induced in the liver shortly after birth. Mrp3 polypeptide is recognized to be 190–200 kD by antibodies and is reduced in size to 155–165 kD after treatment with endoglycosidases (Ortiz et al., 1999). Mrp3 mRNA levels tend to be increased, but not significantly, in normal rat-liver slices during 18-hour incubation in the absence of stimulants (Aoki et al., 2008b). During pregnancy, liver Mrp3 mRNA and protein expression decreases by gestational days 20–21 in rats. Immunofluorescent staining of Mrp3 is more evident in hepatocytes surrounding the central vein, indicating the differential regulation of Mrp isoforms during pregnancy (Cao et al., 2002). During development, liver Mrp3 mRNA expression is high at birth and gradually decreases toward adult levels in SD rats (de Zwart et al., 2008).

Mrp3 expression is detectable in small hepatocytes and decreases as small hepatocytes mature (Oshima et al., 2008). Mrp3 protein expression is also present in periportal progenitor cells (Ros et al., 2003b).

Regulation of hepatic MRP3/Mrp3 expression by xenobiotics

Treatment of rats or mice with CAR activators diallyl sulfide, polychlorinated biphenyl 99, phenobarbital, and TCPOBOP results in the induction of hepatic Mrp3 mRNA and protein levels (Ogawa et al., 2000; Cherrington et al., 2002; Xiong et al., 2002; Cherrington et al., 2003; Slitt et al., 2003; Staudinger et al., 2003; Chandra et al., 2005a; Maher et al., 2005a; Wagner et al., 2005; Merrell et al., 2008). In mice, Mrp3 mRNA expression is also induced by TCDD given in combination with TCPOBOP, but not by treatment with either compound alone (Petrick and Klaassen, 2007). Treatment of primary human hepatocytes with phenobarbital does not change MRP3 expression (Jigorel et al., 2006).

Treatment of human primary hepatocytes, rats, or mice with oltipraz induces hepatic MRP3/Mrp3 mRNA and protein levels (Slitt et al., 2003; Maher et al., 2005a; Jigorel et al., 2006; Merrell et al., 2008). Other Nrf2 activators, such as butylated hydroxyanisole and ethoxyquin, also induce Mrp3 expression in C57BL/6 mice (Maher et al., 2005a). In SD rats, oltipraz, but not ethoxyquin, slightly activates liver Mrp3 mRNA expression (Cherrington et al., 2002).

In acetaminophen-overdosed patients, MRP3 mRNA and protein levels do not change significantly (Barnes et al., 2007). However, toxic treatment with acetaminophen increases Mrp3 expression in male C57BL/6J mice (Aleksunes et al., 2005, 2006, 2007, 2008a). Kupffer cell depletion increases basal Mrp3 expression and further increases Mrp3 induction by acetaminophen in mice (Campion et al., 2008). By contrast, Mrp3 mRNA and protein levels are decreased after treatment with doses of carbon tetrachloride that produce pronounced hepatotoxicity (Aleksunes et al., 2005, 2006).

In mice, as well as SD or WK rats, treated with transstilbene oxide (a synthetic proestrogen) for 4 days, hepatic Mrp3 expression increases, with no changes detected at 3–12 hours after treatment (Cherrington et al., 2003; Slitt et al., 2003, 2006a, 2006b).

In patients treated with omeprazole, liver MRP3 expression is increased (Hitzl et al., 2003). In primary human hepatocytes, MRP3 expression is induced by rifampicin, while in mice, hepatic Mrp3 expression is induced by the PXR activators, pregnenolone 16 α -carbonitrile, dexamethasone, atorvastatin, RU486, and spironolactone (Staudinger et al., 2003; Teng et al., 2003; Maher et al., 2005a; Teng and Piquette-Miller, 2005; Wagner et al., 2005; Jigorel et al., 2006; Wang et al., 2008). However, in SD rats, Mrp3 expression is not changed by pregnenolone 16 α -carbonitrile, spironolactone, or dexamethasone (Cherrington et al., 2002). In rats, liver Mrp3 protein levels are increased by dexamethasone treatment for 4 days (Chandra et al., 2005a).

In C57BL/6 mice treated with TCDD, polychlorinated biphenyl 126, or β -naphthoflavone, hepatic Mrp3 expression is increased (Maher et al., 2005a). However, in primary human hepatocytes treated with TCDD in culture, MRP3 expression does not change (Jigorel et al.,

2006). In SD rats treated with the AhR ligands, TCDD, indole-3-carbinol, β -naphthoflavone, or polychlorinated biphenyl 126, hepatic Mrp3 expression does not change, either (Cherrington et al., 2002).

In mice, Mrp3 expression is induced by the PPAR α ligands, clofibric acid, CPFEB, clofibrate, diethylhexylphthalate, and perfluorodecanoic acid (Maher et al., 2005a; Moffit et al., 2006; Maher et al., 2008). Mrp3 exhibits significant induction in all zones after treatment with perfluorodecanoic acid (Maher et al., 2008). Immunohistochemistry analysis shows that Mrp3 is induced in midzonal and periportal regions in clofibrate-treated mice (Moffit et al., 2006). By contrast, in rats treated with clofibric acid, diethylhexylphthalate, or perfluorodecanoic acid, hepatic Mrp3 levels do not change (Cherrington et al., 2002; Chandra et al., 2005a).

Treatment of SD rats or mice with α -naphthyl isothiocyanate induces hepatic MRP3 mRNA and protein expression (Ogawa et al., 2000; Cui et al., 2009; Tanaka et al., 2009). In mice treated with α -naphthyl isothiocyanate, enhanced immunofluorescence staining of Mrp3 is prominent in basolateral membranes of hepatocytes throughout the liver (greatest in centrilobular hepatocytes) (Cui et al., 2009; Tanaka et al., 2009).

Exposure of C57BL/6J mice to allyl alcohol results in transient increases in Mrp3 mRNA at 12 and 24 hours after treatment, regardless of Kupffer cell function status (Campion et al., 2009).

In SD rats treated with CYP2E1 inducers, isoniazid, acetylsalicylic acid, or streptozotocin, hepatic Mrp3 mRNA expression does not change (Cherrington et al., 2002). In male SD rats after oral administration of the phase II enzyme inducer, 1,7-phenanthroline, for 3 days, hepatic Mrp3 mRNA levels are significantly increased (Wang et al., 2003). In male SD rats, liver Mrp3 mRNA and protein were induced more prominently in the 14-day docosahexaenoic-acid-fed group, in comparison to the linoleic-acid-fed group. Mrp3 induction is even higher in animals fed docosahexaenoic acid with low vitamin E ingestion (docosahexaenoic acid-lowVE), although this difference is not statistically significant (Kubo et al., 2006). In male SD rats, hepatic Mrp3 is also upregulated after treatment with the phenolic acids gentisic acid, gallic acid, ferulic acid, and p-coumaric acid (Yeh and Yen, 2006). Treatment of rats with 17 α -ethynylestradiol increases Mrp3 mRNA and protein expression levels, but treatment with spironolactone does not change Mrp3 expression. Spironolactone does not enhance the effect of 17 α -ethynylestradiol on Mrp3 expression, either (Ruiz et al., 2006, 2007). In rats fed diosgenin in diet (1%; wt/wt) for 7 days, treated with ethinyl estradiol for 5 days, or treated with ethinyl estradiol and diosgenin, hepatic Mrp3 mRNA expression is markedly increased by these three treatments (Kamisako and Ogawa, 2005). Treatment of rats with dehydroepiandrosterone results in a significant increase in liver Mrp3 expression in females and a slight increase in males (Rost et al., 2005).

In pregnant Long-Evans rats exposed to the commercial penta mixture, DE-71, Mrp3 mRNA increases at postnatal days 4 and 21. All responses are reversible by postnatal day 60 (Szabo et al., 2009).

In normal slices and gadolinium-chloride-pretreated rat liver slices, treatment with 3-(4-morpholinyl) sydnonimine hydrochloride (SIN) increases Mrp3 mRNA levels. In both sets of slices treated with SpNO, hepatic Mrp3 mRNA levels are not affected (Aoki et al., 2008b).

Regulation of hepatic MRP3/Mrp3 expression in nonhepatic diseases

In rats with chronic kidney disease, liver Mrp3 mRNA and protein increases. Mrp3 mRNA expression correlates positively with severity of chronic kidney disease (Sun et al., 2006; Lu and Klaassen, 2008). In rats with arthritis induced by adjuvant for 7 and 21 days, Mrp3 mRNA levels do not change (Uno et al., 2009). In mice with collagen-induced arthritis, liver Mrp3 mRNA levels are decreased (Kawase et al., 2007). In Wistar rats undergoing intestinal ischemia-reperfusion, hepatic Mrp3 mRNA levels are not significantly altered (Ogura et al., 2008).

Regulation of hepatic MRP3/Mrp3 expression in diabetes

In obese Zucker rats, basal Mrp3 expression levels are higher than in lean Zucker rats (Xiong et al., 2002). In both obese Zucker rats and lean SD rats, Mrp3 mRNA levels are similar (Kim et al., 2004; Geier et al., 2005a). In ob/ob mice, liver Mrp3 mRNA and protein expression is increased in males, but no differences are found between ob/ob and wild-type females (Beilke et al., 2008).

Regulation of hepatic MRP3/Mrp3 expression in cancers and tumors

In human hepatocellular carcinomas, MRP3 is localized to the basolateral membrane of carcinoma cells. Double-label immunofluorescence microscopy indicates that carcinoma cells express both MRP2 and MRP3 isoforms simultaneously. MRP3 and MRP2 mRNA expression levels are at least 10-fold higher than for MRP1 (Nies et al., 2001). In human hepatocellular carcinomas, MRP3 mRNA levels are higher, in comparison to the surrounding normal tissue. MRP3 expression is detectable in K7/K19(+) tumor cells (Vander Borgh et al., 2008a). In both neoplastic and perineoplastic hepatocellular carcinoma tissues, MRP3 expression increases, although the mean value falls within the normal range (Bonin et al., 2002; Mizukoshi et al., 2008). Zollner et al. observed that MRP3 expression tended to decrease in hepatocellular carcinomas, and that it was highly variable among individual tumors (Zollner et al., 2005). Vander Borgh et al. also observed that MRP3 gene expression significantly decreased in hepatocellular carcinomas, in comparison to surrounding nontumorous liver. The magnitude of decrease in MRP3 expression may be associated with the differentiation grade of hepatocellular carcinomas and tumor diameters (Vander Borgh et al., 2008a). In patients with hepatoblastomas, the expression patterns and intensity of liver MRP3 before chemotherapy are similar to the normal livers. There are no clear changes in these expression patterns after chemotherapy (Vander Borgh et al., 2008b).

There is no difference in MRP3 mRNA expression between metastatic tumors and hepatocellular carcinomas (Hinoshita et al., 2001). MRP3 expression is increased in cholangiolocellular carcinoma (Komuta et al., 2008).

In male F344 rats given 0.03% or higher doses of piperonyl butoxide, a hepatocellular tumor-promoting agent for nongenotoxic hepatocarcinogenesis, liver Mrp3 is upregulated (Muguruma et al., 2007, 2009).

Regulation of hepatic MRP3/Mrp3 expression in cholestasis

In patients with cholestasis, MRP3 mRNA levels are slightly elevated, in comparison to nonjaundiced patients and in patients with preoperative biliary drainage (Schaap et al., 2009).

In patients with various forms of cholestasis, liver MRP3 levels are frequently increased in proliferative cholangiocytes, with sometimes additional staining in the basolateral membranes of hepatocytes. This is especially evident in patients with type III progressive familial intrahepatic cholestasis (Scheffer et al., 2002b). However, Keitel et al. observed that in progressive familial intrahepatic cholestasis, MRP3 mRNA and protein levels did not change significantly (Keitel et al., 2005). In specimens from patients with cholestatic livers, either well drained or poorly drained, MRP3 mRNA levels are increased. Immunostaining of MRP3 is observed in the epithelia of intrahepatic bile ducts in livers of both control subjects and cholestatic patients, and in the epithelia of proliferated bile ductules and the hepatocytes surrounding the portal tracts in the cholestatic liver (Shoda et al., 2001). In patients with biliary obstruction, liver MRP3 protein levels are significantly higher and increase further after inchinkoto treatment. However, MRP3 mRNA levels do not change (Watanabe et al., 2009).

During obstructive cholestasis, rat liver Mrp3 expression is transiently induced (Ortiz et al., 1999). In rats without hyperbilirubinemia, Mrp3 mRNA and protein expression levels are induced in both the nonobstructed and obstructed lobes (Kanno et al., 2003). In neonatal rats from mothers with obstructive cholestasis during pregnancy (OCP) and from OCP mothers treated with ursodeoxycholic acid, liver Mrp3 mRNA levels are elevated (Macias et al., 2006).

Regulation of hepatic MRP3/Mrp3 expression in primary biliary cirrhosis

In patients with primary biliary cirrhosis, hepatic MRP3 expression is increased (Ros et al., 2003a; Zollner et al., 2003b; Barnes et al., 2007). However, Kojima et al. observed that in patients with primary biliary cirrhosis stage I–III, MRP3 mRNA levels and immunostaining intensities did not change (Kojima et al., 2003). By contrast, MRP3 expression is noticeably lower in both cirrhosis and chronic extrahepatic cholestasis (Bonin et al., 2002). In patients with inflammation-induced icteric cholestasis or in anicteric cholestasis caused by primary biliary cirrhosis at stages I and II, MRP3 mRNA expression remains unchanged (Zollner et al., 2001). In a novel mouse model of xenobiotic-induced hepatobiliary injury and biliary fibrosis by cholangiopathy by feeding 3,5-diethoxycarbonyl-1,4-dihydrocollidine, hepatic Mrp3 mRNA and protein expression are significantly increased over time 1 week after treatment (Fickert et al., 2007).

Regulation of hepatic MRP3/Mrp3 expression by bile acid treatment

Treatment of mice by feeding cholic and ursodeoxycholic acid stimulates hepatic Mrp3 expression (Zollner et al., 2003a). In mice, the feeding of a 1% cholic-acid diet induces liver Mrp3 expression. Pretreatment with phenobarbital or TCPOBOP enhances this induction (Guo et al., 2003). In C57BL/6 mice, Mrp3 mRNA expression is significantly increased when pretreated with TCPOBOP for 3 days, followed by cotreatment with lithocholic acid for an additional 4 days, while no changes are seen with phenobarbital/lithocholic acid or oltipraz/lithocholic acid cotreatments, in comparison to lithocholic-acid treatment only. Mrp3 protein levels also remain unchanged among these treatment groups (Beilke et al., 2008). In male and female C57L/J mice, MRP3 is overexpressed, in comparison to AKR/J mice. Mrp3 expression is reduced in male C57L/J mice fed a lithogenic diet for 14 days (Liu et al., 2008). In mice, Mrp3 mRNA levels are increased by a cholic-acid-supplemented diet. Mrp3 mRNA expression levels are significantly higher with cholic acid in combination with pregnenolone 16 α -carbonitrile treatment, in comparison to mice fed cholic acid alone (Teng and Piquette-Miller, 2007).

Treatment of rats with hydrophilic bile salts increases Mrp3 expression (Asamoto et al., 2001). In SD rats treated with bilirubin, hepatic Mrp3 mRNA levels are increased. However, Mrp3 protein levels do not change (Ogawa et al., 2000).

Regulation of hepatic MRP3/Mrp3 expression by bile duct ligation

In bile duct-ligated rats, hepatic Mrp3 expression is significantly increased at 1–7 days after surgery (Ogawa et al., 2000; Kamisako and Ogawa, 2005; Villanueva et al., 2008). Soroka et al. observed that Mrp3 is upregulated from 1 to 14 days in isolated hepatocytes from rats with common bile duct ligation (Soroka et al., 2001). Mrp3 protein levels are increased at 48 hours and are further enhanced at 72 hours after bile duct ligation. In cholestatic rat liver produced by common bile duct ligation, Mrp3 is expressed in periportal hepatocytes. However, there is a preponderance of Mrp3 expression in the pericentral area of the liver lobule (Donner and Keppler, 2001).

In bile duct-ligated mice, liver Mrp3 mRNA levels are also increased (Bohan et al., 2003; Wagner et al., 2003; Mennone et al., 2006; Stedman et al., 2006; Lickteig et al., 2007b; Slitt et al., 2007). However, Mrp3 protein levels do not change in mice at 7 days after common bile duct ligation (Mennone et al., 2006). In endotoxin-sensitive C57BL/6J mice, common bile duct ligation does not change Mrp3 mRNA expression, while in endotoxin-resistant C3H/HeJ mice, common bile duct ligation significantly increases Mrp3 mRNA expression, in comparison to their respective sham-operated controls (Wagner et al., 2007).

Regulation of hepatic MRP3/Mrp3 expression in response to infection and inflammatory process

In patients infected with hepatitis viruses, liver MRP3 expression are reduced, in comparison to the noninfected patients (Hinoshita et al., 2001). However, in patients with chronic hepatitis C virus infection, liver MRP3 expression levels are increased (Ros et al., 2003a).

Treatment of primary human hepatocytes with IL-1 β downregulates MRP3 mRNA expression (Le Vee et al., 2008). Treatment of human hepatocytes with TNF- α or IL-6 for 48 hours does not change MRP3 mRNA levels or protein localization, but it induces MRP3 protein expression levels (Vee et al., 2009).

In rats treated with LPS and/or aminoguanidine, hepatic Mrp3 mRNA levels do not change significantly (Donner et al., 2004; Aoki et al., 2008a, 2008b); however, Mrp3 protein expression is increased (Donner et al., 2004). Cherrington et al. observed that in SD rats, LPS treatment increased liver Mrp3 mRNA levels. Pretreatment with dexamethasone, which decreases the release of cytokines, prevented the LPS-mediated increase in Mrp3, whereas pretreatment with aminoguanidine or gadolinium chloride, an inhibitor of inducible nitric oxide synthetase and a Kupffer cell toxicant, respectively, had no effect on the LPS-induced changes in Mrp3 (Cherrington et al., 2004).

In mice treated with IL-6, IL-1 β , TNF- α , turpentine, or LPS, Mrp3 mRNA levels are downregulated (Hartmann et al., 2002; Siewert et al., 2004; Geier et al., 2005b; Teng and Piquette-Miller, 2005). LPS-mediated downregulation of Mrp3 is abolished at later time points (24 hours) (Siewert et al., 2004). Lickteig et al. observed that LPS treatment upregulated Mrp3 mRNA levels in mice (Lickteig et al., 2007b). In male C57BL/6 mouse treated with lipoteichoic acid (TLR2 ligand, the Gram-positive bacterial component), Mrp3 levels are not significantly affected during 0–16 hours of treatment (Ghose et al., 2009). Gadolinium chloride pretreatment does not change basal or perfluorodecanoic-acid-induced Mrp3 mRNA expression (Maher et al., 2008).

Regulation of hepatic MRP3/Mrp3 expression during ischemia-reperfusion

In male SD rats undergoing reperfusion following 60 minutes of partial hepatic ischemia, Mrp3 mRNA and protein expression levels are not altered at 0–48 hours after perfusion (Tanaka et al., 2008).

Regulation of hepatic MRP3/Mrp3 expression in fatty liver

In rats with SFL induced by feeding a high-fat (HF) diet for 8 weeks, liver Mrp3 mRNA and protein levels do not change significantly. In rats with nonalcoholic steatohepatitis (NASH) induced by feeding a methionine- and choline-deficient (MCD) diet, liver Mrp3 mRNA and protein levels are increased strikingly (Lickteig et al., 2007a).

In rats fed the standard diet, Mrp3 protein expression levels are significantly higher in female than males. In rats fed a high-lipid and high-sucrose diet (HF1 diet), no significant changes in liver Mrp3 protein levels are detected (Osabe et al., 2008).

Regulation of hepatic MRP3/Mrp3 expression in hepatitis

In Long-Evans Cinnamon rats, a model for hepatitis, Mrp3 expression levels are not significantly different from Wistar rats (Chiba et al., 2007).

Regulation of hepatic MRP3/Mrp3 expression during liver regeneration

In patients with submassive liver necrosis, liver MRP3 expression levels are increased. Strong MRP3 reactivity is seen in regenerating human bile ductules at the interface of portal tracts and necrotic areas, in comparison to patients with normal livers (Ros et al., 2003a).

In rats, 90% hepatectomy increases Mrp3 mRNA expression, while 70% hepatectomy does not (Chang et al., 2004). No changes in Mrp3 mRNA expression were detected, either, in proliferating hepatocytes freshly isolated from male Wistar rats 24 hours after partial hepatectomy (Ros et al., 2003b). In rats treated by 2-acetylaminofluorene coupled with partial hepatectomy, liver Mrp3 mRNA levels are significantly increased, with higher Mrp3 levels found in pericentral hepatocytes throughout the entire hepatic acinus. In hepatic progenitor cells, Mrp3 is expressed at high levels and the staining signal is more intense than in bile ductular cells. In cholangiocytes, Mrp3 protein does not change (Ros et al., 2003b). In freshly isolated Thy-1-positive cells, cholangiocytes, and cultured RLF phi 13 progenitor cells, Mrp3 mRNA expression levels are relatively high, while in freshly isolated hepatocytes, Mrp3 mRNA expression levels are very low (Ros et al., 2003b).

Regulation of hepatic MRP3/Mrp3 expression in genetic disease and gene-knockout animal models

In patients with Dubin-Johnson syndrome, liver MRP3 expression is increased (Konig et al., 1999; Corpechot et al., 2006). In 2 patients with Dubin-Johnson syndrome who were deficient in MRP2, particularly strong MRP3 protein expression was observed in basolateral membranes of hepatocyte (Konig et al., 1999). In *Mrp2*-deficient mutant TR⁻ rats, liver Mrp3 protein expression is strongly enhanced and its zonation is lost. The Mrp3 immunostaining of cholangiocytes is preserved in the cholestatic and in the *Mrp2*-deficient mutant liver (Donner and Keppler, 2001). In male TR⁻ rats, liver Mrp3 expression is higher than in normal Wistar rats (Kim et al., 2003; Johnson et al., 2006). Similarly, in Eisai hyperbilirubinuria rats, liver Mrp3 expression is observed (Hirohashi et al., 1998; Ortiz et al., 1999) and is higher than in normal SD rats (Kuroda et al., 2004; Nishiya et al., 2006). Treatment of Eisai hyperbilirubinuria rats with tienilic acid induces marked Mrp3 mRNA expression. Slight induction of Mrp3 mRNA is detected in SD rats by the same treatment, but this induction is much smaller than that in Eisai hyperbilirubinuria rats (Nishiya et al., 2006). In *Mrp2*-knockout mice, hepatic Mrp3 protein levels are significantly higher than in wild-type mice (Nezasa et al., 2006), while Chu et al. observed that in male or female *Mrp2*-knockout mice, Mrp3 mRNA levels do not change significantly from wild types or between genders (Chu et al., 2006).

In *Rdx*-knockout mice, liver Mrp3 expression is increased, and this may due to the lack of Mrp2 (Fukumoto et al., 2007).

In *Mrp4*-knockout mice undergoing common bile duct ligation, liver Mrp3 protein significantly increases while Mrp3 mRNA level does not change. In contrast, in wild-type mice, liver Mrp3 protein expression is unchanged after common bile duct ligation, despite increases in Mrp3 mRNA. Mrp3 mRNA and protein levels are not different between sham-operated *Mrp4*-knockout mice and wild types (Mennone et al., 2006). In *Mrp6*-knockout

mice, Mrp3 mRNA levels are not significantly different from wild-type mice (Li et al., 2007). In *Abcg2*-knockout mice, hepatic Mrp3 protein levels do not change, either (Nezasa et al., 2006). In *Osta*-knockout mice, liver Mrp3 mRNA expression increases (Ballatori et al., 2008). In *Mdr2*-knockout mice, Mrp3 mRNA expression does not change, in comparison to wild types. Treatment with norursodeoxycholic acid for 4 weeks does not induce significantly Mrp3 mRNA and protein. However, ursodeoxycholic acid treatment induces significantly only protein levels (Fickert et al., 2006). In *Spgp*-knockout mice, Mrp3 is increased to only a moderate extent (Lam et al., 2005).

In saline-treated *Tnfr1*-knockout mice, Mrp3 mRNA expression levels are significantly increased, in comparison to wild types. In *Tnfr1*-knockout mice undergoing bile duct ligation, liver Mrp3 mRNA levels increase, in comparison to sham-operated mice. LPS treatment increases Mrp3 mRNA in *Tnfr1*-knockout mice, but the induction is not statistically significant (Lickteig et al., 2007b). By contrast, Bohan et al. showed that bile duct-ligated *Tnfr1*-knockout mice had no significant changes in Mrp3 expression (Bohan et al., 2003).

In saline-treated *Il6*-knockout mice, Mrp3 mRNA levels are higher than in wild-type mice. In *Il6*-knockout mice undergoing bile duct ligation, liver Mrp3 mRNA levels increase significantly. LPS treatment increases Mrp3 mRNA in *Il6*-knockout mice, but the induction is not statistically significant (Lickteig et al., 2007b). Treatment of *Il6*-knockout mice with turpentine significantly upregulated Mrp3 mRNA expression (Siewert et al., 2004).

In saline-treated *Il1r1*-knockout mice, Mrp3 mRNA levels are slightly elevated over those in wild-type mice. In *Il1r1*-knockout mice undergoing bile duct ligation or LPS treatment, liver Mrp3 mRNA levels increase significantly (Lickteig et al., 2007b).

In *IKK β* (f/f) and *IKK β* hep mice, no differences in Mrp3 mRNA expression are observed, while bile duct ligation upregulates Mrp3 mRNA expression in *IKK β* (f/f) by 2-fold, but not in *IKK β* hep mice. LPS treatment has no effect on Mrp3 mRNA expression in *IKK β* (f/f) or *IKK β* hep mice (Lickteig et al., 2007b).

Icam-knockouts, Fas receptor-deficient *lpr* mice, and endotoxin-sensitive wild types of the C57BL/6J strain show no differences in Mrp3 mRNA expression by sham operation, while common bile duct ligation significantly induces Mrp3 mRNA levels 3 days after surgery in these C57BL/6J *Icam*-knockouts and *lpr* mice, as well as Mrp3 protein levels in C57BL/6J *lpr* mice. Between *lpr* mice and the endotoxin-resistant wild types of the C3H/HeJ strain, there are no differences in Mrp3 mRNA and protein expression after sham operation or common bile duct ligation. Common bile duct ligation significantly induces Mrp3 mRNA, but does not change protein levels in these C3H/HeJ mice (Wagner et al., 2007).

In *Hnf1 α* -knockout mice, there are no differences in basal Mrp3 mRNA levels in livers, in comparison to wild types (Maher et al., 2006b).

In *Pxr*-knockout mice, basal Mrp3 mRNA levels are 4-fold higher than in wild-type mice (Staudinger et al., 2003; Teng and Piquette-Miller, 2007). Treatment with a cholic-acid–

supplemented diet induces Mrp3 mRNA levels, in comparison to standard diet controls. Mrp3 induction still occurred in *Pxr*-knockout mice treated with cholic acid in combination with pregnenolone 16 α -carbonitrile, but the induction is significantly less than that seen in wild-type mice (Teng and Piquette-Miller, 2007). Treatment with pregnenolone 16 α -carbonitrile, rifampicin, RU486, dexamethasone, or TCPOBOP does not induce Mrp3 expression. Treatment of *Pxr*-knockout mice with phenobarbital significantly increases Mrp3 gene expression, while Mrp3 level is increased only slightly in wild-type mice (Staudinger et al., 2003; Teng et al., 2003; Teng and Piquette-Miller, 2005; Cheng and Klaassen, 2006; Wang et al., 2008). Cui et al. showed that basal Mrp3 mRNA expression is similar in wild-type and *Pxr*-knockout mice. Treatment with α -naphthyl isothiocyanate induced liver Mrp3 mRNA expression in *Pxr*-knockout mice over time (Cui et al., 2009).

In *Car*-knockout mice, constitutive liver Mrp3 expression does not differ from male C57BL/6 mice (Cherrington et al., 2003; Slitt et al., 2006a). Treatment with phenobarbital equally induces Mrp3 in both wild-type and *Car*-knockout mice (Cherrington et al., 2003).

In wild type, *Fxr*-knockout, *Pxr*-knockout, and *Pxr/Fxr*-double knockout mice with bile duct ligation, Mrp3 mRNA is modestly induced and similar to the expression detected in sham-operated animals at 6 days after surgery (Stedman et al., 2006).

In *Fxr*-knockout mice, no obvious difference of basal Mrp3 mRNA expression levels is detected from wild types (Schuetz et al., 2001; Kitada et al., 2003; Wagner et al., 2003; Cui et al., 2009), similar to the effects of treatment with 1% lithocholic acid supplement in the diet (Schuetz et al., 2001; Kitada et al., 2003). In both *Fxr*-knockout and wild-type mice that underwent common bile duct ligation for 7 days, Mrp 3 mRNA and protein expression is induced (Wagner et al., 2003). Treatment of *Fxr*-knockout mice with α -naphthyl isothiocyanate also induces liver Mrp3 mRNA expression over time (Cui et al., 2009).

In *Ppara*-knockout mice, basal Mrp3 mRNA levels are the same as in wild types. Induction of Mrp3 by perfluorodecanoic acid is lower in *Ppara*-null mice. A similar profile is observed for protein changes (Maher et al., 2008). In male *Ppara*-knockout mice, treatment with clofibrate prevents the induction of Mrp3 mRNA and protein (Moffit et al., 2006).

In *Rxra*-knockout mice, constitutive Mrp3 expression is significantly reduced. Treatment with phenobarbital, diallyl sulfide, trans-stilbene oxide, and oltipraz induces hepatic Mrp3 mRNA levels in these null mice (Cherrington et al., 2003). WY-14643-mediated Mrp3 induction is greater in *Rxra*-knockout hepatocyte than in wild-type mouse livers (Gyamfi and Wan, 2009).

In mice deficient in the hepatocyte-specific glutamate-cysteine ligase catalytic subunit, marked liver Mrp3 mRNA and protein induction and increase in nuclear Nrf2 protein levels are observed in livers (Maher et al., 2007). In *Nrf2*-knockout mice, basal Mrp3 mRNA levels are lower than in wild types (Maher et al., 2008; Tanaka et al., 2009). Treatment with α -naphthyl isothiocyanate tends to increase Mrp3 mRNA and protein levels (Tanaka et al., 2009). Treatment with perfluorodecanoic acid also induces Mrp3 mRNA levels. The magnitude of fold induction is greater in *Nrf2*-knockout mice than in wild types, although induced Mrp3 mRNA levels are lower than that in wild types (Maher et al., 2008). Mrp3

mRNA and protein expression is increased at 48 hours after acetaminophen (400 mg/kg) treatment in wild types, but not in *Nrf2*-knockout mice (Aleksunes et al., 2008b). The induction of Mrp3 in mice treated with the Nrf2 activators, oltipraz and butylated hydroxyanisole, is Nrf2-dependent (Maher et al., 2007). In *Keap1*-knockdown mice, basal liver Mrp3 expression is much higher than in wild types (Okada et al., 2008).

The Gunn rat is an animal model for Crigler-Najjar syndrome (CNS) type I that develops jaundice due to defects in bilirubin conjugation. Bilirubin UDP-glucuronosyltransferase (UGT1A1), which plays a critical role in bilirubin glucuronidation, has been reported to be deficient in CNS type I. In Gunn rats, hepatic Mrp3 mRNA and protein is higher than in normal SD rats (Ogawa et al., 2000; Higuchi et al., 2004).

In rat hepatocytes, which are deficient in Atp8b1, using adenoviral and oligonucleotide small interfering RNAs, Mrp3 basal mRNA levels are upregulated (Cai et al., 2009).

There is no difference in hepatic Mrp3 mRNA levels in liver-specific I κ B kinase β (IKK β)-deficient (*IKK β hep*) mice and *IKK β* -floxed [*IKK β /f/f*] mice after saline treatment, LPS treatment, or sham operation of bile duct ligation. Bile duct ligation increases hepatic Mrp3 mRNA levels in *IKK β /f/f* mice (Lickteig et al., 2007b).

Regulation of hepatic MRP3/Mrp3 expression in cell lines by xenobiotics

In HepG2 cells, MRP3 expression levels are lower than in normal human livers (Hilgendorf et al., 2007). Treatment of HepG2 cells with cisplatin, rifampicin, clotrimazole, β -naphthoflavone, or phenobarbital increases MRP3 mRNA expression. Treatment of HepG2 cells with the carcinogen 2-acetylaminofluorene increases MRP3 mRNA and protein expression. Treatment with dexamethasone or tamoxifen does not change MRP3 mRNA expression (Kiuchi et al., 1998; Stockel et al., 2000; Lee and Piquette-Miller, 2001; Schrenk et al., 2001; Hitzl et al., 2003; Teng et al., 2003). In both HepG2 and g2car-3 cells, MRP3 levels are induced, to a similar extent, by phenobarbital treatment, but not by TCPOBOP (Xiong et al., 2002). Treatment of HepG2 cells with a redox-active compound, tert-butylhydroquinone, significantly elevates MRP3 mRNA levels in a dose- and time-dependent manner (Adachi et al., 2007). Lee et al. observed that MRP3 mRNA is expressed in HepG2 cells, but its expression was not altered by treatment with 250 mM of buthionine sulfoximine or 80 mM of t-butylhydroquinone for 18 hours (Lee et al., 2001). Induction of MRP3 in HepG2 cells is less than half that in hepatocellular carcinoma cell line Huh7 cells by clotrimazole or rifampicin treatment (Teng et al., 2003).

In Huh7 cells, MRP3 mRNA expression is detectable (Lee and Piquette-Miller, 2001; Teng et al., 2003). In a cisplatin-resistant cell line Huh7 subclone, MRP3 mRNA expression increases, in comparison to the parental cells (Wakamatsu et al., 2007). Treatment of Huh7 cells with various PXR activators, such as clotrimazole, rifampicin and RU486, metyrapone, nifedipine, pregnenolone 16acarbonitrile, or the bile acid, lithocholic acid, results in the upregulation of MRP3 mRNA levels in a time- and dose-dependent manner. Maximal induction of MRP3 is seen at 24 hours after treatment (Teng et al., 2003). Treatment of Huh7 cells with IL-6 (10 ng/mL; 24 hours) results in a 1.8-fold increase in the MRP-

mediated efflux of 5-carboxyfluorescein, with a corresponding 1.5-fold induction in MRP3 mRNA levels (Lee and Piquette-Miller, 2001).

Among a human normal liver cell line (L-02), the hepatocarcinoma cell line BEL and its adriamycin-resistant counterpart BEL/ADM cells, MRP3 mRNA expression shows statistically significant differences, with BEL/ADM >BEL>L-02 (Zhang et al., 2008).

In proliferating, confluent, and 1.5% dimethyl-sulfoxide (DMSO)-treated HepaRG cells, a human hepatoma cell line, MRP3 mRNA expression is higher than in primary hepatocytes (Le Vee et al., 2006) and treatment of HepaRG cells with IL-1 β downregulates MRP3 mRNA (Le Vee et al., 2008).

In four human intrahepatic cholangiocarcinoma cell lines (KKU-100, KKU-M156, KKU-M214, and KKUOCA17 cells), MRP3 mRNA is highly expressed. MRP3 is not detected in the KKU-M055 cell line (Tepsiri et al., 2005).

In HPCT-1E3 cells, a fusion cell line between primary rat hepatocytes and Fao Reuber hepatoma cells (H35), the Mrp3 expression profile is almost identical to that found in the rat liver (Halwachs et al., 2005).

Treatment of Hepa-1 cells with 1-chloro-2,4-dinitrobenzene, oltipraz, ethoxyquin, sulforaphane, tert-butylhydroquinone, or catechol results in a dose-dependent induction of Mrp3. At optimal concentrations of chemicals, Mrp3 expression is induced by 1- to 4-fold (Maher et al., 2007).

In the rat hepatoma/human fibroblast hybrid cell line (WIF-B9) cells, Mrp3 mRNA is highly expressed, in comparison to rat livers. Rat Mrp3 mRNA levels in cisplatin-resistant reversal subclone WIF-B9/Rev cells is lower than in cisplatin-resistant subclone WIF-B9/R cells. No human MRP3 expression is detected in WIF-B9, WIF-B9/R, or WIF-B9/Rev cell lines, in contrast to human livers (Briz et al., 2007).

In activated hepatic stellate cells from rats, Mrp3 levels are comparable to normal hepatocytes. Cytokines or oxidative stress does not change Mrp3 expression (Hannivoort et al., 2008).

In nonpolarized Fao cells, Mrp3 is expressed at higher levels, in comparison to normal rat liver. In derived polarized line Can 10 cells, Mrp3 is increased, in comparison to parental Fao cells (Cassio et al., 2007).

Mechanism of regulation of hepatic MRP3/Mrp3 expression

Human MRP3 gene expression has been demonstrated to be controlled by a TATA-less promoter, which may involve SP1-binding sites in transcription. The human MRP3 gene promoter contains consensus sequences for transcription factors AP1, AP2, N-myc, and SP1. Transcription starts at -25 and -27 nt upstream of the translation initiation codon. The region from -127 to -23 nt is important for MRP3 expression. SP1 binds to the sequence between -92 and -58 nt (Fromm et al., 1999; Takada et al., 2000). A SP1-binding GC-box motif at -113 to -108 nt upstream from the MRP3 translation start site has been identified,

and RXR α :RAR α specifically reduces binding of SP1 to this site. Cotransfection of the transcription factor, SP1, stimulates MRP3 promoter activity, and the addition of RXR α :RAR α abrogates this activation in a dose-dependent manner. Since RXR α :RAR α expression is diminished by cholestatic liver injury, loss of RXR α :RAR α may lead to the upregulation of MRP3/Mrp3 expression in these disorders (Chen et al., 2007). Disruption of microtubules is also involved in the regulation of MRP3 gene expression (Stockel et al., 2000).

In the human MRP3 gene promoter, the putative bile-salt-responsive elements is mapped in the region –229/–138 bp, where two α -1 fetoprotein transcription factor (FTF)-like elements are identified, and FTF binds specifically to FTF-like elements in response to chenodeoxycholic acid treatment, activating MRP3 gene promoter activity. FTF thus might play a key role not only in the bile-salt synthetic pathway in hepatocytes, but also in bile-salt excretion pathways in enterocytes through the regulation of MRP3 expression (Inokuchi et al., 2001).

Mrp3 gene upregulation after bile duct ligation is due to the TNF- α -dependent induction of Lrh-1. Lrh-1 and Mrp3 are significantly induced after bile duct ligation in wild-type, but not *Tnfr1*-knockout, mice. The binding of Lrh-1 to the Mrp3 promoter increases after bile duct ligation in wild-type, but not in *Tnfr1*-knockout, mice. TNF- α treatment of HepG2 cells also upregulates cholesterol-7 α -hydroxylase promoter factor (human, FTF), and TNF- α also increases the binding of cholesterol-7 α -hydroxylase promoter factor FTF to the MRP3 promoter and upregulates its activity (Bohan et al., 2003). Induction of the adaptive transporter responses by common bile duct ligation is independent of the degree of the inflammatory response. Rather, the retention of biliary constituents may determine transporter expression in common bile duct ligation (Wagner et al., 2007). Cytokines are key mediators in regulating hepatic expression of anion transporters in inflammatory cholestasis, whereas bile acids likely play a minor role (Hartmann et al., 2002).

Multiple genetic polymorphisms in MRP3 exist in Caucasians. The –211C>T promoter polymorphism appears to be associated with decreased hepatic MRP3 mRNA expression by affecting the binding of nuclear factors. The polymorphic position –211 resides between two FTF-like elements (–222/–218 and –199/–195). Putative transcription-factor binding sites for Egr-1, BRF1, and Pax5 exist with –211C and disappears with –211T. However, the identity of the binding nuclear factor(s) needs to be investigated experimentally (Lang et al., 2004).

The rat Mrp3 gene also contains a TATA-less promoter. Regulatory regions between –157 and –106 bp relative to the translation start site are crucial for Mrp3 promoter activity. Within this sequence, putative binding sites for C/EBP and SP1 have been identified and shown specific binding of SP1, SP3, C/EBP α , C/EBP β , and C/EBP δ and are essential for the transcription of the rat Mrp3 gene in Mrp3-expressing cells. Both SP1 and SP3 transactivate the Mrp3 minimal promoter (pWT-157) in *Drosophila* SL2 cells. C/EBP transcription factors modulate the basal and tissue specific activity of the Mrp3 gene promoter by recognition of the C/EBP (–157/–140 nt) element and through functional cooperation with

factors interacting with the SP1 and *cis*-acting elements for SP1 (–140/–106 nt) (Tzeng et al., 2005).

Mouse Mrp3 gene also contains a TATA-less promoter. McCarthy et al. identified a candidate VDR response element (VDRE) between –1,028 and –1,014 bp of the Mrp3 promoter. Activation of Mrp3 gene in response to VD3 or lithocholic acid treatment is mediated by binding of VDR to a DR3 response element present within the promoter region of the Mrp3 gene. This mechanism most likely contributes to the protection of colon cells from the toxic effects of lithocholic acid, but because of the extremely low levels of VDR expression in the liver, it is not likely to account for the adaptive increase in hepatic Mrp3 expression during cholestasis, as shown *in vivo* by modulation of the Mrp3 gene in the colon, but not in the liver, by treatment with VD3 or lithocholic acid in mice (McCarthy et al., 2005).

Specific agonists and gene-knockout mouse studies show that liver MRP3/Mrp3 gene expression is activated by CAR activators in a species-specific manner. However, CAR does not play a key role in the regulation of human MRP3 gene expression (Xiong et al., 2002; Cherrington et al., 2003; Slitt et al., 2006a; Uno et al., 2009). Studies also show that AhR plays a minimal role in the regulation of MRP3/Mrp3 gene expression in rat or human liver. PXR plays a role in the regulation of Mrp3 expression in both *Pxr*-dependent and -independent pathways. SP1 may be involved in MRP3 induction by PXR activation (Staudinger et al., 2003; Teng et al., 2003; Teng and Piquette-Miller, 2007; Jiang et al., 2009). MRP3 gene expression is also regulated by PPAR α activation.

Specific activators and gene-knockout mouse studies show that the Keap1-Nrf2 pathway plays a key role in the regulation of liver Mrp3 gene expression in response to oxidative stress resulting from xenobiotic treatment and/or disease state(s). Nrf2 is physically associated to the Mrp3 regulatory region (Slitt et al., 2006a; Maher et al., 2007; Okada et al., 2008).

Induction of Mrp3 is found in pericentral hepatocytes, where Mrp2 expression is low. This suggests that Mrp3 is inversely regulated to Mrp2 in a zonal pattern and may compensate for the LPS-induced loss of Mrp2 in the perivenous area (Donner et al., 2004). However, the mechanism of this zonal pattern of regulation is not known.

Short-term regulation of Mrp3/MRP3 by cAMP and PKC is demonstrated in sandwich-cultured rat and human hepatocytes (Chandra et al., 2005b).

Conclusions

Hepatic MRP3 is expressed at high levels in humans and mice, in comparison to rats, and it is localized to the basolateral membrane domain of hepatocytes. It is also present in bile duct epithelial cells. Hepatic MRP3/Mrp3 gene expression is induced by CAR agonists and Nrf2 activators in rodents. MRP3/Mrp3 gene expression is induced by PXR agonists in humans and mice at the transcriptional level, but not in rats. MRP3/Mrp3 expression is also induced by AhR agonists in mice, but not in rats or humans, and by PPAR α agonists in mice, but not

in rats. Decosahexaenoic acid, phenolic acids, 17 α -ethynylestradiol, dehydroepiandrosterone, and SIN also induce MRP3/Mrp3 gene expression.

Hepatic MRP3/Mrp3 expression is induced in chronic kidney disease, cholestasis, primary biliary cirrhosis, by bile-acid treatment, bile duct ligation, during hepatocarcinogenesis, 90% partial hepatectomy-induced liver regeneration, in hepatic progenitor cells, nonalcoholic steatohepatitis, Crigler-Najjar syndrome type I, Dubin-Johnson syndrome, and in *Mrp2*-, *Rdx*-, *Osta*-, *Tnfr1*-, *Il6*-, *Pxr*-, *Gclc*-, and *Keap1*-knockout rodents, as well as *Atp8b1*-knockdown rats. By contrast, MRP3/Mrp3 expression is suppressed in collagen-induced arthritis and *Nrf2*- and *RXR α* -knockout mice. Hepatic MRP3/Mrp3 expression does not change in adjuvant-induced arthritis, hepatoblastoma, during 70% hepatectomy-induced liver regeneration, simple fatty liver, liver ischemia-reperfusion, in hepatitis, diabetes in mice, or in *Mrp4*-, *Mrp6*-, *Abcg2*-, *Mdr2*-, *Il1r*-, *IKK β* -, *Icam*-, *Car*-, *Fxr*-, *Ppara*-, and *Hnf1 α* -knockout mice. Inconsistent observations of MRP3/Mrp3 expression are reported under inflammatory conditions and in hepatocellular carcinomas.

Human, rat, and mouse MRP3/Mrp3 genes have TATA-less promoters. Human MRP3 gene promoter contains consensus sequences for the transcription factors, AP1, AP2, N-myc, and SP1, and α -1 fetoprotein transcription factor (FTF)-like elements. Transcription factors SP1, *RXR α* , and RAR are involved in MRP3 gene expression. Certain SNPs affect MRP3 gene expression. The rat *Mrp3* gene also contains putative binding sites for C/EBP and SP1. SP1, SP3, and C/EBP are involved in the regulation of *Mrp3* gene expression. The mouse *Mrp3* gene contains a VDR response element (VDRE), while its expression is minimally regulated by VDR in the liver. Induction of MRP3/Mrp3 gene expression by PXR and PPAR α activation is species specific. The Keap1-Nrf2 pathway plays a key role in the regulation of liver MRP3/Mrp3 in response to oxidative stress. *Mrp3*/MRP3 gene expression is transiently regulated by cAMP and PKC. Coordinated expression of MRP2 and MRP3 genes in cholestasis has been observed; however, the mechanism remains to be investigated further.

Regulation of hepatic ABCC4/MRP4 transporters by xenobiotics and in disease states

Function of MRP4/Mrp4

MRP4 is an efflux pump found in polarized cells in tissues, such as prostate, kidney, liver, choroid plexus, brain capillary endothelium, testis, ovary, adrenal gland, various blood cells, and neurons. Its substrates include endogenous compounds, such as cyclic nucleotides, eicosanoids, urate, and conjugated steroids involved in cell signaling and a variety of drugs and their metabolites, such as antiviral, cytostatic, antibiotics, and cardiovascular drugs. Thus, MRP4 plays important physiological and pharmacological roles. The versatile transport function of MRP4 and its potential as a new therapeutic target to modulate various pathophysiological signalling processes are reviewed (Borst et al., 2007; Russel et al., 2008).

MRP4/Mrp4 expression in normal liver

Human liver MRP4 is expressed at very low levels. The human MRP4 gene was mapped by using fluorescence *in situ* hybridization to chromosome band 13q32 (Kool et al., 1997; Lee

et al., 1998). MRP4 mRNA and protein expression is highly variable, with 38- and 45-fold variability, respectively (Gradhand et al., 2008). In rats, liver Mrp4 mRNA and protein are expressed at very low levels (Chen and Klaassen, 2004; Halwachs et al., 2005; Donner et al., 2007; Lu and Klaassen, 2008), and protein is equally distributed in pericentral and periportal hepatocytes (Donner et al., 2007). Mouse liver Mrp4 expression is also very low (Maher et al., 2005b; Aleksunes et al., 2008a; Campion et al., 2008; Maher et al., 2008; Tanaka et al., 2009). MRP4 is localized to the basolateral membrane of human, rat, and mouse hepatocytes (Rius et al., 2003).

In mice, a gender difference in hepatic Mrp4 expression is noted, with higher levels in females than in males (Maher et al., 2005b; Petrick and Klaassen, 2007; Cheng et al., 2008). During development in mice from prenatal day -2 to 45 days after birth, Mrp4 expression is maximal at birth and decreased by about 70% by 2 weeks of age, but is relatively constant thereafter (Maher et al., 2005b).

In WK rats, liver basal Mrp4 mRNA expression is slightly higher in males than in females (Slitt et al., 2006b). In SD rats, liver Mrp4 mRNA expression levels are very low and do not change with gender (Lu and Klaassen, 2008).

Regulation of hepatic MRP4/Mrp4 expression by xenobiotics

In patients with gallstones scheduled for cholecystectomy, treatment with either rifampicin for 1 week or ursodeoxycholic acid for 3 weeks does not change MRP4 mRNA expression significantly. Treatment of these patients with rifampicin does not change MRP4 protein expression, while treatment with ursodeoxycholic acid induces protein expression (Marschall et al., 2005). In patients with primary biliary cirrhosis III or IV, MRP4 mRNA and protein expression do not change by ursodeoxycholic acid (Zollner et al., 2007). However, in mice fed ursodeoxycholic acid, liver Mrp4 mRNA and protein levels are induced significantly. This Mrp4 induction is independent of FXR (Zollner et al., 2006).

Among patients with biliary obstruction due to bile duct carcinoma treated with or without inchinkoto, there is no difference in MRP4 mRNA or protein expression from normal livers (Watanabe et al., 2009).

In patients after toxic acetaminophen ingestion, MRP4 mRNA and protein levels are significantly increased (Barnes et al., 2007). Similarly, in male C57BL/6J mice, Mrp4 mRNA levels are induced markedly by 400 mg/kg of acetaminophen. Mrp4 mRNA does not change significantly with lower doses of acetaminophen (200–300 mg/kg). Maximal Mrp4 induction occurs at 48 hours after toxic acetaminophen treatment and upregulation is seen selectively in proliferating hepatocytes (Aleksunes et al., 2005, 2006, 2007, 2008a; Campion et al., 2008). Kupffer cell depletion prevents Mrp4 induction in mice treated with toxic doses of acetaminophen (Campion et al., 2008). When acetaminophen-pretreated mice (400 mg/kg) are challenged 48 hours later with a second, higher dose of acetaminophen (600 mg/kg), these mice exhibit dramatic increases in Mrp4 expression as well as enhanced hepatocyte proliferation. Inhibition of hepatocyte replication by colchicine not only restores sensitivity to acetaminophen toxicity, but also blocks Mrp4 induction. Staining for Mrp4 and proliferating cell nuclear antigen (PCNA) in acetaminophen-pretreated and challenged mice

is very strong and colocalizes to centrilobular hepatocytes. Colchicine interferes not only with hepatocyte proliferation, but also with Mrp4 induction (Aleksunes et al., 2008a).

In male C57BL/6J mice treated with carbon tetrachloride (25 μ L/kg), liver Mrp4 mRNA and protein levels are also induced significantly. Double immunofluorescence imaging demonstrates upregulation of Mrp4 protein in hepatocytes adjacent to the central vein. Treatment with a lower dose of carbon tetrachloride (10 μ L/kg) also increases Mrp4 mRNA levels (Aleksunes et al., 2005, 2006). Similarly, hepatic Mrp4 mRNA is increased in rats by carbon tetrachloride treatment (Okumura et al., 2007).

In sexually dimorphic male and female WK rats, Mrp4 mRNA is induced by the Nrf2 activator, oltipraz, without any apparent difference between genders (Merrell et al., 2008). In Sasco SD rats treated with streptozotocin and the Nrf2 activator, ethoxyquin, liver Mrp4 mRNA is induced (Chen and Klaassen, 2004). Induction of Mrp4 expression is also observed in sandwich-cultured primary rat hepatocytes after buthionine sulfoximine exposure (Lee et al., 2008). Similarly, in C57BL/6 mice, liver Mrp4 expression is induced by Nrf2 activators butylated hydroxyanisole, oltipraz, or ethoxyquin treatment. This induction was shown to be Nrf2-dependent (Maher et al., 2005a, 2007).

In C57BL/6 mice treated with the PPAR α ligands, clofibrate, diethylhexylphthalate, and ciprofibrate, there is no induction of liver Mrp4 mRNA (Maher et al., 2005a). However, treatment with PPAR α agonists perfluorodecanoic and perfluorooctanoic acids induce hepatic Mrp4 mRNA and protein expression. Pronounced induction of Mrp4 is observed in centrilobular hepatocytes after perfluorodecanoic-acid exposure (Maher et al., 2008). In male CD-1 mice treated with the PPAR α agonist, clofibrate, for 10 days, Mrp4 mRNA and protein levels are induced significantly, in comparison to controls. The same changes are observed in sv/129 male mice. Mrp4 is induced dramatically in hepatocytes immediately surrounding the central vein (Moffit et al., 2006). However, in Sasco SD rats treated with clofibrate, diethylhexylphthalate, and perfluorodecanoic acid, Mrp4 mRNA does not change (Chen and Klaassen, 2004).

In Sasco SD rats, liver Mrp4 mRNA is not changed by aryl hydrocarbon receptor ligands TCDD and β -naphthoflavone, PXR ligands pregnenolone 16 α -carbonitrile, spironolactone, and dexamethasone, or the CYP2E1 inducers, isoniazid and acetylsalicylic acid (Chen and Klaassen, 2004). Similarly, in C57BL/6 mice, Mrp4 is not changed by some of the same xenobiotics, except that PXR ligands pregnenolone 16 α -carbonitrile, spironolactone, and dexamethasone tend to decrease Mrp4 mRNA levels (Maher et al., 2005a).

Treatment of mice with 1,4-bis-[2-(3,5-dichloropyridyloxy)] benzene (TCPOBOP) for 3–4 days upregulates liver Mrp4 mRNA levels (Maher et al., 2005a; Wagner et al., 2005; Petrick and Klaassen, 2007). In C57BL/6 mice, Mrp4 mRNA induction by TCPOBOP in females is twice as high as in males. However, treatment with TCPOBOP for 12 hours or TCDD for 4 days does not induce liver Mrp4 mRNA expression, and pretreatment with TCDD, followed by TCPOBOP dosing, resulted in Mrp4 mRNA induction in mice (Petrick and Klaassen, 2007). In mice treated with phenobarbital, liver Mrp4 levels are induced, with no gender differences in mRNA induction. However, protein induction is greater in females (Assem et

al., 2004; Wagner et al., 2005). In rats, liver Mrp4 mRNA is not induced by the CAR activators, phenobarbital and diallyl sulfide (Chen and Klaassen, 2004; Merrell et al., 2008).

In male SD rats, liver Mrp4 mRNA levels are increased at 12 hours and remain elevated 4 days after transstilbene oxide treatment. In WK rats treated with transstilbene oxide, liver Mrp4 mRNA expression is induced, with lower induction detected in females than in males (Slitt et al., 2006b). In male C57BL/6 mice treated with trans-stilbene oxide for 4 days, liver Mrp4 expression is not significantly increased, although there is a trend for induction. In membrane fractions, Mrp4 levels are increased (Slitt et al., 2006a).

In C57BL/6J mice exposed to the periportal hepatotoxicant, allyl alcohol, Mrp4 is induced at 12 and 24 hours after treatment. Enhanced Mrp4 staining is seen exclusively in centrilobular hepatocytes. Removal of Kupffer cells by clodronate liposomes results in higher toxicity and Mrp4 protein expression in response to allyl alcohol exposure, while induction of Mrp4 still occurs in liver regions away from areas of allyl-alcohol damage (Campion et al., 2009).

Regulation of hepatic MRP4/Mrp4 expression in chronic kidney disease

In SD rats with chronic kidney disease induced by 5/6 nephrectomy, liver Mrp4 mRNA expression levels do not change at 7 weeks after surgery (Lu and Klaassen, 2008).

Regulation of hepatic MRP4/Mrp4 expression in diabetes

In SD rats with type 2 diabetes or in obese Zucker rats, Mrp4 protein levels do not change (Geier et al., 2005a; Nowicki et al., 2008). In male ob/ob mice, liver Mrp4 mRNA and protein expression levels are elevated. Mrp4 protein expression is increased markedly in both genders, in comparison to their wild-type counterparts (Cheng et al., 2008).

Regulation of hepatic MRP4/Mrp4 expression in cancers and tumors

In patients with hepatocellular carcinomas, MRP4 mRNA expression is significantly higher in cancerous portions than noncancerous portions (Moustafa et al., 2004).

Regulation of hepatic MRP4/Mrp4 expression in cholestasis

In adult patients with cholestasis, liver MRP4 levels are significantly increased, in comparison to noncholestatic livers (PFIC) (Keitel et al., 2005; Barnes et al., 2007; Gradhand et al., 2008). In patients with primary biliary cirrhosis III and IV, MRP4 protein is induced, whereas mRNA levels remain unchanged in patients with latestage primary biliary cirrhosis, in comparison to noncholestatic controls (Zollner et al., 2007). In children with late-stage cholestasis, MRP4 is increased (Chen et al., 2008). In patients with obstructive jaundice caused by periampullary tumor growth, liver MRP4 mRNA expression is not significantly different from nonjaundiced patients, although levels tend to be elevated in the cholestatic group, in comparison to the control (Schaap et al., 2009).

In a novel mouse model of hepatobiliary injury and biliary fibrosis of cholangiopathy induced by feeding 3,5-diethoxycarbonyl-1,4-dihydrocollidine, Mrp4 mRNA and protein are significantly increased over time after 4 weeks (Fickert et al., 2007).

Regulation of hepatic MRP4/Mrp4 expression by bile acid treatment

Treatment of mice with a cholic-acid-containing diet does not change significantly liver Mrp4 mRNA or protein expression (Zollner et al., 2006). However, Teng and Piquette-Miller observed that Mrp4 mRNA levels are induced in mice fed a cholic-acid-supplemented diet, but no significant changes were seen in mice fed a diet containing cholic acid in combination with pregnenolone 16 α -carbonitrile, in comparison to standard diet controls (Teng and Piquette-Miller, 2007).

Treatment of C57BL/6 mice with a lithocholic-acid-containing diet for 4 days to induce intrahepatic cholestasis increases Mrp4 mRNA and protein expression. Pretreatment with TCPOBOP for 3 days, and then cotreatment with lithocholic acid for additional 4 days, enhances significantly Mrp4 induction by lithocholic acid alone. In contrast, pretreatment with phenobarbital or oltipraz does not enhance Mrp4 mRNA induction by lithocholic acid. However, pretreatment with oltipraz enhances markedly Mrp4 protein induction by lithocholic acid (Beilke et al., 2008). These results suggested that regulation of the induction of Mrp4 expression by TCPOBOP/lithocholic acid could happen at the transcriptional level, while induction by oltipraz/lithocholic acid may involve translational events.

Treatment of human or rat hepatocytes with 6 α -ethyl chenodeoxycholic acid for 24 hours does not change Mrp4 mRNA levels (Cai et al., 2009).

Treatment of mice with ursodeoxycholic acid potently increases hepatic Mrp4 levels and enhances membranous localization of Mrp4 in an Nrf2-dependent manner (Okada et al., 2008).

Regulation of hepatic MRP4/Mrp4 expression by bile duct ligation

In C57BL, endotoxin-resistant strain C3H/HeJ, or an equivalent 129/Sv and C57BL/6 mixed background mice that underwent bile duct ligation for 3–14 days, Mrp4 mRNA levels were induced progressively up to 2.5-fold (Wagner et al., 2003; Mennone et al., 2006; Stedman et al., 2006; Lickteig et al., 2007b; Slitt et al., 2007; Wagner et al., 2007). In endotoxin-sensitive C57BL/6J mice that underwent common bile duct ligation, Mrp4 mRNA expression did not change significantly 3 days after surgery (Wagner et al., 2007).

In male SD rats with bile duct ligation, Mrp4 protein is induced progressively up to greater than 7-fold at 14 days. However, liver Mrp4 mRNA levels have no major changes, except for a marked induction at day 3 after surgery (Denk et al., 2004). In male SD rats, Mrp4 mRNA expression is even observed to decrease to 60% of control values at 3 and 7 days after bile duct ligation (Donner et al., 2007). Progressive upregulation of Mrp4 protein without corresponding changes in Mrp4 mRNA by obstructive cholestasis in rat livers suggests posttranscriptional regulation of Mrp4 expression in bile duct-ligated rats (Denk et al., 2004).

Regulation of hepatic MRP4/Mrp4 expression in response to inflammatory process

Treatment of human hepatocytes with TNF- α for 8, 24, or 48 hours does not change MRP4 mRNA levels. In contrast, exposure of human hepatocytes to IL-6 for 8 or 24 hours reduces MRP4 mRNA levels (Vee et al., 2009). Treatment of primary human hepatocytes with the

proinflammatory cytokine, IL-1 β , results in slight upregulation of MRP4 at 8 hours and reduction at 24 hours after treatment (Le Vee et al., 2008).

In male SD rats that treated with LPS, Mrp4 mRNA expression does not change significantly at 24 hours. Induction of Mrp4 protein in periportal and pericentral hepatocytes by LPS is similar and is not dependent on TNF- α or IL-1 (Donner et al., 2004, 2007).

Regulation of hepatic MRP4/Mrp4 expression during ischemia-reperfusion

In male SD rats that underwent partial hepatic ischemia for 60 minutes and reperfusion, Mrp4 mRNA and protein expression does not change up to 48 hours after this procedure (Tanaka et al., 2008).

Regulation of hepatic MRP4/Mrp4 expression in fatty liver

In male SD rats with nonalcoholic steatohepatitis (NASH) induced by feeding a methionine- and choline-deficient (MCD) diet for 8 weeks (Lickteig et al., 2007a) or mice with steatosis induced by feeding a MCD diet for 2 weeks (Gyamfi et al., 2009), liver Mrp4 mRNA and protein levels are increased strikingly. In SD rats with simple fatty liver induced by feeding a high-fat diet for 8 weeks, liver Mrp4 mRNA and protein levels have no significant changes (Lickteig et al., 2007a).

Regulation of hepatic MRP4/Mrp4 expression during liver regeneration

In Wistar rats treated with 2-acetylaminofluorene coupled with partial hepatectomy, liver Mrp4 mRNA levels are significantly increased, while in rats treated with 2-acetylaminofluorene or partial hepatectomy, Mrp4 does not change. Mrp4 mRNA levels are significantly increased in proliferating hepatocytes freshly isolated from male Wistar rats at 24 hours after 70% partial hepatectomy (Ros et al., 2003b).

Regulation of hepatic MRP4/Mrp4 expression in genetic diseases and gene-knockout animal models

In *Car*-knockout mice, basal hepatic Mrp4 expression tends to be lower than in wild types. Treatment of *Car*-knockout mice with trans-stilbene oxide increases liver Mrp4 expression slightly (Slitt et al., 2006a).

In *Fxr* nullizygous mice, Mrp4 expression is substantially increased and is further elevated by cholic-acid treatment (Schuetz et al., 2001). In *Fxr*-knockout mice, liver Mrp4 mRNA and protein levels are not significantly different from wild types (Kitada et al., 2003; Zollner et al., 2006). Treatment of these null mice with cholic and ursodeoxycholic acids in the diet for 7 days induces Mrp4 significantly (Zollner et al., 2006). There is no difference in liver Mrp4 mRNA expression between *Fxr*-knockout and wild-type mice treated with 1% lithocholic acid in the diet (Kitada et al., 2003). Mrp4 mRNA expression is not altered, either, in *Fxr*-knockout mice treated with α -naphthyl isothiocyanate (Cui et al., 2009). In *Fxr*-knockout mice with common bile duct ligation, Mrp4 mRNA and protein expression is induced at 7 days, in comparison to bile duct-ligated wild types (Wagner et al., 2003). *Fxr*-knockout bile duct-ligated mice showed Mrp4 mRNA levels up to 22-fold higher than sham-operated knockouts at 6 days after surgery. In *Pxr/Fxr*-double-knockout mice with

bile duct ligation, Mrp4 mRNA levels are induced 2–5-fold, which is similar to the value in bile duct-ligated wild-type mice (Stedman et al., 2006).

In *Pxr*-knockout mice, liver basal Mrp4 mRNA levels are not significantly different from wild-type mice. Induction of Mrp4 mRNA levels in these null mice is higher by cholic-acid treatment than by cotreatment with cholic acid and pregnenolone 16 α -carbonitrile (Teng and Piquette-Miller, 2007). Bile duct ligation induces Mrp4 mRNA levels in both PXR knockouts and wild-type mice (Stedman et al., 2006). Treatment with α -naphthyl isothiocyanate does not change Mrp4 mRNA expression in PXR knockouts (Cui et al., 2009).

In *Ppara*-knockout mice, basal hepatic Mrp4 mRNA levels are the same as in wild types. Lack of PPAR α prevents the Mrp4 induction by perfluorodecanoic acid and clofibrate seen in wild-type mice (Moffit et al., 2006; Maher et al., 2008).

In H-RXR α -knockout mice, basal hepatic Mrp4 mRNA levels are not different from wild types. In the mouse with liver steatosis induced by feeding a methionine and choline deficient (MCD) diet for 14 days, induction of hepatic Mrp4 mRNA level in H-RXR α -knockout mice is lower than that in wild-type mice (Gyamfi et al., 2009).

In *Nrf2*-knockout mice, constitutive hepatic Mrp4 mRNA expression is similar to wild types (Aleksunes et al., 2008b; Maher et al., 2008; Tanaka et al., 2009). Treatment with acetaminophen (400 mg/kg) for 48 hours does not result in the induction of Mrp4 expression in *Nrf2* nulls (Aleksunes et al., 2008b). Treatment of *Nrf2*-knockout or wild-type mice with α -naphthylisothiocyanate does not change liver Mrp4 mRNA levels, either (Tanaka et al., 2009). Pretreatment of *Nrf2*-knockout or wild-type mice with gadolinium chloride, a Kupffer cell toxicant, reduces Mrp4 mRNA induction by perfluorodecanoic acid (Maher et al., 2008). In *Keap1*-knockdown mice, basal liver Mrp4 expression is higher than in wild-type mice (Okada et al., 2008).

In hepatocyte-specific glutamate-cysteine ligase catalytic subunit-knockout mice, liver Mrp4 expression increases markedly. Nuclear *Nrf2* protein localization is also increased (Maher et al., 2007). In *Hnf1 α* -null mice, Mrp4 mRNA levels are significantly increased, in comparison to wild-type mice (Maher et al., 2006b).

In a Dubin-Johnson syndrome patient treated with rifampicin or concomitant administration of rifampicin and ursodeoxycholic acid, MRP4 did not change (Corpechot et al., 2006). In *Mrp2*-deficient male rats, liver Mrp4 protein levels do not change, in comparison to control Wistar rats (Johnson et al., 2006). However, in *Mrp2*-knockout mice, basal liver Mrp4 expression levels are increased by approximately 6-fold (Chu et al., 2006).

In *Mrp6*-knockout mice, liver Mrp4 mRNA levels are not significantly different from wild types (Li et al., 2007).

In *Mdr2*-knockout mice, there is no difference in basal Mrp4 mRNA level, in comparison to wild types. Mrp4 expression is induced significantly by 24-norursodeoxycholic acid for 4 weeks, but not by ursodeoxycholic acid treatment (Fickert et al., 2006).

In Atp8b1-deficient rat hepatocytes, Mrp4 basal mRNA levels are upregulated, whereas in ATP8B1-deficient human hepatocytes, MRP4 basal mRNA levels do not change. Treatment of ATP8B1/Atp8b1-knockdown human or rat hepatocytes with 6 α -ethyl chenodeoxycholic acid for 24 hours does not change Mrp4 mRNA levels (Cai et al., 2009).

In *Tnfr1*-knockout, *Il1r1*-knockout, and *Il6*-knockout mice, Mrp4 mRNA levels are similar to those in wild types after saline treatment. LPS treatment does not change Mrp4 mRNA levels in these mice. Bile duct ligation significantly increases liver Mrp4 mRNA levels in *Il1r1*-knockout and *Il6*-knockout mice, but does not change Mrp4 mRNA levels in *Tnfr1*-knockouts. *Il6*-knockout sham-operated mice showed significant decreases in Mrp4 mRNA levels, in comparison to sham-operated wild types (Lickteig et al., 2007b).

There is no difference in hepatic Mrp4 mRNA levels in liver-specific I κ B kinase β (IKK β)-deficient (*IKK β* hep) mice and *IKK β* -floxed [*IKK β /f/f*] mice after saline treatment, LPS treatment, or bile duct ligation. Mrp4 mRNA levels are lower in *IKK β* hep mice than in *IKK β* (f/f) mice after sham operation of bile duct ligation (Lickteig et al., 2007b).

Among *Icam*-knockout mice, endotoxin-sensitive wild types, and Fas-receptor-deficient *lpr* mice of the C57BL/6J strain, there are no differences in Mrp4 mRNA expression after sham operation or common bile duct ligation. Common bile duct ligation does not change Mrp4 mRNA expression significantly 3 days after surgery in all these mice (Wagner et al., 2007). In contrast, Mrp4 mRNA expression levels are significantly induced in endotoxin-resistant wild types and *lpr* mice of the C3H/HeJ strain after common bile duct ligation. There is no difference in Mrp4 mRNA expression between these mice after sham operation or common bile duct ligation (Wagner et al., 2007).

Regulation of hepatic MRP4/Mrp4 expression in cell lines by xenobiotics

In human hepatocellular carcinoma cell line HepG2 cells, MRP4 mRNA is expressed at very low levels (Schrenk et al., 2001), lower than in the human liver (Hilgendorf et al., 2007). MRP4 protein is localized to the basolateral membrane in HepG2 cells (Rius et al., 2003). In the Huh7 cells resistant to cisplatin, MRP4 mRNA expression is increased, in comparison to the parental cells (Wakamatsu et al., 2007). In HepaRG cells, MRP4 has been detected and its expression is increased by the proinflammatory cytokine, IL-1 β (Le Vee et al., 2008).

In Hepa-1 cells, Mrp4 is induced dose-dependently by known Nrf2 activators, such as 1-chloro-2,4-dinitrobenzene, oltipraz, ethoxyquin, and sulforaphane (Maher et al., 2007).

In activated hepatic stellate cells from rats, Mrp4 levels are similar to that in hepatocytes. Cytokines or oxidative stress do not change Mrp4 expression in activated hepatic stellate cells from rats (Hannivoort et al., 2008).

In cultured RLF ϕ 13 cells and freshly isolated cholangiocytes from rats, Mrp4 mRNA is expressed at higher levels than in freshly isolated Thy-1-positive cells or freshly isolated hepatocytes from rats (Ros et al., 2003b).

In HPCT-1E3 cells, a fusion cell line between primary rat hepatocytes and Fao Reuber hepatoma cells (H35), Mrp4 is expressed at low levels (Halwachs et al., 2005).

Mechanisms of regulation of hepatic MRP4/Mrp4 expression

Human, mouse, and rat MRP4/Mrp4 gene promoters reside in GC-rich regions and have no TATA box. Human MRP4 gene basal transcription activity of the promoter-proximal region is constitutively active. The sequence between –116 and –216 bp (relative to translation start site) is required for maximal activity. The basal transcription activity in the liver is controlled by transcription-activating factors and repressive factors. The activating factors might include NRF1, NFE2L2, SP2, STAT1, KLF10, and TFAP2A; the repressive factors might include HES1, KLF15, and ZFP161. HES1 appears to be a major repressive factor in the promoter-proximal region. NRF1 is a transactivator for human MRP4 gene expression in response to oxidative stress. NF- κ B p65 does not appear to be involved in the transcriptional activation of human MRP4 gene (Gu and Manautou, 2009).

Specific agonists and gene-knockout mice studies indicate that PXR and FXR are not likely involved in the regulation of MRP4/Mrp4 gene expression, while PPAR α is. Activation of MRP4/Mrp4 gene expression is dependent on the nuclear receptor, CAR. CAR is required for the activation of mouse Mrp4 promoter, and activation of CAR upregulates Mrp4 in murine and human liver cells (Assem et al., 2004; Donner et al., 2007).

Studies using Nrf2 activators and gene-knockout mouse indicate that Nrf2, an effector in response to oxidative stress, is a transactivator of Mrp4 gene expression. Chromatin immunoprecipitation assays show that Nrf2 binds to an antioxidant response element in the promoter region of mouse Mrp4 gene (–3,767 bp) in Hepa1c1c7 cells treated with the Nrf2 activator, tert-butylhydroquinone (Maher et al., 2007; Okada et al., 2008).

MRP4 mRNA and protein expression and its cellular localization do not appear to be significantly associated with known polymorphisms (Gradhand et al., 2008).

Conclusions

MRP4/Mrp4 expression in liver is low and localized to the basolateral membrane of human, rat, and mouse hepatocytes. Liver MRP4/Mrp4 expression in humans and rats is refractory to treatment with ligands for PXR, FXR, AhR, PPAR α , and CAR. MRP4/Mrp4 expression is suppressed by PXR agonists, while being induced by FXR, PPAR α , and CAR agonists in mice.

Hepatic MRP4/Mrp4 expression is upregulated by Nrf2 activators and hepatotoxicants, such as acetaminophen and carbon tetrachloride, which produce oxidative stress with acute treatment at high doses. MRP4/Mrp4 expression also increases under certain disease states, such as conditions leading to late-stage cholestasis, late-stage primary biliary cirrhosis, hepatocellular carcinoma, steatosis, diabetes in mice, liver regeneration, chronic kidney disease, and by bile duct ligation. Mrp4 expression is also increased in *Gclc*-knockout mice and *Atp8b1*-knockdown rat hepatocytes. By contrast, MRP4/Mrp4 expression is decreased in *Il6*- and *Car*-knockout mice and does not change during liver ischemia-reperfusion, diabetes in rat, simple fatty liver, in *Fxr*-, *Pxr*-, *Ppara*-, H-RXR α -, *Nrf2*-, *Mrp2*-, *Mrp6*-, *Mdr2*-, *Icam*-, and Fas-knockout mice, as well as ATP8B1-knockdown human hepatocytes.

The human, mouse, and rat MRP4 promoters reside in GC-rich regions and have no TATA box. The human MRP4 gene promoter is constitutively active in the liver. The basal transcription activity is controlled by both transcription-activating and -repressive factors. The activating factors might include NRF1, NFE2L2, SP2, STAT1, KLF10, and TFAP2A; the repressive factors might include HES1, KLF15, and ZFP161. HES1 appears to be a major repressive factor in the promoter-proximal region. NRF1 is a transactivator for human MRP4 gene expression in response to oxidative stress. PXR and FXR are not likely involved in the regulation of MRP4/Mrp4 gene expression, while PPAR α and CAR are. The Keap1-Nrf2 pathway is involved in mouse Mrp4 gene expression under oxidative stress. MRP4 mRNA and protein expression and cellular localization are not significantly associated with known polymorphisms in the human MRP4 gene.

Regulation of hepatic ABCC5/MRP5 transporters by xenobiotics and in disease states

Function of MRP5/Mrp5

MRP5 is found in every tissue in low levels. Substrates for MRP5 include cyclic nucleotide or nucleotide analogs. The precise physiological function of MRP5 remains unknown (Borst et al., 2007).

MRP5/Mrp5 expression in normal liver

In human livers, MRP5 is expressed at low levels (Kool et al., 1997). In C57BL/6 mice, liver Mrp5 mRNA expression is relatively moderate. No gender-specific expression patterns have been observed (Maher et al., 2005b). A similar expression profile has been observed in SD and WK rats (Maher et al., 2006a; Lu and Klaassen, 2008).

Regulation of hepatic MRP5/Mrp5 expression by xenobiotics

In SD rats fed fat-free total parenteral nutrition for 4 days, Mrp5 mRNA levels are significantly increased, in comparison to rats fed a normal diet or total parenteral nutrition with 20% of the calories derived from fat (soybean oil emulsion) (Nishimura et al., 2005).

In WK rats, liver Mrp5 mRNA expression is induced by phenobarbital in females, but not males (Merrell et al., 2008). By contrast, treatment of SD rats with phenobarbital slightly represses Mrp5 expression (Maher et al., 2006a).

In C57BL/6J mice treated with allyl alcohol, Mrp5 mRNA expression increases transiently at 12 and 24 hours, while protein expression shows persistently higher levels at 24 hours after exposure. Kupffer cell function is not required for these changes (Campion et al., 2009).

In male SD rats, Mrp5 mRNA expression does not change by treatment with 2,3,7,8-tetrachlorodibenzop-dioxin (TCDD), indole-3-carbinol, β -naphthoflavone, diallyl sulfide, pregnenolone-16 α -carbonitrile, spironolactone, dexamethasone, clofibrate, diethylhexylphthalate, perfluorodecanoic acid, oltipraz, ethoxyquin, isoniazid, acetylsalicylic acid, streptozotocin (Maher et al., 2006a), or trans-stilbene oxide (Slitt et al.,

2006b). In WK rats, oltipraz treatment does not change Mrp5 mRNA expression (Merrell et al., 2008). In acetaminophen-overdose patients, liver MRP5 mRNA levels are not significantly changed, but protein expression is significantly increased (Barnes et al., 2007).

Regulation of hepatic MRP5/Mrp5 expression in chronic kidney disease

In SD rats with chronic kidney disease induced by nephrectomy, Mrp5 mRNA expression levels do not change (Lu and Klaassen, 2008).

Regulation of hepatic MRP5/Mrp5 expression in diabetes

In SD rats with type 2 diabetes induced by feeding a high-fat diet, followed by a single dose of streptozotocin, hepatic Mrp5 protein levels are decreased dramatically (Nowicki et al., 2008).

In ob/ob female mice, liver Mrp5 mRNA and protein expression does not differ significantly from in wild types. In contrast, in ob/ob male mice, liver Mrp5 mRNA expression is slightly lower than that in wild types, and Mrp5 protein expression is even higher, in comparison to wild types (Cheng et al., 2008).

Regulation of hepatic MRP5/Mrp5 expression in cancers and tumors

In patients with hepatocellular carcinoma, MRP5 mRNA expression is very low or nondetectable in cancerous and noncancerous portions of the liver (Moustafa et al., 2004)

Regulation of hepatic MRP5/Mrp5 expression in primary biliary cirrhosis and by bile-acid treatment and bile duct ligation

In primary biliary cirrhosis patients, both MRP5 mRNA and protein levels are significantly increased, in comparison to normal liver specimens (Barnes et al., 2007).

In C57BL/6 mice with intrahepatic cholestasis induced by lithocholic acid treatment for 4 days, Mrp5 mRNA and protein expression is increased. Pretreatment with TCPOBOP or phenobarbital for 3 days, and then cotreatment with lithocholic acid for additional 4 days, tends to suppress Mrp5 mRNA and protein expression. Pretreatment with oltipraz, followed by cotreatment with oltipraz and lithocholic acid, suppresses Mrp5 mRNA expression, but does not change Mrp5 protein, in comparison to lithocholic-acid treatment alone (Beilke et al., 2008). Treatment of mice with bile acid does not affect Mrp5 expression (Zollner et al., 2006).

In bile duct–ligated male rats, Mrp5 expression tends to increase 3 days after surgery. However, this increase is not statistically significant (Maher et al., 2006a). In mice with bile duct ligation, Mrp5 mRNA expression is increased up to 6-fold at 14 days after surgery (Slitt et al., 2007).

Regulation of hepatic MRP5/Mrp5 expression in response to inflammatory process

In rats treated with LPS, liver Mrp5 mRNA expression is induced strongly (Donner et al., 2004), while Cherrington et al. observed that SD rats treated with LPS had slightly lower liver Mrp5 mRNA levels immediately after treatment, followed by a biphasic increase and

decrease. None of these changes were statistically significant. Dexamethasone, aminoguanidine, or gadolinium chloride did not change the effect of LPS on Mrp5 mRNA levels (Cherrington et al., 2004).

Regulation of hepatic MRP5/Mrp5 expression in fatty liver and steatosis

In rats with simple fatty liver induced by feeding a high-fat diet for 8 weeks, liver Mrp5 mRNA levels are increased. In rats with NASH induced by feeding an MCD diet, Mrp5 mRNA levels do not change (Lickteig et al., 2007a).

Regulation of hepatic MRP5/Mrp5 expression in genetic diseases and gene knockout animal models

In male or female *Mrp2*-knockout mice, Mrp5 mRNA expression levels are very low (Chu et al., 2006). In *Mrp6*-knockout mice, there are no significant differences in Mrp5 mRNA levels, in comparison to wild types (Li et al., 2007).

In *Hnf1 α* -null mice, MRP5 mRNA expression is significantly increased, in comparison to wild types (Maher et al., 2006b).

Basal Mrp5 mRNA levels are the same between *Fxr*-knockout and wild-type mouse livers. Treatment with bile acid does not change Mrp5 expression in *Fxr*-knockouts (Zollner et al., 2006).

In *Il6*-, *Il1r1*-, and *Tnfr1*-knockout mice, basal Mrp5 mRNA levels are lower than in wild-type mice after saline treatment. LPS treatment increases Mrp5 mRNA levels in all these knockouts. Bile duct ligation increases Mrp5 mRNA levels in all these strains of knockouts and in wildtype mice, in comparison to the sham operation. After sham operation, Mrp5 mRNA levels in *Tnfr1*-knockout mice are higher than in wild types, but not statistically significant (Lickteig et al., 2007b).

In *IKK β* hep mice, Mrp5 mRNA levels are decreased relative to those in *IKK β* (f/f) mice. Bile duct ligation or LPS treatment significantly decreases Mrp5 mRNA expression in *IKK β* hep mice (Lickteig et al., 2007b).

Regulation of hepatic MRP5/Mrp5 expression in cell lines by xenobiotics

In HepG2 cells, MRP5 expression is detectable (Schrenk et al., 2001), and its levels are lower than those in the normal human liver (Hilgendorf et al., 2007). Treatment of HepG2 cells with 2-acetylaminofluorene, cisplatin, and rifampicin induces MRP5 expression, while treatment with dexamethasone and tamoxifen does not change MRP5 mRNA expression (Schrenk et al., 2001).

Among human normal liver (L-02) cells, the human hepatocarcinoma cell line, BEL, and its adriamycin-resistant counterpart (BEL/ADM cell line), MRP5 mRNA expression shows significant differences, with BEL/ADM>BEL>L-02 (Zhang et al., 2008). In the hepatocellular carcinoma cell line (Huh7 cells) resistant to cisplatin, MRP5 mRNA expression increases, in comparison to the parental cells (Wakamatsu et al., 2007).

In the hepatocytoma fusion cell line, HPCT-1E3, Mrp5 expression is detectable (Halwachs et al., 2005). However, in both activated rat hepatic stellate cells and rat hepatocytes, Mrp5 is not detectable (Hannivoort et al., 2008).

Conclusions

MRP5/Mrp5 is expressed at low levels in the liver across species. Hepatic MRP5/Mrp5 expression is relatively stable and resistant to xenobiotic treatments. However, it is induced by total parenteral nutrition and toxic allyl alcohol treatment. Hepatic MRP5/Mrp5 expression is also induced in chronic kidney diseases, simple fatty liver, cholestasis, type-2 diabetes in mice, and by bile duct ligation. *Hnf1 α* -knockout mice have higher Mrp5 expression than wild-type mice. MRP5/Mrp5 expression is lower in *Il6-Il1r1*-, and *Tnfr1*-knockout mice and in rats with type 2 diabetes. Inconsistent observations of MRP5/Mrp5 expression are reported under inflammation induced by LPS. Overall, little is known about the mechanisms of MRP5/Mrp5 gene expression and regulation.

Regulation of hepatic ABCC6/MRP6 transporters by xenobiotics and in disease states

Function of MRP6/Mrp6

MRP6 is primarily expressed in normal liver, predominantly in the basolateral membrane of hepatocytes. MRP6 is also highly expressed in the kidney, whereas very low levels of expression are found in a variety of other tissues. Mutations leading to loss of function in MRP6 cause *Pseudoxanthoma elasticum*, which is an autosomal recessively inherited multiorgan disorder that is associated with the accumulation of mineralized and fragmented elastic fibers in the skin, Bruch's membrane in the retina, and blood vessel walls. This condition is characterized by a variety of cardiovascular complications. MRP6 protein has been postulated to be involved in the active transport of intracellular molecules essential for extracellular matrix deposition or turnover of connective tissue at specific sites in the body. The precise physiological function and natural substrate(s) transported by MRP6 are unknown and remain to be elucidated (Ringpfeil et al., 2001; Hu et al., 2003; Bergen et al., 2007).

MRP6/Mrp6 expression in normal liver

In humans, MRP6 is expressed in livers and kidneys at high levels and in few other tissues at low to very low levels (Belinsky and Kruh, 1999; Kool et al., 1999; Scheffer et al., 2002a). MRP6 expression is also detected in primary hepatocytes cultured for 3 days (Jigorel et al., 2006).

In rodents, Mrp6 mRNA expression is highest in the liver among all tissues tested (Matsuzaki et al., 2005; Maher et al., 2006a; Douet et al., 2007). In the normal rat liver, Mrp6 is equally distributed to pericentral and periportal areas (Donner et al., 2004), and there are no gender differences in liver Mrp6 mRNA expression (Maher et al., 2006a; Merrell et al., 2008). However, de Zwart et al. showed that the mean Mrp6 levels in female adult rats are only 60% of that in males (de Zwart et al., 2008). In SD rats, liver Mrp6 mRNA expression during development prior to birth is about 3- (in males) and 6-fold (in

females) higher than the adult levels and decrease gradually to adult levels at day 26 after birth (de Zwart et al., 2008).

In rats at days 20–21 of pregnancy, liver Mrp6 mRNA expression is decreased by 60% (Cao et al., 2002). Mrp6 mRNA and protein expression is first detected at embryonic day 16 (E16). The adult phenotype of polarized Mrp6 expression is achieved at approximately postnatal day 12 (Gao et al., 2004).

In mice, liver Mrp6 mRNA expression is much higher than in any other tissue. Gender-specific expression patterns for Mrp6 are not observed. During development from –2 (prenatal day) to postnatal day 45, liver Mrp6 expression is not detectable from days –2 to 5. On postnatal day 10, liver Mrp6 mRNA levels are highest. Mrp6 expression decreases about 60% at postnatal day 15 and remains relatively constant thereafter (Maher et al., 2005b). In mice, liver Mrp6 mRNA expression is significantly higher in females than in males (Cheng et al., 2008).

Regulation of hepatic MRP6/Mrp6 expression by xenobiotics

In primary human hepatocytes, treatment with TCDD, rifampicin, or oltipraz does not change significantly MRP6 mRNA expression, while treatment with phenobarbital decreases it (Jigorel et al., 2006)

In mice treated with the AhR ligands, TCDD, polychlorinated biphenyl 126, and β -naphthoflavone, the CAR ligands, TCPOBOP and phenobarbital, or the Nrf2 activators, oltipraz, ethoxyquin, and butylated hydroxyanisole, liver Mrp6 expression is induced (Maher et al., 2005a).

In rats treated with phenobarbital or α -naphthylisothiocyanate, liver Mrp6 expression does not change significantly (Ogawa et al., 2000; Hagenbuch et al., 2001; Maher et al., 2006a; Merrell et al., 2008). In SD rats, liver Mrp6 mRNA expression is slightly repressed by dexamethasone or isoniazid treatment, while expression does not change with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), indole-3-carbinol, polychlorinated biphenyl 126, β -naphthoflavone, polychlorinated biphenyl 99, diallyl sulfide, pregnenolone-16 α -carbonitrile, spironolactone, clofibrate, diethylhexylphthalate, perfluorodecanoic acid, ethoxyquin, acetylsalicylic acid, and streptozotocin treatment (Maher et al., 2006a). Treatment of rats with oltipraz does not change liver Mrp6 mRNA expression, either (Maher et al., 2006a; Merrell et al., 2008).

Similarly, treatment of male mice or male SD rats with trans-stilbene oxide does not alter liver Mrp6 expression (Slitt et al., 2006a, 2006b).

In patients with acetaminophen overdose, MRP6 mRNA and protein levels do not change significantly, in comparison to normal liver specimens (Barnes et al., 2007), while treatment of rats with carbon tetrachloride decreases Mrp6 mRNA expression (Okumura et al., 2007).

In C57BL/6J mice treated with the periportal hepatotoxicant, allyl alcohol, Mrp6 mRNA expression is transiently decreased at 24 hours after treatment, while protein levels are not changed. Kupffer cell function is not required for these changes (Campion et al., 2009).

In male SD rats treated with fat-free total parenteral nutrition or total parenteral nutrition with 20% calories derived from fat (soybean oil emulsion), liver Mrp6 mRNA levels are significantly decreased, in comparison to rats fed a normal oral diet (Nishimura et al., 2005).

Regulation of hepatic MRP6/Mrp6 expression in chronic kidney disease and diabetes

In nephrectomy rats, Mrp6 mRNA is expressed highest in liver among all tissues tested and remains unchanged after surgery (Lu and Klaassen, 2008).

In ob/ob female mice, Mrp6 mRNA levels are decreased to 62% wild-type controls. In male ob/ob mice, no significant difference in Mrp6 mRNA expression is observed, in comparison to wild types. At protein level, Mrp6 expression in ob/ob male and female mice is decreased to 63% and 36% of their respective wild types (Cheng et al., 2008).

Regulation of hepatic MRP6/Mrp6 expression in primary biliary cirrhosis

In patients with primary biliary cirrhosis, liver MRP6 mRNA and protein levels have no significant changes, in comparison to normal liver specimens (Barnes et al., 2007). In patients with primary biliary cirrhosis I–III, MRP6 immunostaining intensity and mRNA levels are similar to liver samples from patients with fatty liver, metastatic liver tumor, rheumatoid diseases, and autoimmune hepatitis (Kojima et al., 2003).

Regulation of hepatic MRP6/Mrp6 expression by bile-acid treatment and bile duct ligation

In C57BL/6 mice pretreated with either phenobarbital, oltipraz, or TCPOBOP for 3 days, followed by cotreatment with lithocholic acid (which induces intrahepatic cholestasis) for 4 additional days, Mrp6 mRNA expression does not change, while Mrp6 protein is decreased in comparison to corn-oil controls (Beilke et al., 2008).

In rats with cholestasis induced by bile duct ligation, liver Mrp6 mRNA expression does not change (Ogawa et al., 2000; Maher et al., 2006a).

Regulation of hepatic MRP6/Mrp6 expression in response to inflammatory process

In rats treated with LPS, liver Mrp6 mRNA is upregulated, while Mrp6 protein expression is not significantly altered. LPS treatment does not change the staining pattern for Mrp6 either (Donner et al., 2004). However, Cherrington et al. showed that liver Mrp6 mRNA levels were dramatically decreased in SD rats, beginning approximately 6 hours after LPS administration. Dexamethasone, aminoguanidine, or gadolinium chloride did not change the effect of LPS on Mrp6 mRNA levels (Cherrington et al., 2004).

Regulation of hepatic MRP6/Mrp6 expression in fatty liver

In rats with simple fatty liver induced by feeding a high-fat diet for 8 weeks, Mrp6 mRNA levels are decreased in liver. In rats with NASH induced by feeding an MCD diet, Mrp6 mRNA levels are decreased strikingly (Lickteig et al., 2007a).

Regulation of hepatic MRP6/Mrp6 expression during liver regeneration

In rats with partial hepatectomy to induce liver regeneration, liver Mrp6 mRNA expression is downregulated at 3–48 hours following surgery (Dransfeld et al., 2005). However, in

proliferating hepatocytes freshly isolated from male Wistar rats 24 hours after 70% hepatectomy, Mrp6 mRNA levels do not change (Ros et al., 2003b).

Regulation of hepatic MRP6/Mrp6 expression in genetic diseases and gene-knockout animal models

In *Tnfr1*-knockout mice, liver Mrp6 mRNA expression is not significantly different from wild-type mice after saline treatment. LPS treatment significantly decreases liver Mrp6 mRNA expression in *Tnfr1*-knockout mice. Bile duct ligation also decreases liver Mrp6 mRNA expression. In sham-operated *Tnfr1*-knockout mice, Mrp6 mRNA expression is significantly increased, in comparison to sham-operated wild-type mice (Lickteig et al., 2007b).

In *Il1r1*-knockout mice, Mrp6 mRNA expression does not change significantly, in comparison to wild-type mice, by saline treatment, and it is significantly reduced in knockouts after LPS treatment, in comparison to saline treatment. Bile duct ligation in *Il1r1*-knockout mice results in decreased Mrp6 mRNA expression, in comparison to sham-operated animals. In sham-operated *Il1r1*-knockout mice, Mrp6 mRNA expression levels do not change significantly, in comparison to wild types (Lickteig et al., 2007b).

In *Il6*-knockout mice, basal liver Mrp6 mRNA expression levels are significantly reduced, in comparison to wild types, after saline treatment and are further reduced after LPS treatment. Bile duct ligation does not change liver Mrp6 mRNA expression in *Il6*-knockout mice, in comparison to the sham-operated animals. Further, in sham-operated *Il6*-knockout mice, Mrp6 mRNA expression levels do not change significantly, in comparison to the sham-operated wild types (Lickteig et al., 2007b).

In *Hnf1a*-null mice, liver Mrp6 mRNA levels are significantly decreased, in comparison to wild types (Maher et al., 2006b).

In *Fxr*-knockout mice, basal hepatic Mrp6 expression is not different from wild types. Treatment with bile acid does not change Mrp6 levels between *Fxr*-knockouts and wild-type mice (Zollner et al., 2006).

In male or female *Mrp2*-knockout mice, Mrp6 mRNA levels are not significantly altered (Chu et al., 2006). In Eisai hyperbilirubinuria rats, hepatic Mrp6 mRNA expression is not significantly different from normal SD rats, either (Kuroda et al., 2004).

There is no difference in hepatic Mrp6 mRNA levels in liver-specific I κ B kinase β (IKK β)-deficient (*IKK β* hep) and *IKK β* -floxed [*IKK β* (f/f)] mice after saline treatment, LPS treatment, bile duct ligation, or sham operation (Lickteig et al., 2007b).

Regulation of hepatic MRP6/Mrp6 expression in cell lines by xenobiotics

In Huh7 or HepG2 cells, MRP6 is detected. Treatment with rifampicin and other PXR activators does not alter MRP6 mRNA levels (Lee and Piquette-Miller, 2001; Teng et al., 2003). MRP6 expression in HepG2 cells is lower than the human liver (Hilgendorf et al., 2007).

In freshly isolated rat hepatocytes, Mrp6 mRNA is expressed at higher levels than in cultured RLF phi 13 cells, freshly isolated Thy-1 positive cells, or freshly isolated cholangiocytes. In freshly isolated Thy-1-positive and cultured RLF phi 13 progenitor cells, Mrp6 mRNA is only minimally expressed (Ros et al., 2003b).

In the hepatocytoma fusion cell line HPCT-1E3, and rat liver, Mrp6 expression is detected at very low levels (Halwachs et al., 2005).

In activated hepatic stellate cells from rats, no Mrp6 expression is detected (Hannivoort et al., 2008).

Mechanisms of regulation of hepatic MRP6/Mrp6 expression

Alignment of the 5'-flanking regions of the human, rat, and mouse MRP6/Mrp6 genes shows that murine and rat Mrp6 genes share extensive sequence identity (63%), while lower conservation is found between the human and rodent sequences. A CpG island region is present in the promoter-proximal region of the human MRP6 gene, but not in rodent sequences, although this proximal region is GC-rich (60.5%) in rodents and shares 78% identity between the mouse and rat. Despite reduced conservation in that region, highly conserved short DNA segments have been identified in all three species, suggesting that these segments could contain common regulatory elements involved in MRP6/Mrp6 gene regulation (Aranyi et al., 2005; Douet et al., 2006).

The mutation detection rate in the MRP6 coding region of bona fide *Pseudoxanthoma elasticum* (PXE) patients is only approximately 80%, which suggests that polymorphisms or mutations in the regulatory regions may contribute to the development of the disease (Aranyi et al., 2005). The transcription initiation site is found to be located at -37 bp relative to the translation initiation site. Two evolutionarily conserved sequence elements (R1 and R2) are embedded in CpG islands. R1 is -450 bp long and is located between -9.4 and -8.9 kb from the translation start site of the human MRP6 gene. No clearcut conserved response elements for transcription-factor binding sites could be detected in the R1 and R2 regions. The fragment from -145 to +72 bp confers partial transcriptional activity. The presence of a transcriptional activator sequence between -332 and +72 bp has also been identified, while the region between -718 and -332 bp contains repressor elements. The proximal promoter of the MRP6 gene is implicated in tissue-specific transcriptional regulation. Studies on DNA methylation of MRP6 and its pseudogenes have demonstrated a correlation between the methylation of the CpG islands in the proximal promoter and MRP6 expression levels. A DNA methylation-dependent activator sequence in the MRP6 promoter has been identified (Aranyi et al., 2005).

A specific NF- κ B-like sequence between -235 and -226 bp confers a high level of MRP6 expression in HepG2 hepatoma cells, inferring liver specificity. Transforming growth factor beta (TGF- β) upregulates, while TNF- α and interferon (IFN)-gamma downregulates MRP6 promoter activity in HepG2 cells. The responsiveness to TGF- β is shown to reside primarily within a SP1/Sp3 cognate-binding site from -58 to -49 bp. The activity of the MRP6 promoter is also shown to be markedly enhanced by SP1. Further, four additional transcription factors (AP2, USF-1, NF- κ B, and the epidermal growth receptor) are shown to

bind to a 2.6-kb promoter fragment, which contains their cognate-binding sequences. Collectively, human MRP6 displays tissue-specific gene expression, which can be modulated by proinflammatory cytokines (Jiang et al., 2006).

MRP6 gene expression is upregulated by retinoids, which act as agonists of the retinoid X receptor (RXR), rather than the retinoid A receptor (RAR) (Ratajewski et al., 2006).

Overexpression of ATF5, C/EBP α , C/EBP β , C/EBP γ , C/EBP ϵ , C/EBP δ , COUP-TFI, COUP-TFII, ETS2, FOXA1, FOXO1, HNF-1 α , HNF-4 α , HSF1, LEF1, LRH1, MEF2C, MYC, MZF1, NFATc4, NF- κ B, NFYA, NFYB, NFYC, p65/RelA, SATB1, STAT1, STAT3, STAT5A, STAT5B, STAT6, USF2, WT1, YY1, ZNF202, or ZNF35 in HepG2 cells does not change MRP6 reporter gene (–1313/+72) expression, while overexpression of PLAG1, PLAGL1, or SP1 increases it. Overexpression of GATA3 decreases MRP6 reporter gene (–1313/+72) expression. This suggests that PLAG1, PLAGL1, SP1, and GATA3 are involved in the regulation of MRP6 gene expression (Ratajewski et al., 2008).

MRP6 is a target gene for transcriptional induction by PLAG1 and PLAGL1 that are transcription factors from the PLAG family of cell-cycle progression-related DNA-binding proteins. Both these factors are shown to bind to the same single consensus-binding element in the MRP6 proximal promoter in cell lines of hepatic and renal origin. PLAG-mediated MRP6 transactivation may play an important role in determining the level of tissue-specific expression of this gene (Ratajewski et al., 2008).

The only polymorphism known to be common in PXE patients is located within one of the PLAG transcription-factor binding sites. This mutation negatively influences PLAG-mediated induction of MRP6 promoter in a reporter gene system. Moreover, site-directed mutagenesis of an analogous sequence within another PLAG-binding site both reduces PLAG binding and specifically represses MRP6 promoter activity in liver cells. Thus, a novel sequence determinant of liver-specific transcription of the MRP6 gene with direct relevance for at least some PXE patients has been identified (Ratajewski et al., 2009).

In silico analysis shows that in the mouse *Mrp6* gene promoter region, several GC-boxes corresponding to putative SP1 binding sites and a CCAAT-box at –94 bp from 5' ATG exist. Additional putative *cis*-acting liver-specific elements are identified as members of the HNF family and the leucine zipper C/EBP family (CCAAT/enhancer binding protein). Other potential transcription-factor binding sites for GATA-1 and NF-E2 (nuclear-factor erythroid 2) have been found. These transcription factors are known to regulate the expression of genes involved in erythroid development and maturation. Several putative environmental response *cis*-elements for NF- κ B, MTF-1 (metal transcription factor), SRF (serum response factor), and USF (upstream stimulating factor) are also present (Douet et al., 2006).

The promoter of mouse *Mrp6* gene is a TATA-less promoter requiring an intact CCAAT-box and SP1 binding for its basal activity. HNF-4 α and NF-E2 have been shown to enhance *Mrp6* promoter activity, using reporter-gene and chromatin immunoprecipitation assays. The involvement of both HNF-4 α and NF-E2 in *Mrp6* gene regulation suggests that *Mrp6* might be involved in detoxification processes related to hemoglobin or heme (Douet et al., 2006). The level of methylation of the mouse *Mrp6* promoter is associated with tissue-specific

expression. High and moderate levels of methylation correlate with low Mrp6 expression levels. CpG methylation of the Mrp6 proximal promoter region interferes with the binding of SP1, thereby inhibiting SP1-dependent transactivation (Douet et al., 2007). Aberrant splicing in the 3' end of the MRP6 mRNA results in a premature termination codon and thus low mature MRP6 protein expression (Matsuzaki et al., 2005).

Conclusions

MRP6/Mrp6 is expressed in liver at high levels across species. Its basal expression is regulated developmentally. Hepatic MRP6 expression is stable during exposure to xenobiotics that are agonists of AhR, PXR, and Nrf2 activators in human and rats. Hepatic MRP6/Mrp6 expression is suppressed in human hepatocytes by CAR agonists, whereas it is induced in mice by CAR agonists. However, Hepatic MRP6 expression is altered in liver diseases that involve liver-specific or physiological factors. Hepatic MRP6 expression is decreased in simple fatty liver, nonalcoholic steatohepatitis, liver regeneration, diabetic mice, and *Il6*- and *Hnf1α*-knockout mice. Hepatic MRP6 expression does not change in chronic kidney disease, by bile duct ligation, in primary biliary cirrhosis, or in *Tnfr1*-, *Il1r1*-, *Fxr*-, and *Mrp2*- knockout rodents. However, the association between changes in hepatic MRP6/Mrp6 gene expression and several disease states remains relatively understudied. Hepatic MRP6 expression has been reported to be differentially regulated under inflammatory conditions.

The human, rat, and mouse MRP6/Mrp6 gene promoters are TATA-less and GC-rich. Highly conserved DNA segments are short across all three species. The human MRP6 gene promoter has two regions containing CpG islands. It contains a specific NF-κB-like sequence, a SP1/Sp3 cognate-binding site, and cognate-binding sequences for AP2, USF-1, NF-κB, and epidermal growth receptor. PLAG1, PLAGL, SP1, and GATA3 regulate MRP6 gene expression. Human MRP6 gene expression is regulated in a tissue-specific manner at the transcriptional level and is modulated by proinflammatory cytokines. Methylation of the CpG island in the proximal promoter regulates MRP6 expression levels. An SNP within one of the PLAG transcription-factor binding sites represses human MRP6 gene expression.

The mouse Mrp6 gene promoter region contains several GC-boxes, a CCAAT-box, and putative *cis*-acting liver-specific elements for HNF and C/EBP, GATA-1 and NF-E2, NF-κB, MTF-1, SRF, and USF. An intact CCAAT-box and SP1 binding are required for its basal activity. The level of methylation of the mouse Mrp6 promoter is associated with tissue-specific expression. Aberrant splicing in the 3' end of the MRP6 mRNA results in low mature MRP6 protein expression.

Regulation of hepatic ABCC7/CFTR transporters by xenobiotics and in disease states

Function and expression of CFTR/Cftr

The ABCC7/CFTR (cystic fibrosis transmembrane conductance regulator) is a chloride channel and an atypical ABC protein. CFTR is found only in metazoans, where it plays a critical role in epithelial salt and fluid homeostasis. The passive bidirectional diffusion of

small inorganic anions it mediates is simpler, compared to the vectorial transport of other ABC transporters, but the control of the permeation pathway is more stringent than in the case of the ABC transporters (Aleksandrov et al., 2007).

Human CFTR mRNA is expressed in the gastrointestinal tract at high levels, whereas kidneys and lungs contain relatively low levels. The CFTR gene is also expressed in lymphocytes and lymphoblast cell lines. Variable inframe deletions of either exons 4, 9, or 12 are observed in CFTR mRNA. The complete loss of single exons occurs in 1–40% of all investigated tissues and cell lines with large donor-to-donor variation. Alternative splicing may produce various CFTR forms of different function and localization (Bremer et al., 1992).

During development, human CFTR is principally expressed in less-differentiated cells of endodermal origin. The highest levels of expression are seen in specific areas of the developing pancreas, liver, gallbladder, and intestine, with lower, but significant, levels detected in lung and trachea. Cell-specific localization of CFTR mRNA shows developmentally regulated expression in human fetal tissues (Tizzano et al., 1993).

The CFTR plays a pivotal role in normal epithelial homeostasis. Dysfunction of CFTR results in destruction of exocrine tissues, including those of the gastrointestinal tract and lung. Mutations of CFTR gene leads to the autosomal-recessive disorder of cystic fibrosis. Liver disease associated with cystic fibrosis has been increasingly diagnosed and recognized as one of the major causes of death in cystic fibrosis. In the liver, CFTR is found in biliary epithelial cells, and over- or underexpression of CFTR in the liver may play an important role in the development of cystic fibrosis liver disease (CFLD).

In rats, only intrahepatic biliary cells express Cftr, which is detected as a 150–165-kD protein. Immunoperoxidase staining confirms the localization of Cftr to bile duct cells, but not hepatocytes. These findings suggest that Cftr may participate in the control of fluid and electrolyte secretion by bile duct epithelial cells. Abnormal ductular secretion with dysfunctional Cftr may contribute to the pathogenesis of cholestatic liver disease in cystic fibrosis (Fitz et al., 1993).

The mouse Cftr gene is expressed in intestine, lung, stomach, kidney, and salivary glands. In contrast to human CFTR, mouse Cftr transcripts are very low in liver, pancreas, and other tissues. During development, mouse Cftr transcripts are observed as early as embryonic day 13 (Kelley et al., 1992). CFTR is expressed in larger, but not in small, bile ducts. Further, CFTR is expressed only in large cholangiocytes in mice (Glaser et al., 2009).

Regulation of hepatic CFTR/Cftr expression by xenobiotics and in disease states

In patients with progressive familial intrahepatic cholestasis, hepatic CFTR expression is decreased, in comparison to the normal liver. In the human biliary epithelial cell line, Mz-ChA-2, a significant decrease in CFTR expression is associated with ATPase ATP8B1-deficiency by siRNA (Demeilliers et al., 2006).

In bile duct–ligated male C57BL/6 mice, Cftr expression increases in larger bile ducts 3 and 7 days after surgery, in comparison to large ducts in normal liver sections, while staining for

Cftr remains negative in small bile ducts. Cftr is expressed only in large cholangiocytes from normal and bile duct-ligated mice (Glaser et al., 2009). In rats with bile duct ligation, liver Cftr mRNA and protein levels are also increased. Immunohistochemistry staining also demonstrates an increased intensity of Cftr staining in liver tissue sections of bile duct-ligated rats (Shen et al., 2005).

No clear relationship between CFTR expression and fibrosis or inflammation in liver is evident in cystic fibrosis patients. Immunological mechanisms are unlikely to be involved in the initiation of CF-associated liver disease (Kinnman et al., 2000). However, Cftr expression is increased by proinflammatory cytokines in isolated bile duct units from male SD rats (Spirli et al., 2001).

After partial hepatectomy, rat liver Cftr mRNA increases in a specific biphasic manner, with mRNA levels peaking at 2 and 24 hours after surgery. Cftr gene expression is clearly regulated during the regenerative process of the liver (Tran-Paterson et al., 1992).

In bile duct preparations from rats, hypoxia significantly induces Cftr mRNA expression. In the meantime, intracellular cAMP dramatically increases (Fouassier et al., 2007). In rats with complete arterial deprivation, followed by arterial ischemia in liver, hepatic Cftr mRNA levels are significantly increased, in comparison to controls (Fouassier et al., 2007). By contrast, in mice treated with low oxygen *in vivo*, hepatic Cftr mRNA expression is repressed (Guimbellot et al., 2008).

Cftr mRNA levels are not significantly different between *Mrp6*-knockout and wild-type mice (Li et al., 2007).

In the human liver epithelial cell line, BC1, phorbol ester downregulates CFTR mRNA expression in a time- and dose-dependent manner (Kang-Park et al., 1998).

Mechanisms of regulation of hepatic CFTR/Cftr expression

The CFTR gene is located in chromosome 7 (7q31) in humans and is highly conserved within vertebrate species. CFTR gene promoter resides in a GC-rich region without a TATA box. Several potential SP1 and AP1-protein binding sites have been identified. CFTR gene expression *in vivo* is tightly regulated, both developmentally and in a tissue-specific manner, and also demonstrates species-specific regulation. The tight transcriptional regulation of CFTR expression involves the combination of multiple regulatory elements. Some of them are conserved throughout evolution, such as the cAMP-response-element-like element involved in basal levels of transcription, while others are species specific (Vuillaumier et al., 1997). The CFTR gene promoter is important for basal CFTR gene expression, but not for tissue-specific expression. Additional regions in the CFTR gene and transcription factors involved in the regulation of expression, as well as aspects of the regulation of developmental expression of CFTR, are summarized (McCarthy and Harris, 2005). For example, human CFTR transcription is tightly regulated by nucleotide sequences upstream of the initiator sequences. The human homeodomain CCAAT displacement protein/cut homolog (CDP/cut) can bind to the Y-box element (an inverted CCAAT consensus) through

a cut repeat and homeobox. Human histone acetyltransferase GCN5 and transcription factor ATF-1 can potentiate CFTR transcription through the Y-box element (Li et al., 1999).

CFTR gene expression is also regulated post-transcriptionally. In the human liver epithelial cell line, BC1, phorbol ester downregulates CFTR mRNA expression in a time- and dose-dependent manner mediated by protein kinase C alpha and the novel protein kinase C epsilon (Kang-Park et al., 1998).

CFTR gene expression is also regulated by CpG methylation. Hypermethylation of the promoter CpGs of CFTR gene occurs prevalently in hepatocellular carcinomas, in comparison to paired noncancerous tissues, which may correlate with the low expression of CFTR at the mRNA level. This is likely to be specific to the early phases of hepatocellular carcinoma development (Ding et al., 2004; Moribe et al., 2009).

Acute regulation of CFTR protein in response to environmental stimuli occurs at several levels (e.g., ion channel phosphorylation, ATP hydrolysis, and apical membrane recycling). Within each epithelial cell, CFTR interacts with a large number of transient macromolecular complexes, many of which are involved in the trafficking and targeting of CFTR (Ameen et al., 2007).

Regulation of CFTR gene by xenobiotics and in liver disease states deserves further attention.

Regulation of hepatic ABCC8/SUR1 and ABCC9/SUR2 transporters by xenobiotics and in disease states

ABCC8/SUR1 and ABCC9/SUR2 transporters are receptors for the hypoglycemic drugs sulfonylureas that regulate potassium channel function. The sulfonylurea receptors (SURs), ABCC8/SUR1 and ABCC9/SUR2, have no identified transport function and have been matched with the K(+) selective pores, K(IR)6.1/KCNJ8 and K(IR)6.2/KCNJ11, resulting in an assembly of adenosine triphosphate (ATP)-sensitive K(+) channels found in endocrine cells, neurons, and both smooth and striated muscle. Mg-nucleotide binding and/or hydrolysis in the nucleotide-binding domains of SUR antagonize the inhibitory action to stimulate ATP-sensitive K(+) [K(ATP)] channel opening. Mutations in either subunit can alter this function and result in monogenic forms of hyperinsulinemic hypoglycemia and neonatal diabetes in mutations in SUR1/KIR6.2 channels found in neurons and insulin-secreting pancreatic beta cells. Additionally, the subtle dysregulation of K(ATP) channel activity by a K(IR) 6.2 polymorphism has been suggested as a predisposing factor for type 2 diabetes mellitus (Bryan et al., 2007).

Overall, SUR1 functions as a modulator of ATP-sensitive potassium channels and insulin release. Mutations and deficiencies in this protein have been observed in patients with hyperinsulinemic hypoglycemia of infancy, an autosomal recessive disorder of unregulated and high insulin secretion. Mutations have also been associated with noninsulin-dependent diabetes mellitus type II, an autosomal dominant disease of defective insulin secretion.

Alternative splicing of this gene has been observed; however, the transcript variants have not been fully described.

In SUR1 gene, the promoter is G+C rich and has no TATA box. Several E-boxes and potential SP1 sites are present in the promoter. The promoter is completely inactive in the fibroblast cell line, COS7, but shows some activity in HepG2 (liver) and HEK293 (epithelial) cell lines. A short (173-bp) fragment of SUR1 5'-flanking sequence is sufficient for maximal promoter activity (Ashfield and Ashcroft, 1998).

ABCC9 is thought to form ATP-sensitive potassium channels in cardiac, skeletal, vascular, and nonvascular smooth muscles. Protein structure suggests a role as the drug-binding channel modulating the extrapancreatic ATP-sensitive potassium channels. No disease has been associated with this gene, thus far. Alternative splicing of this gene results in several transcripts.

In *Mrp6*-knockout mice, *Sur1* or *Sur2* mRNA levels do not change significantly from wild-type mice (Li et al., 2007).

Regulation of hepatic ABCC10/MRP7, ABCC11/MRP8, and ABCC12/MRP9 transporters by xenobiotics and in disease states

MRP7/ABCC10, MRP8/ABCC11, and MRP9/ABCC12 are recently identified members of the MRP family. Of these proteins, a physiological function has been established only for MRP8, for which a SNP determines wet versus dry earwax type (Kruh et al., 2007).

MRP7 is a lipophilic anion pump and its substrates include the glucuronide E₂17βG and several natural product anticancer agents, such as the taxane docetaxel (Kruh et al., 2007).

Mouse *Mrp7* is expressed at moderate to low levels in liver, with slightly higher expression in females than males (Maher et al., 2005b). In mice, liver *Mrp7* expression is induced by TCDD, polychlorinated biphenyl126, β-naphthoflavone, phenobarbital, TCPOBOP, diallyl sulfide, and butylated hydroxyanisole (Maher et al., 2005a). In male or female *Mrp2*-knockout mice, *Mrp7* mRNA levels are very low (Chu et al., 2006). In *Tnfr1*-, *Il1r1*-, and *Il6*-knockout mice, *Mrp7* mRNA levels are not different from those in wild types after saline treatment. LPS treatment induces *Mrp7* mRNA levels. Bile duct ligation also increases liver *Mrp7* mRNA levels in these knockouts. *Mrp7* mRNA levels in these knockout mice tend to be lower than in wild types after sham operation. Hepatic *Mrp7* mRNA levels tend to be lower in liver-specific IκB kinase β (*IKKβ*)-deficient (*IKKβ* hep) mice than in *IKKβ*-floxed [*IKKβ*(*f/f*)] mice after saline treatment or sham operation of bile duct ligation. LPS treatment or bile duct ligation increases *Mrp7* mRNA levels in *IKKβ* hep mice (Lickteig et al., 2007b). Alternative splicing of this gene results in multiple transcript variants; however, not all variants have been fully described.

MRP8 is also a lipophilic anion pump, able to transport a diverse range of lipophilic anions, including cyclic nucleotides, E₂17βG, steroid sulfates, such as dehydroepiandrosterone and E₁S, glutathione conjugates, such as leukotriene C₄ and dinitrophenyl-S-glutathione, and monoanionic bile acids. However, the constituent of ear-wax that is susceptible to transport

by MRP8 has not been identified. MRP8 also effluxes nucleoside-based therapeutic agents, such as 9'- (2'-phosphonylmethoxyethyl) adenine (PMEA; the active metabolite of the prodrug, adefovir dipivoxil) and 5-fluorouracil (5-FU), an antimetabolite used in the treatment of a variety of cancers (Kruh et al., 2007).

MRP8 gene is expressed at moderate levels in normal breast and testis and at very low levels in liver, brain, and placenta. MRP8 is highly expressed in breast cancer. It is also expressed in the kidneys, lungs, and several fetal tissues. The gene has two major transcripts of 4.5 and 4.1 kb. The 4.5-kb transcript is very abundant in breast cancer. The predicted protein sequence of the 4.5-kb transcript indicates that MRP8 has high homology to MRP5. The smaller 4.1-kb transcript of MRP8 is found in testis, and its transcription may initiate within intron 6 of the gene. Multiple alternatively spliced transcript variants have been described for this gene. One splice variant lacking the exon 28 corresponded to about 25% of total MRP8 gene transcripts (Bera et al., 2001; Yabuuchi et al., 2001). An SNP, 538G>A in the MRP8 gene, is responsible for the determination of earwax type (Yu et al., 2007b).

Human MRP9 is located on human chromosome 16q12.1 and, tandemly, in a tail-to-head orientation with MRP8. MRP8 and MRP9 are derived by duplication based on phylogenetic analysis and are most closely related to the MRP5 gene. Since MRP8 and MRP9 were mapped to a region harboring gene(s) for paroxysmal kinesigenic choreoathetosis, the two genes represent positional candidates for this disorder. MRP9 gene expression is detected in various adult human tissues, including liver, lung, kidney, and in several fetal tissues. Many alternative splicing variants of MRP9 gene have been identified and may represent diverse biological functions (Tammur et al., 2001; Yabuuchi et al., 2001).

Mouse *Mrp9* is almost solely expressed in testes at very high levels. In mice, liver *Mrp9* expression is extremely low and is repressed by ethoxyquin, ciprofibrate, and diethylhexylphthalate. Rat *Mrp9* mRNA is also expressed at low levels in the liver (Maher et al., 2005a, 2005b; Lu and Klaassen, 2008). In *Tnfr1*-knockout mice, liver *Mrp9* mRNA levels tend to be lower than in wild types after saline treatment. LPS treatment does not change liver *Mrp9* mRNA levels in *Tnfr1*-, *Il1r1*-, and *Il6*-knockout mice. Bile duct ligation tends to increase liver *Mrp9* mRNA levels in *Tnfr1*- and *Il1r1*-knockout mice. Liver *Mrp9* mRNA levels are significantly lower in *Tnfr1*- and *Il1r1*-knockout mice than in wild types after sham operation. Hepatic *Mrp9* mRNA levels tend to be lower in liver-specific I κ B kinase β (IKK β)-deficient (*IKK β* hep) mice than in *IKK β* -floxed [*IKK β (f/f)*] mice after saline treatment. LPS treatment or bile duct ligation does not change hepatic *Mrp9* mRNA levels in *IKK β* hep mice or *IKK β (f/f)* mice, in comparison to respective saline treatment or sham operation (Lickteig et al., 2007b). MRP9 mRNA levels are not changed in *Mrp2*-knockout mice (Chu et al., 2006). The functional characteristics of MRP9 are currently unknown (Kruh et al., 2007).

Regulation of hepatic ABCC13/MRP10 transporters by xenobiotics and in disease states

Human MRP10/ABCC13 gene is a pseudogene located on chromosome 21q11.2, spanning 90 kb, with the highest similarity to MRP2, but without transporting activity. At least 28

exons are found, and the major MRP10 transcript in humans consists of only six exons, with a total length of 1.1 kb. The open reading frame of this transcript is capable of encoding a polypeptide of only about 300 amino acids, compared to the more than 1,500 amino acids of related ABC transporters. The truncated MRP10 transcript shows tissue-specific expression, highest in fetal liver, bone marrow, and colon. The expression of human MRP10 is related to hematopoiesis (Yabuuchi et al., 2002; Annilo and Dean, 2004).

In humans, chimpanzees, and gorillas, an 11-bp deletion in the last exon of the MRP10 transcript results in a frameshift, but it is not found in monkeys. The human MRP10 gene contains two other frameshift indels in the exons that encode the second nucleotide-binding domain, indicating that MRP10 is not capable of encoding a functional ABC protein. In all apes, MRP10 has accumulated inactivating mutations, while in the mouse and rat genomes, the coding exons of the gene have degraded so much that the genes are difficult to recognize. A functional MRP10 gene has only been found in the macaque and dog genomes. Chicken and zebrafish also have an ortholog of the mammalian MRP10 (Annilo and Dean, 2004).

Conclusions and perspectives

ABCC transporters are differentially expressed in a variety of tissues. Zonal patterns of expression are also common within specific tissues. These transporters play important roles in chemical detoxification, disposition, and cell physiology. These pathways, in concert with other biological processes, ensure the proper flux of chemicals in different biological systems. Changes in expression of ABCC proteins by xenobiotics and in disease states can disturb cellular homeostasis by altering normal transport and disposition of xenobiotics and endogenous molecules with critical cell functions. This is particularly true for the liver, which is the most prominent organ involved in chemical biotransformation and detoxification, and in some biosynthetic functions. This review article has provided a comprehensive review on our current knowledge on the expression and regulation of liver ABCC transporters. ABCC transporters are subjected to regulation by chemicals historically associated with modulating the expression of phase I and II drug-metabolizing enzymes and their function. It is not surprising that ABCC transporters are similarly and, at times, coordinately regulated with drug-metabolizing enzymes, because membrane transport is the final effector in the biotransformation and disposition machinery for many chemicals and their metabolites.

This review has highlighted the role of xenobiotic-sensing receptors, such as CAR and PXR, in the basal and inducible expression of ABCC transporters. The use of selective activators and knockout animal models for nuclear hormone receptors and transcription factors has been instrumental in determining the role of these sensing molecules in regulating ABCC transporters. As an example, CAR activation has been demonstrated to regulate hepatic Mrp3 and 4 expression. Similarly, the redox sensing complex Nrf2/Keap1 has recently emerged as an important regulator of ABCC transporters by chemicals with antioxidant properties and toxicants that produce oxidative and/or electrophilic stress. This is well illustrated in studies where the hepatotoxicant, acetaminophen, induces Mrp3 and 4 expression in an Nrf2-dependent manner. Proinflammatory and protective cytokines are also

known to mediate regulation of ABCC proteins in the liver under a variety of conditions, such as tissue inflammation and chemical-induced injury.

Certain chemical treatments, surgical interventions, and genetic conditions leading to cholestasis are known to induce the expression of ABCC proteins. However, the interplay between the different signaling pathways leading to these changes is not well characterized. Induction of ABCC proteins also occurs during compensatory cell proliferation in response to acetaminophen or carbon tetrachloride toxicity (e.g., Mrp4) or after partial hepatectomy (e.g., Mrp1). Although some of the regulatory features behind these changes have been investigated, the precise role of ABCC transporters in tissue defense and liver regeneration is not known. This is an important area of investigation, since drug-induced liver injury continues to be a significant human health problem. A better understanding of the molecular regulation of ABCC transporters during development and recovery from liver injury could provide new therapeutic avenues for accelerating tissue repair and regeneration in patients with idiosyncratic drug liver reactions.

It has been postulated that induction of ABCC transporters during cholestasis is an attempt to cope with the accumulation of dangerous bile acids by providing alternative routes for their elimination when their canalicular secretion fails. What is not clear is whether this compensatory induction in ABCC proteins during cholestasis is just aimed at preventing liver bile-acid retention or whether this is also a mechanism to deal with the potential generation of toxic mediators resulting from bile-acid accumulation. This latter possibility has received considerably less attention.

In addition to transcriptional regulation, ABCC proteins are regulated post-translationally. This is evidenced by multiple examples in the literature showing changes in ABCC protein expression in the absence of changes in mRNA levels. This review discussed current data showing how changes membrane fluidity associated with changes in lipid content and composition can affect transporter expression and function in the absence of changes in gene transcription. Retrieval of ABCC proteins from their membrane localization and their degradation under certain conditions and/or treatments is another example of transcriptional- and translational-independent ABCC regulation.

Although a considerable number of ABCC genetic polymorphisms are known to exist, only a handful are associated with clinical or toxicological relevant phenotypes.

Strain-, gender-, and tissue- selectivity in the expression of ABCC are also well documented. However, detailed information on the molecular basis for these differences is lacking. The promoter regions of just a few ABCC genes and the regulatory elements controlling their activity have been somewhat characterized. ABCC promoter activity data rely heavily on studies investigating one or two transcription factors cotransfected with a gene-reporter construct. This approach has provided important information on the ability of individual factors to either activate or repress ABCC gene-promoter activity. Studies that take into consideration the complex interaction among transcription factors and the influence of coactivators or corepressors in ABCC gene expression are scarce. Further, selective expression and/or abundance of transcription factors among different tissues and strains of

rodents could also influence ABCC expression. New studies addressing these unknowns may provide an explanation for tissue-, gender-, and species-selective expression of certain ABCC proteins. Some recent, exciting results have shown that epigenetic events, such as DNA methylation, also control expression and function of ABCC proteins. In summary, many challenges still exist in the field of hepatic ABCC regulation. In addition, many questions remain unanswered regarding the functional consequences for changes in the expression of some members of the ABCC subfamily of transporters.

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