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The heteromeric organic solute transporter, OST α –OST β /SLC51: A transporter for steroid-derived molecules

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Abstract

The organic solute transporter alpha-beta (OST -OST) is one of the newest members of the solute carrier family, designated as SLC51, and arguably one of the most unique. The transporter is composed of two gene products encoded by *SLC51A* and *SLC51B* that heterodimerize to form the functional transporter complex. *SLC51A* encodes OST , a predicted 340-amino acid, 7-transmembrane (TM) domain protein, whereas *SLC51B* encodes OST , a putative 128-amino acid, single-TM domain polypeptide. Heterodimerization of the two subunits increases the stability of the individual proteins, facilitates their post-translational modification, and is required for delivery of the functional transporter complex to the plasma membrane. There are no paralogues for *SLC51A* or *SLC51B* in any genome that has been examined. The transporter functions via a facilitated diffusion mechanism, and can mediate either efflux or uptake depending on the electrochemical gradient of its substrates. Overall, characterization of the transporter's substrate specificity, transport mechanism, tissue distribution, subcellular localization, and transcriptional regulation as well as the phenotype of the recently generated *Slc51a*-deficient mice have revealed that OST -OST plays a central role in the transport of bile acids, conjugated steroids, and structurally-related molecules across the basolateral membrane of many epithelial cells. In particular, OST -OST appears to be essential for intestinal bile acid absorption, and thus for dietary lipid absorption.

Keywords

OST -OST ; Bile acids; FGF15; FXR; *Slc51a*^{-/-} mice; Steroids

1. Introduction

OST -OST was identified in 2001 using a comparative animal model, the little skate *Leucoraja erinacea*, and a unique expression cloning strategy (Wang et al., 2001). This study by Wang et al. (2001) provides the only example to date of the successful use of expression cloning to simultaneously identify two interacting gene products. The mouse and human orthologues were subsequently identified, cloned and characterized by Seward and coworkers in 2003. OST and OST are encoded by the *SLC51A* and *SLC51B* genes on

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different chromosomes, exhibit no sequence identity with each other, and appear to lack paralogues (Table 1) (Ballatori et al., 2009). Heterodimerization of OST α and OST β is required for the delivery of the functional transporter complex to the plasma membrane (Li et al., 2007). Interestingly, the human and mouse proteins are able to complement each other, as well as those from the little skate; that is, all nine OST α –OST β pairs made using the human, mouse, and skate genes generate robust transport activity (Seward et al., 2003). This high degree of functional conservation suggests that these proteins evolved from an evolutionarily ancient common ancestor. A phylogenetic analysis of human OST α and OST β is shown in Fig. 1; however, this does not include the skate proteins due to the restrictions placed on the analysis. Human OST α shares 45% and 83% amino acid identity with skate and mouse OST α , respectively. Human OST β shares less similarity with skate and mouse OST β , exhibiting only 25% and 62% amino acid identity, respectively.

Strong evidence for a role of OST α –OST β in sterol homeostasis was provided by recent studies in *Slc51a*-deficient mice (Ballatori et al., 2008; Rao et al., 2008). These mice display a defect in intestinal bile acid and conjugated steroid absorption; a decrease in bile acid pool size and serum bile acid levels; altered intestinal, hepatic and renal disposition of known substrates of the transporter; and altered serum triglyceride, cholesterol, and glucose levels. Taken together, these observations indicate that OST α –OST β is essential for bile acid and sterol disposition, and confirm its critical role in the enterohepatic circulation of bile acids (Fig. 2A).

In the enterohepatic circulation, hepatocytes metabolize cholesterol to bile acids, which are subsequently transported across the canalicular membrane into bile by the bile salt export pump, BSEP/ABCB11, with a minor contribution from the multidrug resistance-associated protein-2, MRP2/ABCC2 (Fig. 2B). Bile acids travel to the gallbladder during the interdigestive phase for storage, and to the descending part of the duodenum via the common bile duct during digestion. Bile acids facilitate biliary secretion of endogenous metabolites and xenobiotics, and are required for the emulsification and intestinal absorption of dietary lipids. Approximately 90–95% of the bile acids delivered into the duodenum are actively reabsorbed from the small intestine, with most of the absorption occurring in the ileum, the distal portion of the small intestine. Bile acid uptake across the apical membrane of ileal enterocytes is mediated mainly by the apical sodium-dependent bile acid transporter, ASBT/SLC10A2, whereas basolateral efflux is mediated largely by OST α –OST β /SLC51 (Fig. 2B). Venous blood from the ileum goes directly into the portal vein and then into the liver sinusoids, where the bile acids are very efficiently extracted by the hepatocytes. Hepatocellular uptake of bile acids, which completes the cycle, is primarily carried out by the sodium taurocholate co-transporting polypeptide, NTCP/SLC10A1 (Fig. 2B).

2. Tissue distribution and cellular localization

OST α and OST β are expressed in nearly all tissues, but are most abundant in tissues involved in bile acid and steroid homeostasis (Ballatori et al., 2009). In general, tissues with high levels of OST α also have high levels of OST β , indicating co-expression of these genes. In humans, *SLC51A* and *SLC51B* mRNA levels are highest in the small intestine, liver, colon, kidney, testes, ovary, and adrenal gland, and are also detected in mammary gland, uterus, prostate, thyroid, and brain (Seward et al., 2003), consistent with a role in the disposition of steroid-derived compounds. In the brain, *SLC51A* and *SLC51B* are expressed in Purkinje cells and cells in the CA region of the hippocampus (Fang et al., 2010). In support of a role for OST α –OST β in basolateral efflux, both OST proteins are localized to the basolateral membrane of key epithelial cells, including enterocytes, renal tubular cells, hepatocytes, and cholangiocytes (Ballatori et al., 2005; Dawson et al., 2005).

Note that hepatic expression exhibits significant species differences: *SLC51A* and *SLC51B* mRNA are relatively abundant in human liver, whereas mouse and rat liver have very low levels of the corresponding transcripts (Seward et al., 2003; Ballatori et al., 2005). Human OST protein is detected in hepatocytes and cholangiocytes, whereas mouse hepatocytes have no detectable OST protein and mouse cholangiocytes have only moderate expression of this protein (Ballatori et al., 2005). In mice, relatively high levels of *Slc51a* and *Slc51b* message and protein are found in the small intestine, colon, and kidney, and the expression is especially high in the ileum (Dawson et al., 2005). *Slc51a* and *Slc51b* tissue distribution also parallels that of *Slc10a2* (encoding ASBT), consistent with its role in bile acid homeostasis (Dawson et al., 2005).

Additional evidence for a role of the transporter in bile acid disposition comes from the observation that OST –OST tissue distribution pattern overlaps that of the farnesoid X receptor (FXR) and of TGR5/GPBAR1, both bile acid receptors (Ballatori, 2011). *FXR* expressed sequence tag counts are quite abundant in human adrenal gland, are expressed at relatively high levels in the intestine, kidney, liver, and mammary gland, and are also detected in ovary, blood, brain, and connective tissue (Ballatori, 2011).

3. Functional studies

Studies on the mechanism of OST –OST transport in *Xenopus laevis* oocytes revealed that the transporter can mediate either cellular efflux or uptake depending on the particular substrate's electrochemical gradient (Ballatori et al., 2005). OST –OST -mediated transport in the oocytes was unaffected by depletion of intracellular ATP, alterations in transmembrane electrolyte concentration gradients, or changes in the pH gradient, and was *trans*-stimulated by known substrates (Ballatori et al., 2005), consistent with a facilitated diffusion transport mechanism.

Evidence that OST –OST is a multispecific transporter came from the initial characterization of the skate genes in *Xenopus* oocytes (Seward et al., 2003; Wang et al., 2001). Oocytes injected with skate *slc51a* and *slc51b* cRNA were able to transport taurocholate, estrone 3-sulfate, digoxin, and prostaglandin E₂, but not p-aminohippurate or S-dinitrophenyl glutathione (Wang et al., 2001), indicating that this transport system may participate in cellular uptake of bile acids, some endogenous and exogenous steroids, and eicosanoids. In support of this hypothesis, transport was *cis*-inhibited by a variety of bile salts, steroids, and other organic anions. Although inhibition of transport does not necessarily establish that these compounds are transported substrates, it does provide insight into possible substrates. A comparison of the inhibitory potency of bile salts indicates that taurine-conjugated bile salts were in general more effective than the corresponding glycine-conjugated compounds, and unconjugated bile salts were the least effective inhibitors (Wang et al., 2001). The addition of a sulfate group further enhanced inhibitory potency, such that bile salts containing both taurine and sulfate modifications were strong *cis*-inhibitors of taurocholate and estrone 3-sulfate transport. Spironolactone was also an inhibitor of transport, as were sulfobromophthalein, probenecid and indomethacin. Mouse and human OST –OST generally exhibited a similar substrate and inhibitor profile (Seward et al., 2003).

Studies in MDCK cells triply transfected with mouse OST , mouse OST , and human ASBT demonstrated that OST –OST is able to transport a variety of taurine- and glycine-conjugated bile acids (Ballatori et al., 2005; Dawson et al., 2005). The MDCK/ASBT cells expressing both OST subunits mediated significant apical to basolateral taurocholate transport, with minimal transport in the opposite direction (i.e., basolateral to apical), reflecting the appropriate sorting of the proteins and the unidirectional nature of bile acid

transport within these polarized cells (Dawson et al., 2005). For each bile acid species, the taurine-conjugated bile acid appeared to be transported more efficiently than the glycine-conjugated bile acid across the basolateral membrane by OST –OST (Ballatori et al., 2005).

Additional studies have demonstrated that OST –OST also transports dehydroepiandrosterone sulfate (DHEAS) (Ballatori et al., 2005), and pregnenolone sulfate (PREGS) (Fang et al., 2010), and that both of these compounds are high affinity substrates for the transporter (Fang et al., 2010). Because DHEAS and PREGS are also major excitatory neurosteroids, these observations suggest a possible function for OST –OST in the brain. The studies of Fang et al. (2010) also demonstrate that a variety of sulfate- and glucuronic acid-conjugates of steroids can inhibit OST –OST, suggesting that these conjugates are also substrates.

4. Regulation

Bile acids regulate their own synthesis and transport (Eloranta and Kullak-Ublick, 2008), and thus the expression of OST and OST was predicted to be regulated by FXR (encoded by the *NR1H4* gene), the major bile acid-activated transcription factor. Indeed, there is now strong evidence for this hypothesis, including the observations that: (a) *Nr1h4*-null mice have lower ileal expression levels of OST and OST (Zollner et al., 2006); (b) Chenodeoxycholate (CDCA), a natural agonist of FXR, and GW4064, a synthetic agonist, induce expression of *Slc51a* and *Slc51b* mRNA in liver, adrenal, and intestinal cell lines (Boyer et al., 2006; Frankenberg et al., 2006; Landrier et al., 2006; Lee et al., 2006); (c) The bile acid- and GW4064-mediated induction of *Slc51a* and *Slc51b* can be abolished via siRNA targeted knock-down of *Nr1h4* (Landrier et al., 2006); (d) Treatment of *ex vivo* adrenal gland cultures with GW4064 induces expression of *Slc51a* and *Slc51b* in wild type adrenal glands but not in organs from *Nr1h4*-deficient mice (Zollner et al., 2006); and (e) FXR elements are found in the promoters of the *SLC51* genes: two in human *SLC51A* and one in human *SLC51B*, mouse *Slc51a*, and mouse *Slc51b* (Frankenberg et al., 2006; Landrier et al., 2006; Lee et al., 2006).

Dynamic regulation of OST and OST is also achieved through other transcription factors, namely the small heterodimer partner (SHP, encoded by *NR0B2*) and the liver receptor homolog-1 (LRH-1, encoded by *NR5A2*), both orphan nuclear receptors (Fig. 3). Bile acids bind FXR, which in turn dimerizes with the retinoid X receptor- (RXR) and translocates to the nucleus to induce the expression of *Slc51a*, *Slc51b* and *Nr0b2* (Goodwin et al., 2000; Lu et al., 2000; Boyer et al., 2006; Frankenberg et al., 2006; Landrier et al., 2006; Lee et al., 2006) (Fig. 3B). SHP interacts with LRH-1 forming a transcriptional repressor that downregulates OST and OST expression after binding to LRH-1 elements in the promoters of *Slc51a* and *Slc51b* (Fig. 3C) (Frankenberg et al., 2006). One functional LRH-1 response element is present in mouse OST and two in mouse OST (Frankenberg et al., 2006). Taken together, these findings demonstrate that FXR is a master transcriptional regulator involved in both the induction and repression of OST and OST (Fig. 3).

Although FXR, SHP, and LRH-1 appear to be the dominant transcription factors, other nuclear receptors involved in bile acid homeostasis also regulate *Slc51a* and *Slc51b*. These include transcriptional activators such as the liver X receptor- and the hepatic nuclear factor-4 (Okuwaki et al., 2007), and repressors, like the pregnane X receptor (Teng and Piquette-Miller, 2007).

Slc51a and *Slc51b* expression is also regulated via the FXR-FGF15/19-FGFR4 pathway (Fig. 4). In this pathway, bile acids bind FXR in ileal enterocytes and induce the expression of fibroblast growth factor 15/19 (FGF15 in mice and FGF19 in humans) (Holt et al., 2003;

Inagaki et al., 2005). FGF15 can act as a hormone and signals at the liver upon binding the fibroblast growth factor receptor 4 (FGFR4), stimulating the downregulation of *Cyp7a1* in a predominant manner over the FXR-SHP-LRH-1 pathway, which also downregulates *Cyp7a1* (Goodwin et al., 2000; Holt et al., 2003; Inagaki et al., 2005; Kim et al., 2007). Reductions in CYP7A1, the rate-limiting enzyme for the classical pathway of bile acid synthesis, lead to a decrease in the bile acid pool size (Fig. 4) (Ballatori et al., 2008; Rao et al., 2008). The lower bile acid concentrations lead to a decrease in the expression of bile acid efflux transporters, including *Slc51a* and *Slc51b* through reduced FXR signaling (Fig. 3). Although a complete understanding of the role of these transcription factors in the overall regulatory process has not yet been established, these findings reveal that *Slc51a* and *Slc51b* are regulated through complex interconnected pathways.

The altered expression of *SLC51A* and *SLC51B* in certain human diseases, and in particular cholestasis, provides additional evidence for a central role of bile acid-sensitive transcription factors in regulating transporter activity. In human hepatocytes, OST –OST is localized on the basolateral plasma membrane, and is thought to play a role in the sinusoidal efflux of bile acids during conditions of cholestasis (Ballatori et al., 2005; Boyer et al., 2006). Elevated levels of *SLC51A* and *SLC51B* mRNA and protein are found in livers of patients with primary biliary cirrhosis (PBC), a cholestatic disease (Boyer et al., 2006), indicating that *SLC51A* and *SLC51B* may undergo adaptive regulation to protect the liver from accumulating hepatotoxic bile acids. In *Slc10a2*-deficient mice, a model for bile acid malabsorption, *Slc51a* and *Slc51b* are upregulated in the cecum and proximal colon yet downregulated in the ileum where *Slc10a2* is normally expressed (Dawson et al., 2005). The diminished uptake of bile acids into ileocytes is most likely responsible for the decreased expression of *Slc51a* and *Slc51b* in the ileum. The inability of bile acids to be absorbed via ASBT may also promote their conversion from primary to secondary bile acids within the lumen of the small intestine, thus rendering them less water-soluble and in turn more susceptible to apical, passive diffusion in the large intestine. These changes may explain the elevated levels of *Slc51a* and *Slc51b* in the colon of these animals (Dawson et al., 2005).

5. Physiological/pathophysiological implications

As noted above, OST –OST is a key player in the enterohepatic circulation of bile acids (Fig. 2B). Located on the basolateral plasma membrane, OST –OST facilitates the transport of bile acids out of enterocytes and into the portal circulation. The bile acids are returned to the liver, confining the majority of bile acids to the intestine, liver, and gallbladder (the bile acid pool), thus keeping bile acids close to their prime targets and minimizing potential systemic cytotoxic effects. Studies in the *Slc51a*-deficient mouse model have confirmed the importance of OST –OST in the enterohepatic circulation, as well as a potential mechanism to reduce bile acid toxicity during cholestasis (Ballatori et al., 2008; Rao et al., 2008; Soroka et al., 2010).

Slc51a-deficient mice exhibit marked decreases in bile acid levels such that the bile acid pool size is only 10–30% that of wild type mice (Ballatori et al., 2008; Rao et al., 2008). Despite the lower bile acid levels, *Slc51a*-deficient mice excrete comparable amounts of bile acids in feces, consistent with a defect in intestinal bile acid absorption (Ballatori et al., 2008; Rao et al., 2008). In addition to this higher fractional excretion of bile acids in feces, bile acid synthesis is also decreased in *Slc51a*-deficient mice. *Fgf15* mRNA expression is increased in the ileum and *Cyp7a1* mRNA expression is decreased in the liver of *Slc51a*-deficient mice, indicating decreased bile acid synthesis (Ballatori et al., 2008; Rao et al., 2008). When bile acid levels are raised by feeding cholic acid-enriched diets, *Slc51a*-deficient mice excrete more bile acids than wild type mice, and this is partially protective against liver injury (Rao et al., 2008; Soroka et al., 2011). There are also adaptive changes in

Slc51a-deficient mice that partially compensate for the lower bile acid levels. *Slc51a*-deficient mice have heavier and longer small intestines than wild type mice, and their small intestines appear to have more crypts and valleys (Ballatori et al., 2008; Rao et al., 2008; Lan et al., 2011). These physiological changes increase the opportunity for bile acid absorption by other transporters, including the multidrug resistance-associated protein-3 (MRP3/ABCC3), whose substrates include bile acids and conjugated steroids, and whose mRNA expression is upregulated in the small intestine of *Slc51a*-deficient mice (Ballatori et al., 2008; Rao et al., 2008).

As noted earlier, in addition to its critical role in the enterohepatic circulation, OST –OST likely contributes to bile acid efflux during cholestasis. As bile acids begin to build up in the liver, alternate export mechanisms, like OST –OST present on the sinusoidal membrane of hepatocytes, are available to help transport the cytotoxic bile acids out of the cells and into the systemic circulation. *SLC51A* and *SLC51B* mRNA and protein are increased in livers of patients with primary biliary cirrhosis and pediatric biliary atresia, conditions which result in chronic obstructive cholestasis (Boyer et al., 2006; Chen et al., 2008). Increases in liver *Slc51a* and *Slc51b* mRNA and protein are also detected in mouse and rat models of cholestasis (Boyer et al., 2006). Therefore, it might be expected that *Slc51a*-deficient mice would be more sensitive to cholestasis, due to their diminished ability to export bile acids from the hepatocytes into the sinusoidal circulation. Surprisingly, Soroka et al. (2010) reported that cholestasis-induced liver injury is reduced in *Slc51a*-deficient mice, and that this is likely explained by an increase in urinary bile acid excretion. After bile duct ligation, *Slc51a*-deficient mice have increased protein expression of MRP2 and MRP4 on the apical side of proximal tubules, and thus can potentially export more bile acids into urine. Moreover, *Slc51a*-deficient mice display decreased protein expression of ASBT and MRP3 along with absence of OST –OST on the basolateral side of proximal tubular cells, and thus have diminished ability to reabsorb bile acids (Soroka et al., 2010). Overall, this altered transporter profile helps to limit the reabsorption of bile acids by the kidneys, and to enhance urinary excretion of bile alcohol sulfates.

Necrotizing enterocolitis (NEC) is another condition where alterations in the enterohepatic circulation of bile acids may contribute to disease. NEC is a major ailment of newborns, although its pathophysiology is poorly understood and likely multifactorial. The major risk factor for developing this condition is prematurity (Berman and Moss, 2011). In a rat model of NEC, enterocytes were found to have elevated levels of bile acids, which may contribute to the necrosis observed in the ileum during NEC (Halpern and Dvorak, 2008). The expression of ileal OST –OST appears to be maintained or even slightly elevated in the NEC susceptible neonatal rats when compared to both fetal and weanling tissues, whereas ASBT expression is up-regulated at the site of injury, likely contributing to the increased bile acid levels in enterocytes (Halpern and Dvorak, 2008; Halpern et al., 2006). It is possible that the transporters involved in the enterohepatic circulation, including OST –OST are not yet expressed appropriately in premature human infants leaving them susceptible to damage from accumulating bile acids.

OST –OST also appears to have effects outside of the digestive tract (Ballatori, 2011). Bile acids are decreased in serum of *Slc51a*-deficient mice, indicating that OST –OST is important for maintaining systemic bile acid levels and that it may have effects on other tissues (Ballatori et al., 2008). Note that bile acids are increasingly recognized as signaling molecules through their interaction with nuclear receptors such as FXR, pregnane X receptor, vitamin D receptor, G protein-coupled receptors such as TGR5/GPBAR1, and through other cell signaling pathways such as protein kinase C and *c-jun* N-terminal kinase (Lefebvre et al., 2009; Hylemon et al., 2009; Pols et al., 2011). OST –OST can also transport other signaling molecules, including estrone 3-sulfate, PREGS, DHEAS and

prostaglandin E₂ (Ballatori et al., 2008; Fang et al., 2010; Wang et al., 2001). The localization of OST –OST to steroidogenic tissues such as the ovary, testis, uterus, mammary gland, prostate, thyroid, and adrenal gland, and steroidogenic cells in the brain supports additional roles for this transporter (Fang et al., 2010; Seward et al., 2003; Ballatori, 2011).

6. Pharmaceutical aspects

The observation that *Slc51a*-deficient mice have a significantly smaller bile acid pool size (Ballatori et al., 2008; Rao et al., 2008) makes OST –OST an attractive therapeutic target for diseases associated with increased bile acid levels. As discussed above, the elevated bile acid concentrations in cholestatic liver diseases can have serious adverse consequences. OST –OST inhibition could theoretically lower intestinal and renal bile acid reabsorption, as well as diminish hepatic bile acid synthesis, thereby decreasing systemic and hepatic bile acid levels and protecting against cholestatic liver injury (Soroka et al., 2010).

Slc51a-deficient mice also excrete more cholesterol in feces (Rao et al., 2008) and have lower serum cholesterol and triglyceride levels (Ballatori et al., 2008), suggesting that OST –OST may also be an attractive therapeutic target for diseases associated with high lipid levels. Commonly prescribed drugs for dyslipidemia treatment include statins and ezetimibe, which inhibit cholesterol synthesis and dietary cholesterol uptake, respectively, and are often prescribed together (Toth, 2010). An additional treatment for dyslipidemia includes bile acid sequestrants, which bind to and sequester bile acids in the small intestine, therefore rendering them unable to facilitate the emulsification and absorption of dietary lipids (Staels and Kuipers, 2007). However, bile acid sequestrants have been found to increase triglyceride levels, and thus their use is often limited (Scaldaferri et al., 2011). In the *Slc51a*-deficient mouse model, both serum cholesterol and triglycerides are lowered (Ballatori et al., 2008). While the mechanism for this is not yet known, it is likely that the reduced bile acid pool size results in lower bile acid levels in the lumen of the small intestine, thus causing impaired dietary lipid absorption.

Even though *Slc51a*-deficient mice have a much smaller bile acid pool size and lower serum bile acid levels, they have higher expression of *Nr1h4* in the small intestine and liver, and of *Fgf15* in the small intestine (Ballatori et al., 2008; Rao et al., 2008). Although not yet established experimentally, the higher FGF15 levels may increase FGFR4 signaling, and thus contribute to the decreased *Cyp7a1* expression that has been observed in these mice (Ballatori et al., 2008; Rao et al., 2008). Based on these findings, it can be predicted that a putative OST –OST inhibitor would produce some of the same effects as either an FXR agonist, administration of FGF15/FGF19, or as an agonist to FGFR4. In addition to altering bile acid levels, FXR activation has also been implicated in lowering triglyceride levels, improving insulin sensitivity, and in modulating gluconeogenic genes (Cariou et al., 2006; Lefebvre et al., 2009; Zhang et al., 2006). Transgenic mice expressing FGF19, as well as mice injected with FGF19 have decreased adiposity, resistance to high fat diet-induced weight gain, and improved glucose tolerance in diabetic mouse models (Tomlinson et al., 2002; Fu et al., 2004). Studies in *Fgfr4*-deficient mice suggest that FGFR4 is important for maintaining cholesterol and triglyceride levels, and insulin sensitivity (Huang et al., 2007). The alterations in these signaling pathways may contribute to the phenotype of the *Slc51a*-deficient mouse, and suggest that OST –OST may be a valuable therapeutic target for the treatment of insulin resistance, diabetes, and the metabolic syndrome.

7. Summary

Recent studies have demonstrated that OST –OST, one of the newest members of the SLC family, plays an important role in the disposition of steroid-derived molecules, and in

particular, in the enterohepatic circulation of bile acids. Because the steroid-derived class of compounds plays critical roles in human physiology, these findings identify OST α -OST β as a key transporter for the regulation of these important bioactive signaling molecules.

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Fig. 1.

Computer-generated phylogenetic analysis of putative OST and OST amino acid sequences from different organisms. OST (A) and OST (B) phylogeny trees were generated using NCBI BLAST (tree method: fast minimum evolution, 0.6 max sequence difference). The protein accession number for each species in the analysis is listed on the branches of the tree.

Fig. 2.

The major transporters involved in the enterohepatic circulation of bile acids. Bile acids undergo cycling between the liver, their site of production, and the small intestine, where approximately 90–95% of bile acids are reabsorbed and returned to the liver via portal circulation. Roughly 5–10% of the bile acids are excreted in feces, with a minimal contribution from urinary excretion (A). In hepatocytes, bile acid uptake across the sinusoidal membrane is largely mediated by NTCP. Active transport of bile acids across the canalicular membrane into bile is predominantly mediated by BSEP, a monovalent bile acid transporter, and to a minor extent by MRP2, a divalent bile acid transporter. In the small intestine, the bile acids emulsify and aid in the absorption of dietary lipids and fat-soluble xenobiotics, and are then taken up from the lumen of the gut into enterocytes largely via ASBT. OST α -OST β mediates their efflux across the basolateral membrane into blood, where they return to the liver via portal blood (B).

Fig. 3. Transcriptional regulation of transporters participating in the enterohepatic circulation of bile acids. In both hepatocytes and enterocytes, bile acids bind FXR, which then dimerizes with RXR to positively regulate the expression of bile acid efflux transporters and negatively regulate the expression of bile acid uptake transporters (A). The FXR-RXR heterodimer binds FXR response elements (FXREs) in the promoters of mouse *Slc51a*, *Slc51b*, and *Nr0b2* (encoding SHP), leading to their expression. Over time, SHP binds LRH-1, which normally exerts constitutively positive regulation on OST α and OST β (B). On the LRH-1 response elements (LRH-1Es), SHP-bound LRH-1 functions as a transcriptional repressor and downregulates the expression of OST α , OST β , and SHP (C).

Fig. 4.

Dual coordinated regulation of CYP7A1 by the FXR-SHP-LRH-1 and FXR-FGF15/19-FGFR4 pathways. FXR-mediated signaling from the intestine and within the liver regulates *CYP7A1* expression and in turn bile acid synthesis. Bile acid accumulation within hepatocytes provokes negative feedback on their own synthesis via the FXR-SHP-LRH-1 pathway. The accumulation of bile acids within enterocytes as a result of intestinal reabsorption induces the expression of murine FGF15 (FGF19 in humans), which signals at the liver upon binding with the receptor, FGFR4. This signal indicates the extent of intestinal reabsorption so that the level of *de novo* bile acid synthesis within hepatocytes can be appropriately modulated. Both pathways contribute to the downregulation of CYP7A1 with the FXR-FGF15/19-FGFR4 pathway exerting dominance, most likely due to the high efficiency of bile acid recycling. This ultimately leads to decreased bile acid synthesis and transient fluctuations in the bile acid pool size.

SLC51 – transporters of steroid-derived molecules. For detailed information about the SLC gene tables, please visit: <http://www.bioparadigms.org>.

Table 1

Human gene name	Protein name	Human gene locus	Sequence accession ID	Transporter name	Predominant substrates	Transport type	Tissue distribution and cellular/subcellular expression
SLC51A	OST	3q29	NM 152672	OST –OST	Bile acids and steroids	Facilitated diffusion	Basolateral membrane of intestine, and the liver, kidney, testes, ovary, and adrenal gland
SLC51B	OST	15q22.31	NM 178859				