MINIREVIEW

Biology of a Novel Organic Solute and Steroid Transporter, OSTα-OSTβ

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Using a comparative approach, recent studies have identified and functionally characterized a new type of organic solute and steroid transporter (OST) from skate, mouse, rat, and human genomes. In contrast to all other organic anion transporters identified to date, transport activity requires the coexpression of two distinct gene products, a predicted 340–amino acid, seven-transmembrane (TM) domain protein (OSTα) and a putative 128–amino acid, single-TM domain ancillary polypeptide (OSTβ). When OSTα and OSTβ are coexpressed in Xenopus oocytes, they are able to mediate transport of estrone 3-sulfate, dehydroepiandrosterone 3-sulfate, taurocholate, digoxin, and prostaglandin E_2, indicating a role in the disposition of key cellular metabolites or signaling molecules. OSTα and OSTβ are expressed at relatively high levels in intestine, kidney, and liver, but they are also expressed at lower levels in many human tissues. Indirect immunofluorescence microscopy revealed that intestinal OSTα and OSTβ proteins are localized to the basolateral membrane of mouse enterocytes. In MDCK cells, mouse Ostα–Ostβ mediated the vectorial movement of taurocholate from the apical to the basolateral membrane, but not in the opposite direction, indicating basolateral efflux of bile acids. Overall, these findings indicate that OSTα-OSTβ is a heteromeric transporter that is localized to the basolateral membrane of specific epithelial tissues and serves to regulate the export and disposition of bile acids and structurally related compounds from the cell. If confirmed, this model would have important implications for the body's handling of various steroid-derived molecules and may provide a new pharmacologic target for altering sterol homeostasis. Exp Biol Med 230:689–698, 2005

This work was supported in part by National Institutes of Health grants DK067214, DK48823, and ES07026, and by National Institute of Environmental Health Sciences Center grants ES03828 and ES01247.

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1535-3702/05/23010-0689$15.00

Key words: steroid transporter; organic solute transporter; basolateral membrane; bile acid reabsorption

All cell functions ultimately depend on the regulated movement of chemicals across the plasma membrane. Cells must take up specific amounts of nutrients, metabolic precursors, inorganic ions, signaling molecules, and other macromolecules, while also exporting signaling molecules, hormones, electrolytes, metabolic waste products, and xenobiotics. Although recent studies have described some of the genes involved in these transport processes, it is clear that many other essential gene products remain to be identified and characterized.

Some of the major organic solute and drug transporters are shown in Figure 1. Uptake into cells is mediated by several families of ATP-independent proteins, including the Na^+-coupled bile acid transporters, ASBT and NTCP (SLC10A); the Na^+-independent organic anion transporting polypeptides, OATPs (SLC21); and the organic anion, OATs, and organic cation transporters, OCTs (SLC22A) (1–8). The first member of each of these families was identified by expression cloning in *Xenopus laevis* oocytes, and additional members have subsequently been identified by homology screening. All of these transporters consist of single polypeptides, which, when expressed in heterologous systems, are able to mediate organic solute transport.

In contrast to the uptake transporters, all of which are ATP-independent, export of organic solutes from cells appears to be mediated in part by members of the ATP-binding cassette (ABC) superfamily of proteins (Fig. 1). In hepatocytes, for example, the bile salt export pump Bsep/Abcb11, the multidrug resistance proteins Mdr1/Abcb1 and Mdr2/Abcb2, and the multidrug resistance–associated protein-2, Mrp2/Abcc2, are all localized to the canalicular

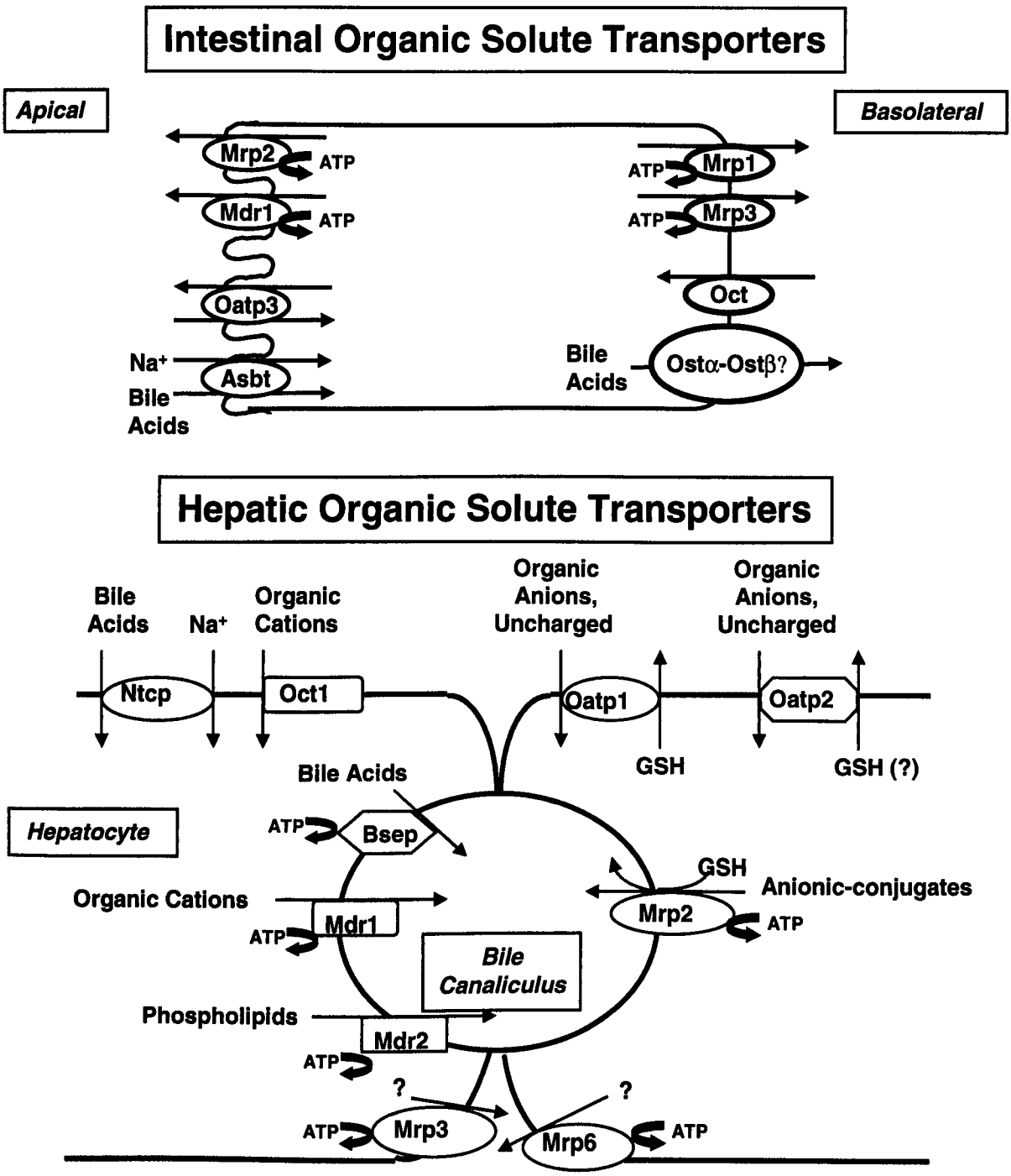

Figure 1. Some of the major intestinal and hepatic organic solute uptake and efflux mechanisms. Uptake into enterocytes and hepatocytes is mediated by several families of ATP-independent proteins, including the Na^+-coupled bile acid transporters, ASBT and NTCP (SLC10A); the Na^+-independent organic anion transporting polypeptides, OATPs (SLC21); and the organic anion, OATs, and organic cation transporters, OCTs (SLC22A) (1–8). In contrast to the uptake transporters, all of which are ATP-independent, export of organic solutes from cells appears to be mediated in part by members of the ATP-binding cassette (ABC) superfamily of proteins. In enterocytes, Mdr1 and Mrp2 are localized to the apical (luminal) membrane, whereas Mrp1 and Mrp3 are on the basolateral membrane. In hepatocytes, the bile salt export pump Bsep/Abcb11, the multidrug resistance proteins Mdr1/Abcb1 and Mdr2/Abcb2, and the multidrug resistance–associated protein-2, Mrp2/Abcc2, are localized to the canalicular membrane and mediate export into bile, whereas Mrp1, Mrp3, Mrp5, and Mrp6 are localized to the sinusoidal membrane and presumably mediate export into blood plasma.

membrane and mediate export into bile, whereas Mrp1, Mrp3, Mrp5, and Mrp6 are localized to the sinusoidal membrane and are thought to mediate export into blood plasma. Although the function of these basolateral Mrp proteins is not clearly defined, there is evidence that they may act as salvage systems in situations in which the function of the canalicular export systems is impaired (6–8).

Likewise, in intestinal epithelial cells, Mdr1 and Mrp2 are localized to the apical (luminal) membrane, whereas Oct1, Oct3, Mrp1, and Mrp3 are on the basolateral

membrane (Fig. 1). Intestinal epithelial cells also express Asbt and Oatp3 on the luminal membrane, and these proteins mediate the absorption of bile acids and other organic anions, respectively, from the intestinal lumen (Fig. 1). However, a key intestinal transporter that has not yet been identified at the molecular level is the basolateral protein that mediated efflux of bile acids and other steroid-derived molecules from the enterocytes into the splanchnic circulation (Fig. 1).

The molecular identification of these membrane transporters has provided the opportunity to study the mechanisms of transport, the role of these proteins in normal cell physiology, their regulation under both physiological and pathophysiological conditions, and their contributions to various human diseases (9–12). Of significance, inherited or acquired defects in membrane transporters often lead to human disease, including Dubin-Johnson syndrome (due to a defective MRP2), pseudoxanthoma elasticum (MRP6), progressive familial intrahepatic cholestasis (PFIC)-type 2 (BSEP), PFIC-type 3 (MDR3), and drug-induced cytotoxicity. It is likely that additional transporter-related human diseases will be described as new transporters are identified and characterized. Indeed, recent estimates indicate that ~6% of the human genome (i.e., ~2000 genes) may encode for membrane transport proteins (13), yet less than one-half of these have thus far been identified. This large number of transport genes is not surprising when one considers the huge number of endogenous and exogenous compounds that our cells are collectively required to import, export, and distribute to various intracellular organelles.

A critical family of compounds that cells must import and export is the steroid-derived class of compounds, including the various steroid hormones, bile acids, and other cholesterol metabolites. Steroid hormones are grouped into five categories: progestins, glucocorticoids, mineralocorticoids, androgens, and estrogens. All of these molecules share the sterol nucleus and are remarkably similar in structure; however, they exhibit marked differences in their physiologic effects. Metabolism of these five categories of steroids yields a wide variety of products that must be transported across cell membranes and across epithelial barriers, but the mechanisms of transport are largely unknown. Although it is often assumed that transport occurs by simple diffusion, this mode of transport would largely preclude the ability to regulate intracellular concentrations of these important bioactive and signaling molecules and is therefore unlikely to play a significant role in their disposition. Indeed, recent studies on the transport of other very hydrophobic compounds, including cholesterol (the precursor of steroid hormones and bile acids), fatty acids, and other membrane lipids, demonstrate that simple diffusion plays only a minimal role in their transport (14–22). Membrane transport of these very hydrophobic compounds is mediated largely if not exclusively by specific proteins, and in particular by members of the ABC superfamily of proteins and by specific members of the OATP and OAT families (3, 23–27). Among the ABC proteins, ABCA1, ABCA2, ABCG1, ABCG5, and ABCG8 are thought to modulate cholesterol and lipoprotein transport (23–27).

As noted above, a key transporter that has not yet been identified is the basolateral bile acid transporter in enterocytes and renal tubular epithelial cells. Efficient intestinal reabsorption of bile acids is essential for the absorption of dietary fats and vitamins, for proper regulation of bile flow and biliary lipid secretion, and for cholesterol homeostasis (6). The initial step in intestinal bile acid reabsorption, namely the uptake from the intestinal lumen into the enterocytes, is mediated in large part by the apical sodium-dependent bile acid transporter (Asbt; Slc10a2 [28]), also referred to as the ileal sodium-dependent bile acid transporter (Isbt and Ibat) (Fig. 1). Both primary and secondary conjugated and unconjugated bile acids are substrates for Asbt (6, 28). Once within the cell, bile acids are then secreted across the basolateral cell membrane into the splanchnic circulation; however, the carrier responsible for this transport step is unknown. Although several bile acid uptake transporters have been characterized (3, 5–8), this critical basolateral efflux transporter had not been identified. Enterocytes do not express Bsep; thus, this ABC transport protein cannot account for the basolateral transport of bile acids in the intestine. As described below, OSTα–OSTβ is a candidate for this important basolateral export step.

Identification of a Heteromeric Organic Solute and Steroid Transporter (OST) from Skate Liver. In order to functionally characterize organic anion transport proteins, our laboratory has used a comparative approach, studying transporters from an evolutionarily ancient vertebrate species, the little skate *Leucoraja erinacea*. The skate is thought to have evolved about 200–400 million years ago, but it displays many physiologic features of modern mammals. The livers of all vertebrates, including the skate, function as the primary site for clearance and metabolism of endogenous and exogenous lipophilic organic substances such as bile salts, steroids, eicosanoids, and natural toxins (29–35). One interesting difference between skate and mammalian liver is that skate hepatocytes take up bile salts from blood plasma largely by Na^+-independent mechanisms, whereas mammals utilize both Na^+-dependent (NTCP) and -independent (OATP) mechanisms (31, 32, 35). Thus, the skate liver expresses orthologues of the OATP transporters, but not of the NTCP transporter (36). Once taken up into skate hepatocytes, these compounds are transported across the canalicular membrane into bile by transport proteins that appear to be functionally and structurally similar to those in mammals (29, 30, 34). We have recently identified the skate BSEP orthologue (37), as well as putative members of the skate MDR and MRP families (38).[1]

In an attempt to identify a novel type of sterol transporter, we used expression cloning in *Xenopus laevis*

[1] S.C. Cai et al., unpublished observations.

oocytes and screened for bile salt ([^{3}H]taurocholate) transport activity using skate liver mRNA (21). These studies took advantage of the fact that the skate liver lacks the NTCP transporter and synthesizes and excretes sulfated bile alcohols rather than bile acids, and therefore, skates may have distinct transporters for these steroid-derived molecules.

Initial expression studies yielded two surprising results. First, [^{3}H]taurocholate transport activity in oocytes injected with size-fractionated skate liver mRNA appeared bimodal: it was highest in Size Fractions 4 (1.2–2.3 kb) and 6 (0.6–1.5 kb) and intermediate in Fraction 5. This bimodal distribution indicated that either two different-sized mRNA molecules are able to stimulate taurocholate transport or that two separate genes from these partially overlapping mRNA size fractions must be coexpressed to generate the transport signal. The second surprise was that the transport activity observed with small-sized mRNA (i.e., 0.6–2.3 kb) was considerably higher than that seen with mRNA of 2–5 kb. The latter is the transcript size for most of the mammalian organic anion transporters. Based on these initial findings, the mRNA from Size Fractions 4 and 6 was combined, a cDNA library was constructed, and the cDNA library was screened for [^{3}H]taurocholate transport activity.

During the initial screening of this library, it was noted that some of the cRNA-stimulated transport activity was not stable and was lost when positive pools of clones were divided into progressively smaller groups of clones. We hypothesized that this loss of transport activity may be due to the segregation into different cDNA pools of two or more genes that may be required to form a heteromultimeric protein complex. We therefore initiated studies to identify the two or more clones that may be interacting to generate the active transport system by making smaller subdivisions of positive pools. After multiple rounds of screening, a pool containing only 13 clones was identified that exhibited strong taurocholate transport activity (21). However, when the cRNA from the individual clones of this pool were injected into oocytes, they failed to stimulate transport, supporting the hypothesis that two or more gene products may be required. To evaluate which of the 13 clones were required, a mixture of cRNA was prepared from either all 13 clones or from 12 clones by sequentially deleting each clone from the mixture. Taurocholate uptake was observed under all conditions, except when Clone 4 or Clone 12 was deleted, indicating that both of these are required for transport. When the cRNA from Clone 4 or Clone 12 was injected individually, there was no transport; however, when they were injected simultaneously in various ratios, there was strong taurocholate transport activity. Clone 4 was denoted organic solute transporter-α (Ostα), and Clone 12 was named Ostβ.

Oocytes injected with skate Ostα and Ostβ cRNA (1 ng each) were able to transport taurocholate, estrone sulfate, digoxin, and prostaglandin E_2, but not *p*-aminohippurate or *S*-dinitrophenyl glutathione, indicating that this transport system may participate in cellular transport of conjugated steroids and eicosanoids (21). Transport was sodium independent and saturable, although the apparent Michaelis constants (K_m) for taurocholate (785 ± 43 μ*M*), estrone sulfate (85 ± 16 μ*M*), and digoxin (148 ± 30 μ*M*) were high when compared to those reported for the OATP transporters, which are generally about one order of magnitude lower. The major skate bile salt scymnol sulfate was a competitive inhibitor of estrone sulfate transport, with a Ki of 145 μ*M*, indicating that this may be an endogenous substrate.

Overall, these studies identified a novel type of organic solute and steroid transporter that is composed of two unique gene products, Ostα and Ostβ. They also provide the only example of the use of expression cloning to simultaneously identify two interacting gene products (21).

Identification of Human and Mouse Orthologues of Skate Ostα and Ostβ. Although the identification of these skate genes was interesting, the human health significance was not immediately obvious. Indeed, when the skate Ostα and Ostβ sequences were initially described, orthologous genes did not appear to be present in the human genome, or in any other sequenced genome, indicating that these genes may be specific to marine elasmobranchs. However, the initial drafts of the human and mouse genome sequences were quite incomplete and continue to be refined to this day. In the spring and summer of 2002, sequences for hypothetical human and mouse proteins were entered into the databases that exhibited significant sequence identity with skate Ostα and Ostβ (39; Table 1). The hypothetical human protein CAC51162 and the hypothetical mouse protein AAH25912 each exhibit 41% predicted amino acid identity with skate Ostα, and they exhibit 83% amino acid identity with each other. Because of the many conserved amino acid substitutions among these three sequences, the extent of similarity is ~70% between skate Ostα and these mammalian orthologues and 89% between the hypothetical mouse and human proteins (Fig. 2). Interestingly, all three deduced amino acid sequences are predicted to have seven-transmembrane (TM) domains and to share a highly unusual cluster of cysteine residues in a predicted hydrophilic loop between TM Domains 3 and 4 (Fig. 2). This relatively high overall amino acid homology, along with the conserved cysteine cluster in the human, mouse, and skate gene products, demonstrates that they are structural orthologues and indicates that they are functional orthologues.

Likewise, putative human and mouse genes were entered into the databases in May 2002 that exhibit significant predicted sequence identity with skate Ostβ (39). The hypothetical human protein XP_058693 and hypothetical mouse protein XP_134984 exhibit 25% and 29% amino acid identity with skate Ostβ, respectively (Fig. 3). These hypothetical human and mouse proteins exhibit 63% amino acid identity with each other: they both have

Table 1. Genomic Context and General Gene Information for Human, Mouse, Rat, and Skate *OSTα* and *OSTβ*

	Gene ID	NCBI Ref. Seq.[a] mRNA	NCBI Ref. Seq.[a] Protein	Chrom. location	DNA strand	Gene size (bp)	No. of exons	mRNA size (bp)	Predicted amino acids
OSTα									
Human	200,931	NM_152672	NP_689885	3q29	+	16,919	9	1,476	340
Mouse	106,407	NM_145932	NP_666044	16B2	–	12,603	9	1,545	340
Rat	303,879	XM_221376	XP_221376	11q22	+	14,194	9	1,398	340
Skate		AY027664	AAK14805					1,228	352
OSTβ									
Human	123,264	NM_178859	NP_849190	15q22.3	+	3,227	3	454	128
Mouse	330,962	NM_178933	NP_849264	9C	–	2,536	3	488	128
Rat	300,790	XM_238546	XP_238546	8q24	–	8,074	4	671	127
Skate		AY027665	AAK14806					882	182

[a] NCBI Ref. Seq., National Center for Biotechnology Information Reference Sequence; Chrom, chromosome; bp, base pairs.

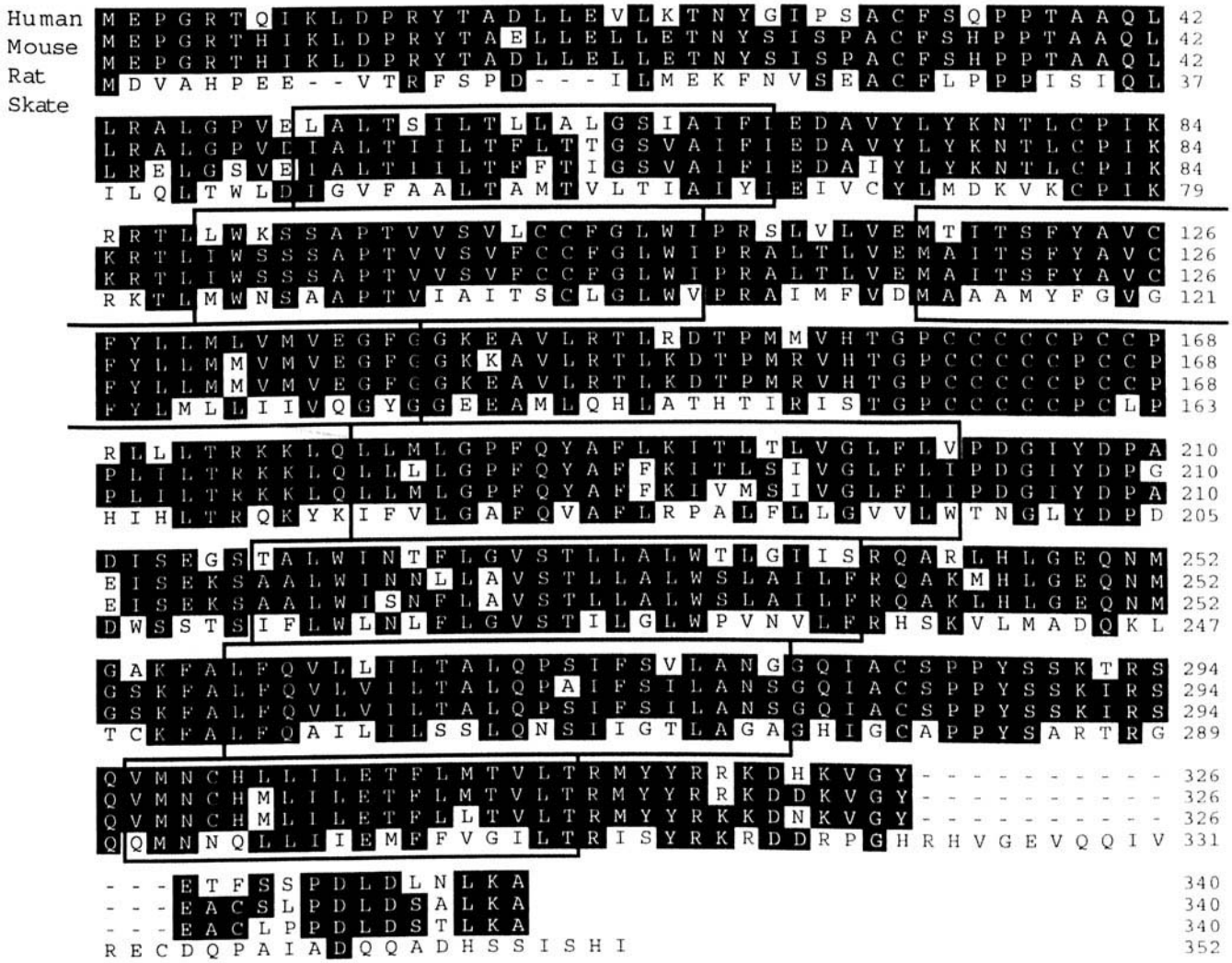

Figure 2. OSTα amino acid alignments. The deduced amino acid sequences for putative OSTα orthologues from human, mouse, rat, and skate were aligned using DNAStar's MegAlign computer program running the Jotun Hein algorithm. Amino acid identity is displayed with black shading, and the predicted TM domains are underlined. The R(R/K)K and RXR motifs are located at amino acid position 318 in the human, mouse, and rat sequences, and at amino acid position 313 in the skate.

128 amino acids, and they are both predicted to have only a single TM domain (Fig. 3).

We therefore designed studies to test whether these mammalian genes are in fact expressed and whether they encode for functional orthologues of the skate gene products, and if so, whether they functionally complement each other's transport activity. As described by Seward *et al.* (39), we cloned the putative human OSTα and OSTβ and mouse Ostα and Ostβ cDNAs from liver mRNA, expressed them in *Xenopus laevis* oocytes, and tested for their ability to functionally complement the corresponding skate genes by measuring transport of [^{3}H]estrone 3-sulfate. None of the genes elicited a transport signal when expressed individually in oocytes; however, all nine OSTα–OSTβ combinations (i.e., OSTα–OSTβ pairs from human, mouse, or skate) generated robust transport activity (39). Overall, the transport function of the heteromeric mammalian protein complex was comparable to the skate transporter. In addition, the cross-species complementation indicated a high degree of functional conservation throughout evolution.

Evolutionarily Conserved Features of OSTα and OSTβ Sequences. The predicted OSTα proteins from human, mice, rats, and skates are comparable in size and are expected to have remarkably similar membrane topologies, namely seven-TM domains, with an extracellular amino terminus and an intracellular carboxy terminus (Fig. 2). Each of the predicted TM domains and hydrophilic loops are similar in length and in relative position within the polypeptide (39). Human OSTα shares 41% amino acid identity with the skate protein, and 82%–83% amino acid identity with mouse and rat Ostα. The mouse and rat proteins share 93.5% amino acid identity. The amino acid identity between the human, mouse, rat, and skate proteins is not restricted to the TM helices but is also seen in putative intracellular and extracellular loops (Fig. 2). Several amino acid regions appear highly conserved in the hydrophilic loops of OSTα, including an unusual stretch of six to seven cysteine residues that reside in a predicted cytosolic loop between TM Domains 3 and 4 [TGPCCCCCPC(C/L)P; Fig. 2]. The significance of this cysteine motif in Ostα is not known, although it may function either as a ligand or as a substrate binding site, a site of interaction with Ostβ, or a site of membrane association. In general, cysteine residues play important roles in protein secondary structure, metal coordination, oligomerization, and posttranslational modifications.

Likewise, the predicted OSTβ proteins from human, mice, rats, and skates are comparable in size and are expected to have similar membrane topologies, namely only one TM domain (Fig. 2). The only exception is skate Ostβ, which has a longer amino terminus; however, the first 27 amino acids of skate Ostβ are predicted to be a signal peptide (21), such that the mature protein may be comparable in length to the human and mouse proteins. Human OSTβ shares 25% amino acid identity with the skate protein, and 63% and 58% amino acid identity with mouse and rat Ostβ, respectively. The mouse and rat proteins share 80% amino acid identity (Fig. 3).

It is also interesting to note that human, mouse, and skate OSTα and OSTβ proteins all appear to have membrane-targeting sequences in their C-terminal, putative cytosolic domains. Skate Ostα and Ostβ and mouse Ostβ have an Arg-X-Arg (RXR) motif, whereas human OSTα and mouse Ostα have an RRK sequence at the corresponding location in the sequence (Figs. 2 and 3). RXR sequences in hetero-oligomeric proteins function as retention or retrieval signals that must be masked before the corresponding protein complexes can be transported from the endoplasmic reticulum (40–42).

A computer-generated phylogenetic analysis of OSTα and OSTβ sequences is illustrated in Figure 4. This analysis included two *Drosophila melanogaster* and two *Caenorhabditis elegans* proteins that may belong to the OSTα gene family, although the function of these proteins is unknown. The results indicate that skate Ostα and Drosophila NP_649079.2 may have arisen from an evolutionarily ancient common ancestor and that the mammalian OSTα proteins diverged more recently from the skate lineage (Fig. 4A). A very similar evolutionary pattern is seen for OSTβ (Fig. 4B), although Drosophila and *C. elegans* homologues of OSTβ have not yet been identified.

Tissue Distribution of Human and Mouse *OSTα* and *OSTβ* mRNA. Quantitative polymerase chain reaction (PCR) analysis of *OSTα* and *OSTβ* mRNA levels in 19 human tissues revealed that these genes are widely expressed in human tissues, with the greatest degree of expression seen in testes, colon, liver, small intestine, kidney, ovary, and adrenal gland; lower levels were measured in heart, lung, brain, pituitary, thyroid gland, uterus, prostate, mammary gland, and fat (39).

Mouse *Ostα* and *Ostβ* mRNAs were also abundant in small intestine and kidney, but in contrast to these genes in the human, the genes were expressed at very low levels in liver (43). These reverse transcription–PCR results are supported by an analysis of expressed sequence tag (EST) counts, which are available from the UniGene EST Profile Viewer Web site of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/UniGene/); these counts are summarized in Table 2. The human tissues with the highest EST counts for *OSTα* are testis, liver, kidney, lung, colon, and eye, whereas in the mouse, *Ostα* is most abundant in kidney and colon, with very low levels in the liver (Table 2).

In both the human and mouse, the tissues that had high levels of *OSTα* mRNA generally also had high levels of *OSTβ* mRNA, indicating coexpression of these genes (39, 43). This parallel tissue expression of OSTα and OSTβ supports the hypothesis that these gene products work together to elicit transport activity. Moreover, the high expression of the genes in the three tissues that are perhaps

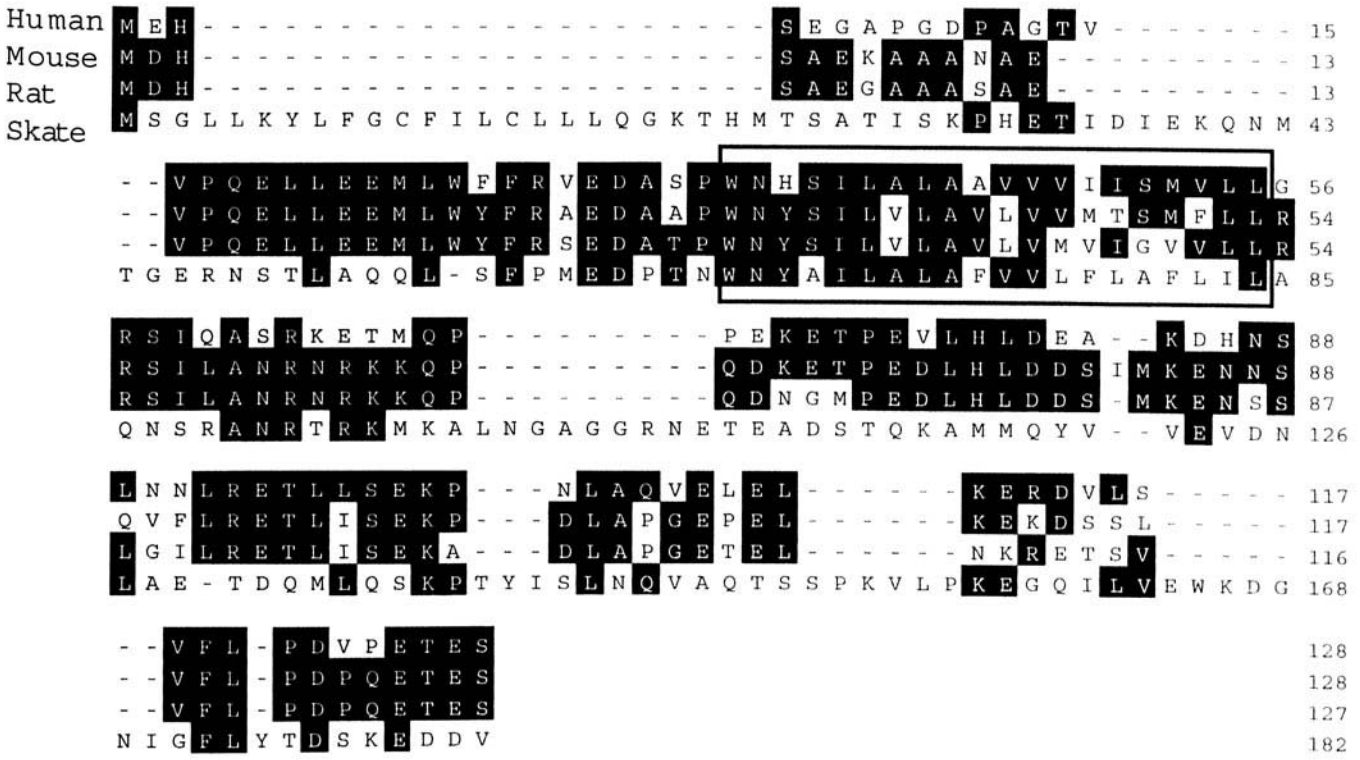

Figure 3. OSTβ amino acid alignments. The deduced amino acid sequences for human, mouse, rat, and skate OSTβ were aligned using DNAStar's MegAlign computer program running the Jotun Hein algorithm. Amino acid identity is displayed with black shading, and the predicted TM domain is underlined. The RXR motif is located at amino acid position 61 in the mouse and rat sequences and at amino acid position 92 in the skate.

the most important in the disposition of bile acids and related steroid compounds (namely the intestine, kidney, and liver) provides strong support for the contention that OSTα–OSTβ is a critical regulator of steroid homeostasis.

OSTα and OSTβ Lack Paralogues in the Human or Mouse Genome. In contrast with most other transporters that are members of large gene families, OSTα and OSTβ are unique genes in the human and mouse genomes. Because individual members of multi–gene transporter families often have overlapping substrate specificities and functions, deletion of a single gene often has no consequences under normal physiologic conditions, although a phenotype may be observed when the animal is stressed. In contrast, OSTα and OSTβ do not have paralogues that may compensate in their absence. The absence of paralogues for OSTα and OSTβ, and the fact that this transporter has survived evolutionary selection, provides support for the hypothesis that these genes play a

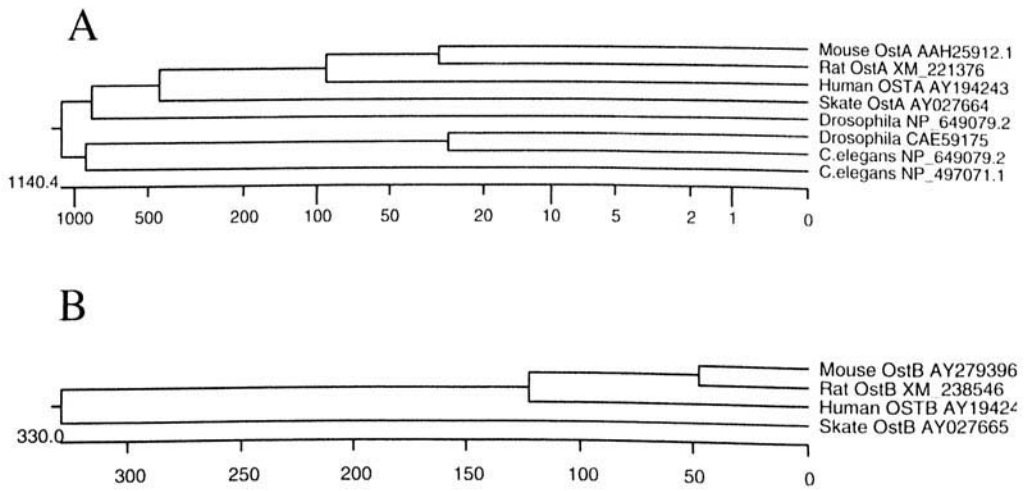

Figure 4. Phylogenetic analysis of OSTα and OSTβ. A computer-generated phylogenetic analysis of putative OSTα and OSTβ amino acid sequences from different organisms.

Table 2. Tissue Expression of Human *OSTα* and *OSTβ* and Mouse *Ostα* and *Ostβ* as Indicated by Analysis of Expressed Sequence Tag (EST) Counts[a]

	Human		Mouse	
	OSTα	*OSTβ*	*Ostα*	*Ostβ*
	(Transcripts per million)			
Testis	95	7	0	0
Liver	30	7	9	0
Kidney	21	64	179	34
Lung	17	3	0	0
Colon	11	33	57	96
Eye	11	11	0	0

[a] Data were obtained from the UniGene EST Profile Viewer Web site of the National Center for Biotechnology Information on May 13, 2005 (http://www.ncbi.nlm.nih.gov/UniGene/).

necessary and perhaps unique physiologic role in humans. In addition, OSTα–OSTβ appears to function as a transporter for steroids such as estrone 3-sulfate, dehydroepiandrosterone 3-sulfate, taurocholate, and digoxin, as well as the eicosanoid PGE_2. Because steroids and eicosanoids are involved in many cellular functions, this transporter may play a central role in regulating these activities. Thus, one possible role of OSTα–OSTβ is to regulate the cellular disposition of signaling molecules.

Evidence that OSTα–OSTβ Is an Efflux Transporter That Is Localized to the Basolateral Membrane of Intestinal Epithelial Cells. Recent studies by Dawson and colleagues (43) demonstrate that mouse Ostα and Ostβ proteins are both localized to the basolateral membrane of enterocytes. These proteins showed a vertical distribution of staining along the crypt-to-villus axis, with maximal staining in the mature villus enterocytes. Staining for a small fraction of Ostβ and Ostα protein was also detected in intracellular membranes of the enterocytes.

The ability of Ostα–Ostβ to function as a basolateral bile acid efflux transporter was examined in triply transfected MDCK cells expressing ASBT, Ostα, and Ostβ (43). MDCK/ASBT cells expressing Ostα, Ostβ, or both Ostα and Ostβ were grown on Transwell filter inserts and assayed for their ability to mediate transcellular transport of taurocholate. The MDCK/ASBT cells expressing Ostα or Ostβ alone exhibited only background levels of apical to basolateral taurocholate transcellular transport, whereas cells expressing both Ost subunits mediated significant taurocholate transcellular transport (43). In contrast to the apical transport, expression of Ostα–Ostβ had no effect on the basolateral to apical transcellular transport of taurocholate, reflecting the appropriate sorting of the proteins in the MDCK cells. Additional preliminary data demonstrate that OSTα–OSTβ can mediate either uptake or efflux when expressed in *Xenopus laevis* oocytes.[2] Taken together, these data indicate that OSTα–OSTβ is the transporter responsible for steroid and bile acid reabsorption in the intestine and perhaps in other tissues.

How Do OSTα and OSTβ Interact to Generate a Functional Transporter? Although the answer to this question is unknown, insight is provided by the studies of Dawson *et al.* (43), which demonstrate that coexpression of Ostα and Ostβ is required for delivery of the individual proteins to the plasma membrane of transfected HEK 293 cells or of the MDCK cells. These studies demonstrated that coexpression of Ostα and Ostβ was required to convert the Ostα subunit to a mature *N*-glycosylated Endo H-resistant form, indicating that coexpression facilitates the movement of Ostα through the Golgi apparatus. This conclusion was also supported by immunolocalization studies that showed that coexpression was necessary for plasma membrane expression of Ostα and Ostβ (43). Thus, stable association of both subunits may be required for transporter function, or the Ostβ subunit may function as a chaperone to promote the egress of Ostα and possibly other proteins from the endoplasmic reticulum. However, additional studies are needed to define the mechanism by which these two proteins interact, their individual roles in generating a functional complex at the plasma membrane, and their roles in solute transport.

Regulation of Gene Expression. To date there is little direct evidence for factors that may regulate *Ostα* or *Ostβ* gene expression. Because Ostα–Ostβ is a bile acid transporter, one might expect that bile acids should modulate expression, and indeed, the work of Dawson and colleagues (43) supports this contention. Their results demonstrate that Ostα and Ostβ expression is decreased in the ileum of the *Slc10a2* null mice that have a diminished capacity to take up bile acids but are induced in the cecum and colon of these animals in response to the 10-fold increased flux of bile acids through their large intestine (43). Thus, one would predict that the nuclear bile acid receptor, the farnesoid X-activated receptor, is involved in this response, and current studies are testing this possibility.

Summary

Using a comparative approach, recent studies have identified and functionally characterized a novel type of organic solute and sterol transporter from the marine skate (21) and have then used this information to identify the mouse and human orthologues (39). This unusual transporter is generated by coexpression of two unique gene products, OSTα and OSTβ. In contrast with most other transporters that are members of gene families, OSTα and OSTβ do not have paralogues in the mouse or human genomes and are able to functionally complement each other across species. Characterization of the transport function and cellular localization of the proteins suggests that OSTα–OSTβ is a critical regulator of the reabsorption of bile acids and other sterols in the intestine, kidney, and

[2] N. Ballatori et al., unpublished observations.

other epithelial tissues. Transported substrates include steroids (estrone 3-sulfate, dehydroepiandrosterone 3-sulfate, taurocholate, digoxin) and prostaglandin E_2, indicating a role in the disposition of key cellular metabolites and signaling molecules. Indirect immunofluorescence microscopy revealed that both proteins are detected in the basolateral membrane of ileal enterocytes (43). Overall, these studies identify a novel heteromeric transporter that is localized to the basolateral membrane of specific epithelial tissues and that may serve to regulate transepithelial movement and disposition of endogenous and exogenous sterols.

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