Functional Complementation between a Novel Mammalian Polygenic Transport Complex and an Evolutionarily Ancient Organic Solute Transporter, OSTα-OSTβ*

Received for publication, January 31, 2003, and in revised form, April 18, 2003
Published, JBC Papers in Press, April 28, 2003, DOI 10.1074/jbc.M301106200

David J. Seward‡§, Albert S. Koh‡, James L. Boyer§§, and Nazzareno Ballatori‴¶

From the ‡Department of Environmental Medicine, University of Rochester School of Medicine, Rochester, New York 14642, §Mount Desert Island Biological Laboratory, Salsbury Cove, Maine 04672, and the ¶Department of Medicine and Liver Center, Yale University School of Medicine, New Haven, Connecticut 06520

These studies identify an organic solute transporter (OST) that is generated when two novel gene products are co-expressed, namely human OSTα and OSTβ or mouse OSTα and OSTβ. The results also demonstrate that the mammalian proteins are functionally complemented by evolutionarily divergent Ostα-OSTβ proteins recently identified in the little skate, Raja erinaceae, even though the latter exhibit only 25–41% predicted amino acid identity with the mammalian proteins. Human, mouse, and skate OSTo proteins are predicted to contain seven transmembrane helices, whereas the OSTβ sequences are predicted to have a single transmembrane helix. Human OSTα-OSTβ and mouse Ostα-OSTβ cDNAs were cloned from liver mRNA, sequenced, expressed in Xenopus laevis oocytes, and tested for their ability to functionally complement the corresponding skate proteins by measuring transport of [3H]estrone 3-sulfate. None of the proteins 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 estrone 3-sulfate transport activity. Transport was sodium-independent, saturable, and inhibited by other steroids and anionic drugs. Human and mouse OSTα-OSTβ also were able to mediate transport of taurocholate, digoxin, and prostaglandin E2, but not of estradiol 17β-d-glucuronide or p-aminohippurate. OSTα and OSTβ were able to reach the oocyte plasma membrane when expressed either individually or in pairs, indicating that co-expression is not required for proper membrane targeting. Interestingly, OSTα and OSTβ mRNAs were highly expressed and widely distributed in human tissues, with the highest levels occurring in the testis, colon, liver, small intestine, kidney, ovary, and adrenal gland. Cellular homeostasis requires the regulated entry and exit of a multitude of compounds 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. Recent studies have described some of the genes involved in these transport processes; however, it is clear that many other genes and gene products remain to be identified and characterized (1–7).

Using a comparative approach, a novel type of organic solute and steroid transporter was recently identified in the liver of an evolutionarily ancient vertebrate, the little skate Raja erinaceae (8). In contrast to all other organic anion carriers, this skate transporter is generated by co-expression of two distinct and novel gene products, Ostα and Ostβ. Substrates for this multispecific transporter include estrone 3-sulfate, taurocholate, digoxin, and prostaglandin E2. Interestingly, the overall predicted membrane topology of skate Ostα-Ostβ is similar to that of the heterodimeric sensory rhodopsins, suggesting that Ostα-Ostβ may have evolved from an ancestral rhodopsin-like molecule but has acquired the ability to transport steroids and eicosanoids, compounds that also function as ligands for some G-protein-coupled receptors. Initially, Ostα and Ostβ orthologues were not identified in the human genome or in any other sequenced genomes, indicating that these genes might be specific to marine elasmobranchs. However, sequences for hypothetical human and mouse proteins have recently been entered into the data bases that exhibit 25–41% predicted amino acid sequence identity with skate Ostα and Ostβ (see “Results”). The present study tested whether these mammalian gene products were expressed, whether they encode for orthologues of the skate gene products, and if so, whether they functionally complement the transport activity of one another.

EXPERIMENTAL PROCEDURES

Materials and Animals—[3H]Taurocholic acid (3.47 Ci/mmol), [3H]estrone 3-sulfate (53 Ci/mmol), [3H]digoxin (19 Ci/mmol), [3H]prostaglandin E2 (200 Ci/mmol), [3H]estradiol 17β-d-glucuronide (50 Ci/mmol), and p-[3H]glycyl-2-3H]aminohippuric acid (4.08 Ci/mmol) were purchased from PerkinElmer Life Sciences. Chemicals were obtained from Sigma or J. T. Baker Inc. Molecular biology reagents were purchased from Invitrogen; Clontech, Palo Alto, CA; Integrated DNA Technologies, Coralville, IA; Qiagen, Valencia, CA; Origene, Rockville, MD; Ambion, Austin, TX; and Promega, Madison, WI. Mature Xenopus laevis were purchased from Nasco, Fort Atkinson, WI.

* The abbreviations used are: Ost/OST, organic solute transporter; hOSTa, human OSTα; mOSTa, mouse OSTα; sOSTa, skate Ostα; ORF, open reading frame; TM, transmembrane; PBS, phosphate-buffered saline; RT-PCR, reverse transcription PCR; SNAP, soluble N-ethylmaleimide-sensitive fusion attachment protein; SNARE, SNAP-25 receptor.

1 The work was supported in part by National Institutes of Health Grants DK48823, ES06484, DK25638, and ES07026 and by National Institute of Environmental Health Sciences Grants ES03628 and ES01247. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AY194243, AY194242, AAH25912, AT279396, AT027664, and AT027665.

[To whom correspondence should be addressed: Dept. of Environmental Medicine, Box EHSIC, University of Rochester School of Medicine, 575 Elmwood Ave., Rochester, NY 14642. Tel.: 585-275-0262; Fax: 585-256-2591; E-mail: Ned_Ballatori@urmc.rochester.edu]

This paper is available on line at http://www.jbc.org

27473
Cloning Strategy—A multistep RT-PCR-based strategy was employed to obtain cDNAs for the putative open reading frames (ORFs) of hypothetical proteins Ostα (GenBank™/EBI accession number CAC51162), Ostβ (GenBank™/EBI accession number XP_058693), and mOSTβ (GenBank™/EBI accession number XP_134984/A2Y79396). Oligonucleotide primers were designed to generate ORFs for each gene based on the cDNA sequences listed on GenBank™ (Table 1). RT-PCR products of predicted sizes were created for putative hOSTα, hOSTβ, and mOSTβ using either human or mouse liver poly(A) RNA as a template (Clontech). The reaction products were isolated via agarose gel electrophoresis and ligated into the pCR-II TOPO vector (Invitrogen). The resulting plasmids were used to transform DH5α Escherichia coli, from which positive clones were selected for ampicillin resistance and blue/white staining. Upon isolation and transformation, a single clone expressing the new plasmid was used as a template in a PCR with primers designed to generate a final product flanked by two unique restriction sites (Table 1). Following isolation and enzymatic digestion, the cDNAs with sticky ends were directionally ligated into the pSP64 poly(A) vector. These plasmids then were used to transform DH5α E. coli, and positive clones were selected for ampicillin resistance. Plasmid purifications were completed using Promega’s DNA Wizard kit. The cDNA for the hypothetical mOstα (GenBank™/EBI accession number BC024441.1 and AAH25912) was obtained from the American Type Culture Collection (ATCC #6994476). This clone was used as a template in a PCR to generate the ORF for GenBank™ sequence BC024441.1. The primers were designed to add a HindIII site to the 5’ end and an XbaI site to the 3’ end of the PCR product (Table 1). The PCR product was purified by agarose gel electrophoresis, and the band of expected size (1060 bp) was excised and enzymatically digested with HindIII and XbaI. The resulting cDNA sequence was then directionally cloned into the pSP64 poly(A) vector and used to transform DH5α E. coli. A positive clone was selected, and the plasmid was isolated with Promega’s DNA Wizard miniprep kit.

Final clones for all four genes were sequenced in both directions with a series of specific oligonucleotide primers at the Mount Desert Island Biological Laboratory DNA Sequencing Core (Salsbury Cove, ME) to confirm sequence identity. The plasmids were then linearized with EcoRI and used to synthesize cRNA via Ambion’s mMessage mMachine kit for injection into Xenopus oocytes.

Real Time Quantitative PCR to Determine Tissue Distribution and Expression Levels of hOSTα and hOSTβ in 19 Human Tissue cDNAs—Human tissue cDNAs were purchased from Origene (human Sure-RACE™ panel). Synthetic oligonucleotide primers were designed to amplify portions of hOSTα, hOSTβ, and human β-actin for use in quantitative real time PCR (Table 1). Reactions were conducted and analyzed on a Roche Real Time Light Cycler from Corbett Research (Hayward, CA). Qiagen’s QuantiTect Sybr Green quantitative RT-PCR kit was used for PCR analysis. Expression levels are reported as a ratio to β-actin within each tissue examined.

Xenopus Oocyte Preparation, Microinjection, and Transport Assays—Isolation of Xenopus oocytes was performed as described by Goldin (10) and employed previously in our laboratory (10–12). Stage V and VI oocytes were obtained from a Xenopus Oocyte Preparation, Microinjection, and Transport Assays—Intact Xenopus oocytes on day 3 after CRNA injection were fixed in methanol-acetone (1:1) for 10 min on ice, followed by four washes of 5 min each at room temperature and an overnight wash at 4 °C in antibody dilution buffer (0.01 M PBS + 0.05% Tween 20, 1% bovine serum albumin, 1% normal goat serum, and 0.01% sodium azide). Oocytes were washed at room temperature with 1 × PBS + 1% Tween 20 for 1 h with 10-min buffer changes followed by a 15-min incubation in blocking solution (3% bovine serum albumin in antibody dilution buffer). Oocytes were then washed with 1 × PBS + 1% Tween 20 for another 1 h with 10-min buffer changes followed by an overnight wash at 4 °C. The oocytes were incubated with either anti-FLAG M2 monoclonal antibody (Sigma; 4.9 mg/ml, diluted 1:200) or monoclonal anti-c-MYC Cy3-conjugated antibody (Sigma; 1.2 mg/ml, diluted 1:100 with antibody dilution buffer) for 1 h. To remove excess antibody, oocytes were washed in 1 × PBS + 1% Tween 20 three times for 5 min each, two times for 10 min each, and then overnight. The oocytes labeled with the anti-FLAG M2 monoclonal antibody were incubated with an Alexa fluor 488 Phalloidin, fragment of goat anti-mouse antibody in the dark for 1 h (Molecular Probes; 2 mg/ml, diluted 1:200). Secondary antibody was removed by washing the oocytes with 1 × PBS + 1% Tween 20 three times for 5 min each, two times for 10 min each, and then overnight. Cells were imaged using a ×10 objective on a Leica TCS-SP laser-scanning confocal microscope.

Kinetic Analysis—Kinetic data from experiments measuring uptake of radiolabeled substrate were fit to the Michaelis-Menten equation by nonlinear least squares regression analysis. Vmax and Km values with standard errors were derived from these curves. Comparison of data measuring initial rates of uptake of radiolabeled substrates in the presence and absence of inhibitors was performed by unpaired Student’s t test and correlated to p < 0.05.

RESULTS

Cloning Putative Human OSTα and Ostβ and Mouse Ostα and Ostβ—Hypothetical human and mouse proteins (GenBank™/EBI accession numbers CAC51162 and AAH25912, respectively) that were recently added to the data bases exhibit 41% predicted amino acid identity with skate Ostα and share 83% amino acid identity with each other (Fig. 1). Because of the many conserved amino acid substitutions, the extent of amino acid similarity is ~70% between skate Ostα and these putative mammalian orthologues and 89% between the hypothetical mouse and human proteins (Fig. 1). Interestingly, all three deduced amino acid sequences share a highly unusual cluster of cysteine residues in a predicted hydrophilic cytosolic loop between transmembrane (TM) domains 3 and 4 (Fig. 1). This relatively high overall amino acid identity along with the conserved cysteine cluster in the human, mouse, and skate gene products suggests that they are functional orthologues.

Likewise, possible human and mouse orthologues of skate Ostβ were recently entered into the data bases, but these sequences exhibit only 25–29% predicted amino acid identity with skate Ostβ (Fig. 2). The hypothetical human protein with GenBank™ sequence XP_058693 and the hypothetical mouse protein with GenBank™ sequence XP_134984/A2Y79396 exhibit 25 and 29% predicted amino acid identity with skate Ostβ, respectively (Fig. 2). These hypothetical human and mouse proteins exhibit 62.5% amino acid identity with each other, both encoding for proteins containing 128 amino acids with a putative single TM domain (Fig. 2).

Synthesis of FLAG Epitope-tagged Skate and Human OSTα and of c-MYC-tagged Skate and Human OSTβ—The FLAG epitope (DYKDDDK) was added in-frame to the 3’ end of skate and human OSTα cDNA, and the c-MYC epitope (EQKLISEEDL) was added to the 3’ end of skate and human OSTβ cDNA. Constructs were created via PCR using skate and human OSTα and OSTβ cDNA as templates with oligonucleotide primers containing the FLAG or c-MYC sequences flanked by unique restriction sites. PCR products of predicted size were identified by agarose gel electrophoresis, excised, and purified with Qiagen’s agarose gel purification kit. The isolated fragments were subcloned into the pSP64 poly(A) vector, and that vector was used to transform DH5α E. coli. Positive clones were grown and plasmid DNA isolated using Promega’s DNA Wizard plasmid isolation kit. After restriction digestion and sequencing to establish clone identity, cRNA was prepared for each of the four constructs using Ambion’s mMessage mMachine kit.

Immunofluorescence Labeling of Intact Oocytes—Intact Xenopus oocytes on day 3 after CRNA injection were fixed in methanol-acetone (1:1) for 10 min on ice, followed by four washes of 5 min each at room temperature and an overnight wash at 4 °C in antibody dilution buffer (0.01 M PBS + 0.05% Tween 20, 1% bovine serum albumin, 1% normal goat serum, and 0.01% sodium azide). Oocytes were washed at room temperature with 1 × PBS + 1% Tween 20 for 1 h with 10-min buffer changes followed by a 15-min incubation in blocking solution (3% bovine serum albumin in antibody dilution buffer). Oocytes were then washed with 1 × PBS + 1% Tween 20 for another 1 h with 10-min buffer changes followed by an overnight wash at 4 °C. The oocytes were incubated with either anti-FLAG M2 monoclonal antibody (Sigma; 4.9 mg/ml, diluted 1:200) or monoclonal anti-c-MYC Cy3-conjugated antibody (Sigma; 1.2 mg/ml, diluted 1:100 with antibody dilution buffer) for 1 h. To remove excess antibody, oocytes were washed in 1 × PBS + 1% Tween 20 three times for 5 min each, two times for 10 min each, and then overnight. The oocytes labeled with the anti-FLAG M2 monoclonal antibody were incubated with an Alexa fluor 488 Phalloidin, fragment of goat anti-mouse antibody in the dark for 1 h (Molecular Probes; 2 mg/ml, diluted 1:200). Secondary antibody was removed by washing the oocytes with 1 × PBS + 1% Tween 20 three times for 5 min each, two times for 10 min each, and then overnight. Cells were imaged using a ×10 objective on a Leica TCS-SP laser-scanning confocal microscope.

Kinetic Analysis—Kinetic data from experiments measuring uptake of radiolabeled substrate were fit to the Michaelis-Menten equation by nonlinear least squares regression analysis. Vmax and Km values with standard errors were derived from these curves. Comparison of data measuring initial rates of uptake of radiolabeled substrates in the presence and absence of inhibitors was performed by unpaired Student’s t test and correlated to p < 0.05.
An RT-PCR-based strategy was employed to obtain cDNAs for the predicted ORFs of putative human OSTα and OSTβ using human liver mRNA and of putative mouse Ostα and Ostβ using mouse liver mRNA. The cDNA for the putative mouse Ostα was obtained from the American Type Culture Collection (ATCC #6994476). Sequence analysis of the synthesized cDNAs for the four genes indicated that both mouse clones were identical to GenBank™ sequences, whereas the human sequences varied by a single nucleotide in the reading frame of each gene. Both differences in the human gene sequences result in amino acid substitutions (Figs. 1 and 2; GenBank™/EBI accession numbers AY194243 and AY194242). It is unclear whether the observed differences are due to polymorphisms or whether they result from mutations introduced during PCR. They are unlikely to be sequence artifacts as both occur in regions of strong sequence data. When compared with the published human genomic DNA data base (www.ncbi.nlm.nih.gov:80/BLAST/), our human OSTα sequence matches perfectly, whereas the GenBank™ cDNA sequence for the protein with GenBank™/EBI accession number CAC51162 contains an adenine at the base in question rather than a guanine (position 604 of the ORF). This difference predicts a valine at position 202 of human OSTα (circled in Fig. 1), whereas the GenBank™ sequence CAC51162 predicts an isoleucine. In contrast, our human OSTβ sequence differs from the genomic DNA sequence at the base in question, containing a cysteine at nucleotide 197 of the ORF instead of an adenine. As a result, the sequence for human OSTβ obtained in the present study predicts a threonine at position 66 (circled in Fig. 2), whereas the GenBank™ sequence XP_058693 predicts a lysine. Because the human liver poly(A)^+ RNA used as a template for RT-PCR in the present study was pooled from four different people, the reaction product containing the inserts used for cloning human OSTβ was sequenced. Upon analysis it was determined that an equal distribution of adenine and cytosine occurred at this nucleotide position (data not shown), indicating that this may be a naturally existing polymorphism.

A search of the human genomic data base (www.ncbi.nlm.nih.gov:80/BLAST/) revealed that human OSTα is located on chromosome 3 and is coded by nine exons in the 3q29 region,
whereas human OST\(\alpha\) is on chromosome 15 and is coded by four exons located in the 15q21 region. Mouse OST\(\alpha\) is located on chromosome 16 and is coded by nine exons at 16B2, whereas mouse OST\(\beta\) is found on chromosome 9 and is coded by four exons located at 9C.

**The Human and Mouse Proteins Function as Organic Solute Transporters**—To assess whether the human and mouse proteins function as organic solute transporters, uptake of [3H]estrone 3-sulfate was measured in *X. laevis* oocytes injected with cRNA synthesized from the human, mouse, or skate genes. As expected, co-expression of skate Ost\(\alpha\) and Ost\(\beta\) was required to generate transport activity (Fig. 3A) (8). When the putative human OST\(\alpha\) and mouse OST\(\alpha\) were expressed individually in oocytes, no transport activity was detected (Fig. 3A); however, when these proteins were co-expressed with skate Ost\(\beta\), a strong transport signal was obtained (Fig. 3A), indicating that human OST\(\alpha\) and mouse OST\(\alpha\) can functionally complement the corresponding skate protein. Likewise, human OST\(\beta\) and mouse OST\(\beta\) did not induce transport activity when expressed individually in oocytes but generated a functional transporter. 

**Fig. 1. OST\(\alpha\) amino acid alignments.** The deduced amino acid sequences for human, mouse, and skate OST\(\alpha\) were aligned using DNASTar's MegAlign computer program running the Jotun Hein algorithm. Amino acid identity is displayed with black shading, and the predicted 7-TM domains are boxed. The shared and conserved stretch of cysteine residues (TGPCCPCPCCLP) is denoted with a dotted underline. The cDNA sequence obtained in the present study for human OST\(\alpha\) predicts a valine at position 202 (circled) (GenBankTM/EBI accession number AY194243), whereas the GenBankTM sequence CAC51162 predicts an isoleucine at this position. This change results from a single nucleotide difference at base 604 of the ORF, where GenBankTM sequence AY194243 contains an adenine and GenBankTM sequence CAC51162 contains a guanine. The RRK and RXR motifs are located at amino acid position 318 in the human and mouse and 313 in the skate.

**Fig. 2. OST\(\beta\) amino acid alignments.** The deduced amino acid sequences for human, mouse, and skate OST\(\beta\) 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 boxed. The cDNA sequence obtained in the present study for human OST\(\beta\) predicts a threonine at position 66 (circled) (GenBankTM/EBI accession number AY194242), whereas the GenBankTM sequence XP_058693 predicts a lysine at this position. This change results from a single nucleotide difference at base 197 of the ORF, where GenBankTM sequence AY194242 contains a cytosine and GenBankTM sequence XP_058693 contains an adenine. The RXR motif is located at amino acid position 61 in the mouse and 92 in the skate.
when co-expressed with skate Ostα (Fig. 3B). Thus, the human and mouse proteins not only are functional orthologues of the skate proteins but also are able to complement each other across species. Moreover, co-expression of the two human (OSTα-OSTβ) or the two mouse (mOSTα-mOSTβ) proteins generated a very strong transport signal, as did the human-mouse α-β pairs (Fig. 3C).

Characteristics of Human OSTα-OSTβ- and Mouse OSTα-OSTβ-mediated Transport—Oocytes injected with human OSTα and OSTβ cRNA (1 ng each) or with mouse Ostα and Ostβ cRNA (1 ng each) were able to transport taurocholate, estrone 3-sulfate, digoxin, and prostaglandin E2 but not estradiol 17β-d-glucuronide or p-aminobipiridate (Fig. 4), indicating that this transport system is multispecific and that it may participate in cellular uptake of conjugated steroids and eicosanoids. This substrate profile is similar to that of skate Ostα-Ostβ (8).

The skate, mouse, and human transporters shared a number of other features as well. Transport was sodium-independent (Fig. 5), saturable (Fig. 6), and inhibited by bile salts, steroids, and other organic anions (Table II). Replacement of the NaCl in the oocyte incubation medium with either choline chloride or lithium chloride had no effect on estrone 3-sulfate uptake (Fig. 5), indicating that transport is not coupled to the sodium electrochemical gradient. Initial rates of estrone 3-sulfate uptake into human OSTα-OSTβ- or mouse OSTα-OSTβ-expressing oocytes were saturable, although the apparent Michaelis constants (Km) were relatively high (320 ± 30 μM and 290 ± 24 μM, respectively; Fig. 6). The Km for estrone 3-sulfate uptake by skate Ostα-Ostβ is lower (85 μM) (8). Uptake of ³H Estrone 3-sulfate in hOSTα-hOSTβ- and mOSTα-mOSTβ-expressing oocytes was inhibited by a variety of bile salts, steroids, and other organic anions (Table II). As reported previously for skate Ostα-Ostβ (8), ³H Estrone 3-sulfate uptake was inhibited by sulfated steroids, including lithocholic acid sulfate and tauro-lithocholic acid sulfate (Table II).

Tissue Distribution of Human OSTα and OSTβ mRNA—OSTα and OSTβ mRNA levels were measured in 19 human tissue cDNAs and were expressed relative to β-actin mRNA levels using quantitative real time PCR analysis. The results revealed that OSTα and OSTβ are widely expressed in human tissues (Fig. 7). Tissues that had high levels of OSTα mRNA generally also had high levels of OSTβ mRNA, indicating co-expression of these genes. Relatively high levels of both mRNAs were found in testis, colon, liver, small intestine, kidney, ovary, and adrenal gland (Fig. 7A); and lower levels were measured in heart, lung, brain, pituitary, thyroid gland, uterus, prostate, mammary gland, and fat (Fig. 7B). The mRNA for OSTα and OSTβ was below our limit of detection in skeletal muscle and peripheral blood leukocytes (data not shown).

Trafficking of OSTα and OSTβ to the Plasma Membrane—To gain insight into the mechanism by which OSTα and OSTβ interact to generate a functional transporter, epitope-tagged
The present results identify a novel mammalian organic solute transporter for which the mRNA is widely distributed and highly expressed in human tissues. This transporter is unique among mammalian organic anion transporters in that it is generated when two distinct gene products are co-expressed, namely a putative 7-TM domain membrane protein, OSTα, and a smaller, single-TM domain polypeptide, OSTβ. Interestingly, the human and mouse proteins were able to complement each other as well as those from an evolutionarily ancient vertebrate, the little skate, indicating a high degree of conservation throughout evolution. Although the physiological functions of this transporter are not known, its broad tissue expression and its ability to transport steroids and prostaglandin E₂ suggest an important role in cellular functions.

Identification of OSTα and OSTβ was made possible by the recent cloning of orthologous genes from the liver of an evolutionarily ancient marine vertebrate, the little skate (8). Surprisingly, when skate Ostα and Ostβ genes were identified in 2001, comparable genes were not present in any of the sequenced genomes, including the human genome, suggesting that they may be unique to elasmobranchs (8). However, because the human genome remains in draft form, many genes have not yet been discovered.

Hypothetical human and mouse genes that recently were entered into the data bases are predicted to encode proteins exhibiting 25–41% amino acid identity with skate Ostα and Ostβ (Figs. 1 and 2). Although this level of amino acid identity is low, it is not insignificant given an evolutionary distance of 200 million years between skates and humans. Thus, despite the modest level of amino acid identity, these skate and mammalian proteins may be carrying out the same biological functions. To test this possibility, the present study assessed the functional conservation throughout evolution.

Fig. 4. Substrate selectivity of human OSTα-OSTβ and mouse OSTα-OSTβ. Oocytes were injected with water, 1 ng of hOSTα cRNA plus 1 ng of hOSTβ cRNA, or 1 ng of mOstα cRNA plus 1 ng of mOstβ cRNA. After 3 days in culture, uptake of radiolabeled compounds ([3H]taurocholate, 20 μM; [3H]estrone 3-sulfate, 50 nM; [3H]digoxin, 0.5 μM; [3H]prostaglandin E₂ (PGE₂), 5 nM; [3H]estradiol glucuronide (estradiol 17β-D-gluc.), 57 nM; or [3H]aminopterin acid (PAH), 1 μM) was measured at 25 °C for 1 h. Uptake values are reported as pmol/oocyte for all of the other compounds. Values are means ± S.E., n = 3 fmol, femtomoles.
generated by proteins that exhibit only 25% amino acid identity, suggesting that only a few conserved amino acids may be required for this interaction. For example, human OSTα was activated equally well by human OSTβ or skate Ostβ (Fig. 3) despite only a 25% amino acid identity for the latter two proteins (Fig. 2). Alternatively, the interaction between OSTα and OSTβ may be determined more by their three-dimensional structures or post-translational modifications than by primary

Table II
Effects of bile salts, steroids, and anionic drugs on human OSTα-OSTβ and mouse Ostα-Ostβ-mediated transport of [3H]estrene 3-sulfate

|                | Human OSTα-OSTβ | Mouse Ostα-Ostβ |
|----------------|-----------------|-----------------|
| **Bile salts** |                 |                 |
| Control        | 100 ± 5         | 100 ± 6         |
| Lithocholic acid, 200 μM | 105 ± 6       | 102 ± 5        |
| Lithocholic acid sulfate, 200 μM | 33 ± 4*     | 34 ± 4*        |
| Glycolithocholic acid, 200 μM | 56 ± 4*     | 71 ± 7         |
| Glycolithocholic acid, sulfate, 200 μM | 56 ± 1*   | 44 ± 3*        |
| Tauro lithocholic acid, 200 μM | 50 ± 8*    | 56 ± 1*        |
| Tauro lithocholic acid sulfate, 200 μM | 40 ± 6*  | 29 ± 8*        |
| **Steroids**   |                 |                 |
| Estrone 3-sulfate, 200 μM | 62 ± 7*    | 57 ± 1*        |
| Spironolactone, 200 μM | 63 ± 2*    | 72 ± 10        |
| Digoxin, 500 μM | 28 ± 5*       | 27 ± 1*        |
| **Other anions** |             |                 |
| Sulfobromophthalein, 100 μM | 75 ± 8*   | 64 ± 3*        |
| Bilirubin ditaurate, 500 μM | 64 ± 5*    | 67 ± 5*        |
| Probenecid, 1 mM | 75 ± 5*    | 57 ± 2*        |
| Indomethacin, 200 μM | 48 ± 1*    | 50 ± 6*        |

* Significantly different from control, p < 0.05.
amino acid sequences or may require the participation of a third, as yet unidentified protein or cofactor.

Interestingly, the predicted transmembrane domain architectures of human, mouse, and skate OSTα are similar, as are those of the three OSTβ proteins (Figs. 1 and 2), providing additional evidence that these proteins carry out the same biological functions. Each of the predicted TM domains and hydrophilic loops in OSTα and OSTβ from human, mouse, and skate are similar in length and relative position within the polypeptides (Figs. 1 and 2). The only significant exception is skate Ostβ, which has a longer amino terminus region; however, the first 27 amino acids of skate Ostβ are predicted to be a signal peptide (8) such that the mature protein may be comparable in length to the human and mouse proteins. The conserved membrane architecture between these evolutionarily divergent proteins indicates that this membrane structure is essential for function.

As indicated above, the amino acid identity between the human, mouse, and skate proteins is not restricted to the TM helices but is also seen in putative intracellular and extracellular loops (Figs. 1 and 2). For OSTα, several amino acid regions appear highly conserved in the hydrophilic loops, including an unusual stretch of 6–7 cysteine residues that reside in a predicted cytosolic loop between TM domains 3 and 4 (TGPCCCCP(C/L)P; Fig. 1). The significance of this cysteine motif in OSTα is not known, although it may function either as a ligand or substrate binding site, a site of interaction with OSTβ, or as a site of membrane association. In general, cysteine residues play important roles in protein secondary structure, metal coordination, oligomerization, and post-translational modifications. Proteins that contain comparable cysteine-rich motifs include the human t-SNARE protein Syntaxin 11 (GenBankTM/EBI accession number NP_003755), a chicken protocadherin isoform (GenBankTM/EBI accession number AAK57196), a candidate gene for human Cat-Eye syndrome (GenBankTM/EBI accession number AAK30049), the cysteine-string proteins, two proteins of unknown function (Chic1 and Chic2), a putative human zine transporter (GenBankTM/EBI accession number NM_017767), and a zebra fish Na/Pi co-transporter (GenBankTM/EBI accession number NM_017767).

**Fig. 7.** Tissue distribution of human OSTα and OSTβ mRNA. cDNAs isolated from different human tissues were subjected to quantitative real time PCR analysis with primers designed to OSTα, OSTβ, or β-actin. Panel A includes tissues with relatively high levels of expression, and panel B includes tissues with a low level of mRNA expression. Data are reported relative to β-actin expression for each tissue. The abbreviation BD indicates below experimental detection limit. Values are means ± S.E., n = 3.

**Fig. 8.** Estrone 3-sulfate transport activity in oocytes expressing epitope-tagged skate Ostα-Ostβ or human OSTα-OSTβ. The FLAG epitope was added to skate Ostα and human OSTα cDNA, and the c-MYC epitope was added to skate Ostβ and human OSTβ cDNA. cRNA from these four constructs was injected into oocytes either individually or together (i.e. skate Ostα-FLAG plus Ostβ-c-MYC or human OSTα-FLAG plus OSTβ-c-MYC). After 3 days in culture, uptake of 50 nM [3H]estrone 3-sulfate was measured for 1 h at 25 °C. Values are means ± S.E., n = 3; each of the three separate experiments was performed in triplicate.
OSTα-OSTβ, a Polygenic Organic Solute Transporter

AF121796). Although each of these proteins contains a short polycysteine motif, they share no additional sequence identity with OSTα. It is interesting to note, however, that many of these proteins are associated with the cell membrane and are either directly or indirectly involved in membrane transport (14–21).

It is also interesting to note that human, mouse, and skate OSTα and OSTβ proteins all appear to have membrane-targeting sequences in their carboxy-terminal, putative cytosolic domains. Skate OSTα and Ostβ and mouse OSTβ have an Arg-X-Arg (RXX) motif, whereas human OSTα and mouse OSTα have an RRK sequence at the corresponding location in the sequence (Figs. 1 and 2). RXX 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 (22–24).

(OSTα and OSTβ) for each species (I and J for skate and K and L for human). Oocytes were labeled either with an anti-FLAG M2 monoclonal antibody (human) or with a monoclonal anti-c-MYC antibody (B, F, and J for skate, C, G, and K for human) or with a monoclonal anti-c-MYC Cy3-conjugated antibody (E, I, and L for human).


The absence of primary structural homologues for OSTα and OSTβ and the fact that this transporter has survived evolutionary selection provide support for the hypothesis that these genes play a necessary and perhaps unique physiological role in humans. The present results indicate that OSTα-OSTβ can function as a transporter for steroids such as estrone 3-sulfate, taurocholate, and digoxin, as well as the eicosanoid prostaglandin E2 (Fig. 4). 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 cellular entry and/or exit of signaling molecules.

Additional evidence for an essential physiological role of these genes is provided by the broad tissue distribution of OSTα and OSTβ mRNA and by the high levels of mRNA found in several human tissues (Fig. 7). mRNA expression was detected in 17 of 19 tissues examined with relatively high levels found in testis, colon, liver, small intestine, kidney, ovary, and adrenal gland. In these tissues, OST mRNA was present at 5–40% of the levels of β-actin mRNA. Because β-actin is a relatively abundant transcript, this indicates that OSTα and OSTβ are expressed at high levels. However, because transcript abundance does not always correlate with high protein expression, additional studies are needed to evaluate relative protein abundance as well as the cellular and subcellular localization of these proteins.

REFERENCES

1. Saier, M. H. (2000) Microbiol. Mol. Biol. Rev. 64, 354–411
2. Borst, P., Evers, R., Kool, M., and Wijnholds, J. (2000) J. Natl. Cancer Inst. 92, 1295–1302
3. Keppler, D., and Konig, J. (2000) Semin. Liver Dis. 20, 265–272
4. Küllak-Ublick, G. A., Stieger, B., Hagenbuch, B., and Meier, P. J. (2000) Semin. Liver Dis. 20, 273–292
5. Suzuki, H., and Sugiyama, Y. (2000) Semin. Liver Dis. 20, 251–263
6. Venet, J. C., Adams, M. D., Myers, E. W., Li, P. W., Mural, R. J., Stutzer, G. G., Smith, H. G., Yandell, M., Evans, C. A., Holt, R. A., Gocayne, J. D., Amantadites, P., Ballew, R. M., Huson, D. H., Wurtman, J. R. et al. (2001) Science 291, 1304–1351
7. Lander, E. S., Linton, L. M., Birren, B., Nusbaum, C., Zody, M. C., Baldwin, J., Devon, K., Dewar, K., Doyle, M., FitzHugh, W., Funke, R., Gage, D., Harris, K., Headford, A., Howland, J. et al. (2001) Nature 409, 860–921
8. Wang, W., Seward, D. J., Li, L., Boyer, J. L., and Ballatori, N. (2001) Proc. Natl. Acad. Sci. 98, 8431–8436
9. Goldin, A. L. (1992) Methods Enzymol. 207, 266–279
10. Ballatori, N., Wang, W., Li, L., and Truong, A. T. (1996) Am. J. Physiol. 270, R1156–R1162
OSTα-OSTβ, a Polygenic Organic Solute Transporter

11. Li, L., Lee, T. K., Meier, P. J., and Ballatori, N. (1998) J. Biol. Chem. 273, 16184–16191
12. Li, L., Meier, P. J., and Ballatori, N. (2000) Mol. Pharmacol. 58, 335–340
13. Kyte, J., and Doolittle, R. F. (1982) J. Mol. Biol. 157, 105–132
14. Edwardson, J. M. (1998) Curr. Biol. 8, R390–R393
15. Hay, J., C., and Scheller, R. H. (1997) Curr. Opin. Cell Biol. 9, 505–512
16. Linial, M. (1997) J. Neurochem. 66, 1781–1792
17. Chamberlain, L. H., and Burgoyne, R. D. (1998) Biochem. J. 335, 205–209
18. Chamberlain, L. H., and Burgoyne, R. D. (2000) J. Neurochem. 74, 1781–1789
19. Cools, J., Bilhou-Nabera, C., Wlodarska, I., Cabrol, C., Talnatt, P., Bernard, P., Hagemoeijer, A., and Marynen, P. (1999) Blood 94, 1820–1824
20. Clerc, P., and Avner, P. (1998) Nat. Genet. 19, 249–253
21. Cools, J., Mentens, N., and Marynen, P. (2001) FEBS Lett. 492, 294–299
22. Ellgaard, L., Molinari, M., and Helenius, A. (1999) Science 286, 1882–1888
23. Zerangue, N., Schwappach, B., Jan, Y. N., and Jan, L. Y. (1999) Neuron 22, 537–548
24. Margeta-Mitrovic, M., Jan, Y. N., and Jan, L. Y. (2000) Neuron 27, 97–106
25. Dantzig, A. H., Heskina, J. A., Tabas, L. B., Bright, S., Shepard, R. L., Jenkins, I. L., Duckworth, D. C., Sportsman, J. R., Mackensen, D., Rosteck, P. R., Jr, et al. (1994) Science 264, 430–433
Functional Complementation between a Novel Mammalian Polygenic Transport Complex and an Evolutionarily Ancient Organic Solute Transporter, OSTα-OSTβ
David J. Seward, Albert S. Koh, James L. Boyer and Nazzareno Ballatori

J. Biol. Chem. 2003, 278:27473-27482.
doi: 10.1074/jbc.M301106200 originally published online April 28, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M301106200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 23 references, 8 of which can be accessed free at http://www.jbc.org/content/278/30/27473.full.html#ref-list-1