An evolutionarily ancient Oatp: insights into conserved functional domains of these proteins

SHI-YING CAI,1 WEI WANG,2 CAROL J. SOROKA,1 NAZZARENO BALLATORI,2,3 AND JAMES L. BOYER1,3

1Liver Center, Yale University School of Medicine, New Haven, Connecticut 06520; 2Department of Environmental Medicine, University of Rochester School of Medicine, Rochester, New York 14642; and 3Mt. Desert Island Biological Laboratory, Salsbury Cove, Maine 04672

Received 31 October 2001; accepted in final form 10 December 2001

Cai, Shi-Ying, Wei Wang, Carol J. Soroka, Nazzareno Ballatori, and James L. Boyer. An evolutionarily ancient Oatp: insights into conserved functional domains of these proteins. Am J Physiol Gastrointest Liver Physiol 282: G702–G710, 2002. First published January 9, 2002; 10.1152/ajpgi.00458.2001.—Cellular uptake of organic solutes is mediated in large part by a gene family of membrane transporters called OATPs (SLC21A). To study the structural determinants and evolutionary development of the SLC21A family, we have cloned and functionally characterized a novel skate Oatp from the liver of the small skate, Raja erinacea. A full-length cDNA (2.3 kb) was obtained that encodes a protein of 689 amino acids. The characteristics of this novel skate Oatp, including tissue expression, subcellular localization, substrate selectivity, Na+ dependence, and inhibitor selectivity, were generally similar to liver-specific human OATP-C and rat Oatp4. However, sequence comparisons with other OATPs indicate that this skate Oatp shares only ~40–50% amino acid identity with the liver-specific OATPs/Oatps and with human OATP-F. Further computer analysis revealed that the highest amino acid identities reside in the first external (78%) and internal loops (75%) and transmembrane domains 2 (76%), 3 (62%), 4 (70%), and 11 (64%). We propose that the conserved regions of the SLC21A transporter family may be critical structural determinants of substrate specificity and function.

A MAJOR FUNCTION OF THE LIVER is to protect the organism from the accumulation of toxic substances by removing them from the circulation and excreting them into bile. The hepatic uptake of these organic compounds is facilitated in large part by a family of proteins known as organic anion transporting polypeptides (OATPs) classified by the Human Gene Nomenclature Committee within the gene superfamily of solute carriers (SLC) as “SLC21A.” Other proteins that may contribute to hepatocellular uptake of organic anions include certain members of the OAT family of proteins and the recently identified skate liver Ost transporter (40), although the quantitative significance of these proteins to hepatic uptake of organic anions has not yet been examined. The Ost transporter is structurally distinct from the OATP and the OAT proteins, but it exhibits an overlapping substrate specificity with some OATP proteins (40). The OATP transport proteins function as organic solute exchangers with broad substrate specificity that includes organic anions, cations, and neutral compounds (5, 28, 36, 38). To date, 14 different Oatps have been cloned from human and rat tissues (1, 2, 9, 17, 19–22, 24–26, 30, 32, 39). Several OATP/Oatps appear to be expressed solely or predominately in liver, including human OATP-C (SLC21A6) (2, 17, 21) and OATP8 (SLC21A8) (21) and rat Oatp1 (Slc21a1) (19) and Oatp4 (Slc21a10) (9), a liver-specific isoform of Lst1 (9, 20). Four human (2, 10, 16, 17, 21, 24, 25) and several rat Oatps (1, 15, 19, 30) have been characterized at the functional level to mediate the cellular uptake of a variety of organic solutes, many of which are drugs and xenobiotics. Sufficient differences in amino acid composition exist among these various Oatps, suggesting that they are not clear orthologues but probably represent different members of the same families or subfamilies of an OATP superfamily of transport proteins (25). Substrate specificities of the OATP family are broad and generally overlapping. Most OATP/Oatps transport sulfobromophthalein (BSP) and [3H]taurocholate, except Oatp2 and OATP-B, respectively (25). Only OATP-A transports the cation tetraethylammonium (38), and only OATP8 and rat Oatp2 transport the neutral drug digoxin (25, 30, 32).

The structural determinants that account for these different substrate profiles are currently unknown. Previous functional studies in lower marine vertebrates indicate that similar transport systems must have evolved early in vertebrate evolution (7). Studies in the free-swimming dog fish shark (Squalus acanthias) and the small skate (Raja erinacea) (7), studies in isolated perfused liver and cell preparations from skate liver (12, 31, 35), and studies in skate liver membrane vesicle preparations (18) all demonstrate carrier-mediated, sodium-independent saturable.
transport systems for the hepatic uptake of a variety of organic anions including BSP (11, 18, 31), bile salts (11, 12, 31, 35), bilirubin (14), and lucifer yellow (3).

Molecular cloning and functional characterization of an ancient Oatp may help elucidate the structural determinants and evolutionary development of this important family of drug and xenobiotic transporters. Here we have now cloned, functionally expressed, and performed tissue localization studies for an ancient member of the OATP SLC21A family. This unique skate Oatp shares closest structural similarity and substrate specificity with human OATP-C, human OATP8, rat Oatp4, and with OATP-F from human brain. Single nucleotide polymorphisms that impair transport function in human OATP-C are conserved over this evolutionary distance of ~200 million years. We propose that the shared regions of these Oatps may be critical determinants of their functional properties.

MATERIALS AND METHODS

Materials

All chemicals were obtained from commercial sources and were the highest degree of purity available. Scytmol sulfate was purified from pooled samples of skate bile by Gert Fricker, University of Heidelberg, Germany. [3H]estrone-3-sulfate (53 Ci/mmol), [3H]estradiol-17β-glucuronide (44 Ci/mmol), [3H]taurocholate (3.47 Ci/mmol), [3H]digoxin (19 Ci/mmol), [3H]prostaglandin E2 (PGE2; 200 Ci/mmol), [3H]leukotriene C4 (LTC4; 165 Ci/mmol), p-[3H]aminohippurate (PAH; 4.1 Ci/mmol), and [14C]tetraethylammonium ([14C]TEA; 2.4 Ci/mol) were obtained from New England Nuclear.

Methods

Skate Oatp gene cloning. To isolate the full length skate OATP, three consensus regions were identified from alignments of the protein sequences of members of the OATP/
Oatp family. Based on these peptide sequences, -(N/D)-GSFEIGN—GETPPIVPLG—WVGAWW-, two forward and two reverse degenerate oligonucleotide primers were synthesized. Forward 1: 5'-AYGGNWSNTTYGARATHGGNAA-3', Forward 2: 5'-GGNGARACNCNATHTGNCCNYTNGG-3', Reverse 1: 5'-CCNARNGGNACDATNGGNGTYTCNCC-3', Reverse 2: 5'-NARCCACANGGNGCNCNACCCA-3'. With the use of reverse transcription and touchdown PCR, a 570-bp band was amplified with the predicted size from 5 μg of skate liver total RNA. DNA sequencing confirmed that this gene fragment encoded for an Oatp. A skate liver cDNA library was prepared as described previously (8) and screened using this 32P-labeled DNA fragment as probe. A strong positive hybridization signal was obtained for ~600 clones from 106 plaques. Thirty positive plaques were analyzed in a second round of screening. The confirmed positive clones were excised from phage to plasmid. Furthermore, Southern blot analysis (33) showed that most of the positive clones contained a 2.3-kb insert. Sequencing of three of these clones was performed by the W. M. Keck Sequencing Facility at Yale University School of Medicine. Species comparisons were performed by BLAST analysis, and phylogenetic development was assessed by DNASTar software (Madison, WI) with Clustal W algorithm. The transmembrane domain was predicted by the HMMTOP2.0 program (http://www.enzim.hu/hmmtop/).

In vitro translation. In vitro translation was performed by incorporating [35S]methionine into the synthesized proteins with a TNT kit (Promega), using 0.5 μg of pBK-CMV-sOatp plasmid or pBK-CMV vector. The reaction products were resolved in 12% SDS-PAGE and transferred to nitrocellulose paper. Protein bands were visualized by phosphoimage scanner or exposed to X-ray film.

cDNA preparation and oocyte injection. pBK-CMV-sOatp plasmid DNA (2 μg) was linearized with XhoI. After proteinase K treatment and phenol/chloroform extraction, 1 μg of DNA was used as a template to generate a capped cRNA with a mMESSAGE mMACHINE T3 kit (Ambion, Austin, TX). Xenopus laevis oocytes were prepared as described (27). Healthy oocytes were microinjected with 5 ng cRNA encoding for skate Oatp or water (50 nl). After incubation for 3 days, the uptake of labeled substrates was assessed for 1–2 h at 25°C as described previously (27).

**Tissue distribution analysis.** Total RNA was isolated from multiple skate tissues using cesium chloride gradient centrifugation. RNA (15 μg) was loaded on a 1% agarose gel that contained formaldehyde. To increase the transfer efficiency, the gel was treated with 0.05 N NaOH to partially hydrolyze the RNA and was neutralized with Tris buffer (pH 7.4) after electrophoresis. The RNA was then transferred to a GeneScreen (NEN Life Science Products) nylon membrane. The blot was probed with either a 32P-labeled skate Oatp gene fragment or skate β-actin gene fragment (8).

**Antibody production and Western blot analysis.** On the basis of the deduced amino acid sequence of the skate Oatp, three peptides were synthesized by the W. M. Keck facility at Yale University. They were: CKTSSGVKSTEFHNCSC, CQLRAKEKHAAPKGDNDVV, and CGPENGDITTKVNANHPESES. The peptides were mixed and conjugated to KHL (Pierce) and injected into rabbits (Chemicon International). A glutathione S-transferase (GST)-sOatp fusion protein construct was made and purified, and crosslinked to agarose beads to affinity purify the anti-skate Oatp antisera, used for the Western blot and immunofluorescence analyses (34). The GST-sOatp fusion protein was also used for titrating the antisera. Western blotting was performed by the following method. Fifty micrograms of skate liver membrane protein was resolved in a 12% gel by SDS-PAGE, and the separated proteins were electrotransferred to a nitrocellulose membrane (Bio-Rad) overnight. The membranes were blocked with 5% nonfat milk for 1 h at room temperature and then incubated with 1:2,000 diluted antisera either overnight at 4°C or 1 h at room temperature. After they were reacted with horseradish peroxidase-conjugated goat anti-rabbit antibody (1:2,500 dilution; Sigma), the membranes were visualized by using an enhanced chemiluminescence kit (Amer sham). For peptide competition experiments, the antisera was incubated with the three synthesized peptides (5 μg/ml for final membrane incubation volume) for 30 min before use.

**Immunofluorescence.** Indirect immunofluorescence was performed on frozen sections from skate liver. Briefly, livers were perfused rapidly with ice-cold elasmobranch Ringer and small cubes of tissue from various lobes were snap-frozen in liquid nitrogen and then stored in liquid nitrogen until cut. Tissue was placed on a pedestal of OCT embedding medium (Miles, Elkhart, IN), and 5- to 7-μm frozen sections were cut.

---

**Fig. 2.** A: Northern blot analysis of skate Oatp. Total RNA (15 μg) from skate tissue were loaded on each lane. Lane 1, brain; lane 2, heart; lane 3, intestine; lane 4, kidney; lane 5, liver; lane 6, pancreas; lane 7, rectal gland; lane 8, spleen; lane 9, stomach; lane 10, testes. B: in vitro translation of skate OATP. Lane 1, pBK-CMV plasmid control; lane 2, Skate Oatp clone in pBK-CMV vector. C: Western blot analysis using anti-skate Oatp peptide antibodies and skate liver plasma membranes. Lane 1, preimmune sera; lane 2, crude serum; lane 3, purified antibody; lane 4, peptide competition using the purified antibody.
and placed on poly-L-lysine-coated glass slides. Sections were treated with acetone at −20°C for 10 min and air dried, and nonspecific sites were blocked with 1% BSA in phosphate-buffered saline containing 0.05% Triton X-100. Polyclonal antibody to skate Oatp or the preimmune serum was diluted to 1:750 in blocking buffer and incubated on the sections for 2 h at room temperature. Counterstaining of the apical membrane was performed with a monoclonal antibody to Mdr1 (C219, Signet Laboratories, Dedham, MA). Competition experiments were carried out by incubating the diluted antibodies with 5 μg/ml of the three peptides used to inject the rabbit. After 1 h incubation at room temperature, the material was centrifuged for 2 min at 13,000 g and placed on the tissue section for 1 h at room temperature. Secondary antibodies were Alexa 594 anti-rabbit IgG and Alexa 488 anti-mouse IgG (Molecular Probes, Eugene, OR). All fluorescent imaging was performed on a Zeiss LSM 510, and digital images were recorded on an Iomega zip disc and processed with Adobe Photoshop.

Statistical analysis. Values for the uptake of substrates are given as the means ± SE of 8–12 individual oocyte measurements and compared with water-injected oocytes using the unpaired Student’s t-test (29). Statistical significance is given as P < 0.05.

RESULTS

On the basis of the aligned sequences of Oatp family members, a set of degenerate oligonucleotides was made, and a 570 bp DNA fragment of Oatp was identified from skate liver total RNA. Using this 32P-labeled DNA fragment as probe, a full-length 2.3 kb Oatp cDNA was cloned from the liver cDNA library of this marine elasmobranch, *R. erinacea*. This complete skate Oatp cDNA includes a 2,070-bp open reading frame, which encodes 689 amino acids designated as skate Oatp, a 76-bp 5′ untranslated region, and a 51-bp 3′
untranslated region. The 3′ untranslated sequence contains a canonical polyadenylation signal (AATAAA) and is followed by a 22-nt poly(A) tail. Computer modeling predicts 12 transmembrane domains, 4 N-glycosylation sites, and 5 protein kinase C phosphorylation sites (Fig. 1A, GenBank accession no. AF449798).

A phylogenic analysis was performed to display the evolutionary relationship with other members of the OATP/Oatp family (Fig. 1B). This demonstrates that this primitive Oatp is most closely related to human OATP-F and to the mammalian liver-specific OATP/Oatps, OATP2, OATP-8, and rat Oatp4.

Northern blot analysis of multiple skate tissues (Fig. 2A) indicates that skate Oatp is expressed predominantly in liver tissue, although weak signals were also obtained in skate brain and testes.

In vitro translation studies showed that this skate Oatp encodes for a 76-kDa polypeptide that is the predicted size (Fig. 2B). However, Western blotting revealed a single band at 100 kDa in total skate liver homogenate that was enriched in a plasma membrane fraction using both crude antisera and purified antibody. This band was not present with the prebleed sera, and competition experiments completely eliminated the staining of the 100-kDa band (Fig. 2C). As another control, the antiskate Oatp sera also did not react with total protein or crude membrane fractions from skate intestine and pancreas (data not shown). Immunofluorescence studies localized the skate Oatp to the basolateral membrane of the skate hepatocyte throughout the hepatic lobule (Fig. 3).

To determine the spectrum of substrates transported by this Oatp, skate Oatp cRNA was injected into X. laevis oocytes. After 3 days, the uptake of a panel of radiolabeled compounds was determined as described (27). Figure 4 illustrates the time-dependent uptake of [3H]estrone-3-sulfate compared with water-injected oocytes and the inhibitory effect of the major skate bile salt, symnol sulfate (200 μM), on this process. Uptake of [3H]estrone-3-sulfate was independent of external sodium gradients, as demonstrated by the lack of effect of either lithium or choline substitutions on the cRNA-mediated uptake of [3H]estrone-3-sulfate in skate OATP-injected oocytes. Oocytes were injected with either water or 5 ng cRNA. After 3 days in culture, 50 nM [3H]estrone-3-sulfate uptake was determined at 25°C in the presence or absence of 200 μM symnol sulfate over 2 h. Values are means ± SE (n = 3).

Fig. 4. Time course of [3H]estrone-3-sulfate uptake in skate OATP injected oocytes. Oocytes were injected with either water or 5 ng cRNA. After 3 days in culture, 50 nM [3H]estrone-3-sulfate uptake was determined at 25°C in the presence or absence of 200 μM symnol sulfate over 2 h. Values are means ± SE (n = 3).

Fig. 5. Substrate selectivity of skate Oatp-mediated uptake of radiolabeled compounds. Oocytes were injected with either water or 5 ng of cRNA. After 3 days in culture, the uptake of radiolabeled compounds [3H]estrone-3-sulfate (50 nM) (A), [3H]estradiol-17β-glucuronide (57 nM) (B), [3H]taurocholate (20 μM) (C), [3H]digoxin (0.5 μM) (D), [3H]PGE2 (50 nM) (E), [3H]LTC4 (10 nM) (F), [3H]PAH (1 μM) (G), and [14C]TEA (0.6 mM) (H) as measured at 25°C for 1 h. Values are means ± SE (n = 3).
dependent uptake in injected oocytes (data not shown). Figure 5 illustrates the capacity for skate Oatp to transport \([\text{H}]\)estrone-3-sulfate, \([\text{H}]\)PGE\(_2\), \([\text{H}]\)LTC\(_4\), PAH, and \([\text{H}]\)TEA in addition to \([\text{H}]\)estrone-3-sulfate, estradiol-17β-glucuronide, and \([\text{H}]\)taurocholate. These results indicate that skate Oatp is unable to transport prototype substrates for OATs (PAH), OCTs (TEA), or the neutral organic compound \([\text{H}]\)digoxin. These transport characteristics are typical of several mammalian OATPs/Oatps expressed in the liver. To demonstrate that the uptake is carrier mediated, saturation kinetics were examined in eRNA-injected oocytes for both \([\text{H}]\)estrone-3-sulfate and \([\text{H}]\)taurocholate (Fig. 6, A and B). The Michaelis-Menten constants (\(K_m\)) for the uptake of these Oatp substrates were 61 ± 11 \(\mu M\) and 85 ± 7 \(\mu M\) for \([\text{H}]\)estrone-3-sulfate and \([\text{H}]\)taurocholate, respectively. Substrate inhibition studies were carried out with \([\text{H}]\)estrone-3-sulfate (Fig. 7).

These findings indicate that a broad range of substrates are capable of cis-inhibiting skate Oatp-mediated uptake of \([\text{H}]\)estrone-3-sulfate, including BSP, bilirubin ditarurate, \([\text{H}]\)digoxin, \([\text{H}]\)taurocholate, scymnol sulfate, tauroliothicholate, and tauroliothicholate sulfate. In contrast, no inhibition was observed with ouabain.

**DISCUSSION**

In this study we report the cloning, functional characterization, and tissue localization of a novel evolutionarily primitive vertebrate member of the SLC21A gene family obtained from the liver of the marine skate, *R. erinacea*. The full-length cDNA is 2.3 kb and encodes for a protein of 689 amino acids. This gene appears to be highly expressed in skate liver, representing ~0.06% of total mRNA, but Northern blot analysis also revealed low levels of expression in brain and testes. Thus this Oatp is essentially liver specific. However, phylogenetic and sequence comparisons with other Oatp genes indicate that skate Oatp is most closely related to OATP-F (SLC21A14) from human brain (50.4% identity), human OATP-A (SLC21A3; 42.6% identity), human OATP-C (SLC21A6; 43.4% identity), human OATP8 (SLC21A8; 44.2% identity), and rat Oatp4, which is also called Lst1 (Slc21a10; 41.2% identity). Human OATP-C and OATP8 and rat Oatp4 are liver-specific Oatps. A BLAST search also demonstrated 48.5% homology to a rat brain-specific anion transporter (Bsat1), an ortholog of human OATP-F.

Antibodies raised to COOH-terminal peptides in skate Oatp clearly localized this Oatp to the basolateral domain of the skate hepatocytes. Several mammalian Oatps have also been localized to this domain by immunofluorescence, including human OATP-B (25),...
OATP-C (21), and OATP8 (22) as well as rat Oatp1 (4) and Oatp2 (32). Western blotting revealed that skate Oatp appeared as a 100-kDa protein in skate liver and was not detectable in intestine or pancreas. This result supports the tissue distribution studies in the Northern blot indicating that skate Oatp is a liver-specific Oatp. Because the predicted size of skate Oatp is 76 kDa, a finding also demonstrated by the in vitro translation studies, the larger size on Western blotting suggests that skate Oatp is glycosylated and possibly also phosphorylated when fully expressed in skate liver. A similar phenomenon was observed for human OATP8, which has a predicted size of 77 kDa but appeared as a 120-kDa band in a Western blot analysis (22).

Uptake studies in skate Oatp cRNA-injected Xenopus oocytes, using a panel of substrates, demonstrate that this skate Oatp is a multispecific transporter whose substrate profile overlaps most closely with human OATP-C (SLC21A6) and rat Oatp4 (Slc21a10) (36), the two liver-specific members of this family (25).

Uptake of [3H]estrone-3-sulfate and [3H]taurocholate was saturable, with apparent $K_m$ of 61 ± 11 and 85 ± 7 μM, respectively. This $K_m$ value for [3H]taurocholate compares favorably with that measured previously in cultured skate hepatocytes (45 ± 12 μM) (12). Uptake of [3H]estrone-3-sulfate in skate Oatp-expressing oocytes was unaffected when sodium was replaced with equimolar concentrations of lithium or choline, indicating that transport is independent of the sodium gradient, in agreement with findings for other OATPs. Because the sodium-dependent [3H]taurocholate transporting polypeptide Ntcp (Slc10a1) is unlikely to be expressed in lower vertebrates based on functional as well as bile acid photo affinity labeling experiments (6, 13), this skate Oatp may also be a major determinant of hepatic bile salt uptake in elasmobranchs.

Inhibition studies on the effects of substrates on [3H]estrone-3-sulfate uptake in cRNA-injected oocytes were also similar to the pattern of inhibition observed with human OATP-C and rat Oatp4. Interestingly, digoxin (500 μM) inhibited [3H]estrone-3-sulfate uptake by ~70%, despite the fact that [3H]digoxin itself was not transported by skate Oatp-expressing oocytes. However, it is well known that it is difficult to infer substrate specificity for Oatps based on inhibition studies (23).

Because the mammalian liver OATP/Oatps exhibit considerable amino acid identity and broad overlapping substrate specificities, it has been difficult to predict which regions are important determinants of function. Given that there is a large evolutionary distance between this skate Oatp and mammalian Oatps, yet skate Oatp exhibits similar substrate specificity to rat Oatp4 and human OATP-C, insights into critical structural determinants of this family of xenobiotic transporters might be gained by comparing conserved amino acid regions. As shown in Fig. 1A, we performed sequence alignments to determine consensus amino acids based on the predicted skate Oatp domains. As shown in Table 1, the highest identities for the liver-specific OATPs/Oatps are seen in transmembrane (TM) domains 1–6, and 11 (50–70% amino acid identity), whereas TM 7, 9, 10, and 12 (19–36% amino acid identity) have the least homology. In the five outer loop regions, loops 1, 5, 7, and part of 9 reveal 57–78% identities, whereas loop 11 is less conserved. On the cytoplasmic side, the NH2 terminus, loop 6, and the COOH terminus show considerable variation. In contrast, loops 2, 4, 8, and 10 demonstrate 54–75% identity. We therefore propose that the highly conserved outer loop regions (loop 1, part of 9) and transmembrane regions 2, 3, 4, and 11 may be the primary determinants of substrate binding and solute translocation, whereas the least homologous regions may play less critical roles in Oatp structure and/or function.

Further examination of sequence alignments reveals that many OATP/Oatp transcripts encode polypeptides with similar NH2-terminal but different COOH-terminal regions or lack part of the COOH-terminal region.

Polymorphisms in human OATP-C have recently been described that affect its transport activity (37). When these single nucleotide polymorphisms for the OATP-C variants are aligned with the skate Oatp and human OATP-C, their amino acid overlap most closely with human OATP-C (SLC21A6) whose substrate profiles have been described that affect its transport activity (37). When these single nucleotide polymorphisms for the OATP-C variants are aligned with the skate Oatp, OATP-F, and mammalian liver-specific Oats, it has been difficult to predict which regions are important determinants of function. Given that there is a large evolutionary distance between this skate Oatp and mammalian Oatps, yet skate Oatp exhibits similar substrate specificity to rat Oatp4 and human OATP-C, insights into critical structural determinants of this family of xenobiotic transporters might be gained by comparing conserved amino acid regions. As shown in Fig. 1A, we performed sequence alignments to determine consensus amino acids based on the predicted skate Oatp domains. As shown in Table 1, the highest identities for the liver-specific OATPs/Oatps are seen in transmembrane (TM) domains 1–6, and 11 (50–70% amino acid identity), whereas TM 7, 9, 10, and 12 (19–36% amino acid identity) have the least homology. In the five outer loop regions, loops 1, 5, 7, and part of 9 reveal 57–78% identities, whereas loop 11 is less conserved. On the cytoplasmic side, the NH2 terminus, loop 6, and the COOH terminus show considerable variation. In contrast, loops 2, 4, 8, and 10 demonstrate 54–75% identity. We therefore propose that the highly conserved outer loop regions (loop 1, part of 9) and transmembrane regions 2, 3, 4, and 11 may be the primary determinants of substrate binding and solute translocation, whereas the least homologous regions may play less critical roles in Oatp structure and/or function.

Further examination of sequence alignments reveals that many OATP/Oatp transcripts encode polypeptides with similar NH2-terminal but different COOH-terminal regions or lack part of the COOH-terminal region.

Polymorphisms in human OATP-C have recently been described that affect its transport activity (37). When these single nucleotide polymorphisms for the OATP-C variants are aligned with the skate Oatp, mutants F73L, I535T, and G488A, which significantly impaired transport activity in OATP-C, are all in regions that are fully conserved between the primitive skate Oatp and human OATP-C. In contrast, other OATP-C variants such as mutations at P155T, D462G, D655G, and E667G were not conserved between the two evolutionary distant species, and these variants
did not alter OATP-C function significantly (37). These findings illustrate the utility of comparative functional genomics in assessing important structural determinants in the OATP/Oatp family. Future studies should be able to test these predictions further using mutational and deletion analyses on human OATP-C.

This work was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grants DK-25636 and DK-48823, the National Institute of Diabetes and Digestive and Kidney Diseases Liver Center Molecular Biology and Imaging Cores P30-34989, and the National Institute of Environmental Health Sciences Marine and Freshwater Biomedical Science Center Grant ES-003828.

REFERENCES


