0026-895X/04/6503-479–487$20.00
MOLECULAR PHARMACOLOGY
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Mol Pharmacol 65:479–487, 2004

Vol. 65, No. 3
2936/1131675
Printed in U.S.A.

MINIREVIEW

The Molecular Pharmacology of Organic Anion Transporters: from DNA to FDA?

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Received September 3, 2003; accepted October 3, 2003

This article is available online at http://molpharm.aspetjournals.org

ABSTRACT

Renal organic anion secretion has been implicated in numerous clinically significant drug interactions and adverse reactions, indicating the importance of a detailed understanding of this pathway for the development of optimum therapeutics. With the cloning of multiple genes encoding organic anion transporters (OATs), the study of organic anion secretion has entered the molecular age. In this review, we focus on various aspects of the molecular biology and pharmacology of the OATs, including discussion of their structural biology, genomic organization in pairs, developmental regulation, toxicology, and pharmacogenetics. We propose functional, pathophysiological, and evolutionary hypotheses to help explain recent experimental and genomic data.

Many prescribed medications or their metabolites exist as organic anions at physiological pH. These compounds are ultimately handled by the organic anion transport systems of the kidney, liver, and choroid plexus, perhaps the best studied of which is the 'classic' multispecific organic anion secretory pathway of the renal proximal tubule. Substrates of this secretory pathway are strikingly diverse and include such clinically important pharmaceuticals as β-lactam antibiotics, probenecid, loop and thiazide diuretics, angiotensin converting enzyme (ACE) inhibitors, nonsteroidal anti-inflammatory drugs (NSAIDs), and methotrexate, among others, as well as various endogenous compounds, including cyclic nucleotides, prostaglandins, folate, neurotransmitter-metabolites, and hormone-conjugates (reviewed in Sweet et al., 2001; Russel et al., 2002).

The results of numerous studies have led to the following picture of this pathway (Fig. 1). Organic anion entry across the basolateral membrane of the proximal tubule occurs through an antiporter in exchange for dicarboxylates (in particular, α-ketoglutarate, an intermediate in the Krebs cycle). The latter are maintained at high intracellular concentrations by a Na^+-dicarboxylate cotransporter, which is, in turn, driven by the *trans*-membrane Na^+ gradient generated by the Na^+/K^+ ATPase. Accordingly, basolateral uptake is referred to as "tertiary" active transport (Sweet et al., 2001; Dantzler, 2002). After uptake, organic anions may be concentrated in cytoplasmic vesicles that move toward the apical surface along the microtubular network (Miller et al., 1993; Miller and Pritchard, 1994). Finally, apical (luminal) efflux (i.e., into urine) occurs through mechanisms that are less well understood but that probably include anion exchange, albeit in a Na^+-independent manner (Sweet et al., 2001; Dantzler, 2002). Although this pathway has been studied as a physiological process for more than a century, only within the last several years, with the cloning of multiple related organic anion transporter (OAT) genes, have its molecular underpinnings begun to be explored. Several excellent and detailed recent reviews have addressed substrates, regulation, physiology, and clinical correlates of the cloned OATs (Berkhin and Humphreys, 2001; Dresser et al., 2001; Sweet et al., 2001; Dantzler, 2002; Russel et al., 2002; You, 2002;

S.A.E. was supported by National Institute of Diabetes and Digestive and Kidney Diseases grant K08-DK064839 and S.K.N. by National Institute of Child Health and Human Development grant R01-HD40011.

ABBREVIATIONS: OAT, organic anion transporter; ACE, angiotensin-converting enzyme; NSAID, nonsteroidal anti-inflammatory drug; OCT, organic cation transporter; MFS, major facilitator superfamily; PKC, protein kinase C; TMD, *trans*-membrane domain; PAH, *para*-aminohippurate; OA, organic anion.

Eraly et al., 2003a; Terlouw et al., 2003). Here, we focus on the molecular biology and pharmacology of OATs, particularly in relation to genomic location, transcriptional regulation, protein structure, ontogeny, knockouts, toxicology, and pharmacogenetics.

The Organic Anion Transporter Family of Genes

The OATs (currently) comprise OAT1 (slc22a6), the prototypical OAT, originally cloned in our laboratory as NKT (GenBank accession number MMU52842) (Lopez-Nieto et al., 1996, 1997; Sekine et al., 1997; Sweet et al., 1997), OAT2 (slc22a7, originally cloned as NLT) (Simonson et al., 1994), OAT3 (slc22a8, originally cloned as ROCT) (Brady et al., 1999; Kusuhara et al., 1999), OAT4 (slc22a11) (Cha et al., 2000), and RST (slc22a12) (Mori et al., 1997). In addition, UST1 (Schomig et al., 1998), UST3 (GenBank accession number AB062418) (Sun et al., 2001), and OAT5 (Sun et al., 2001; Eraly and Nigam, 2002), although not proven to transport organic anions, are predicted to do so on the basis of sequence homology. The recently identified tubular urate transporter URAT1 (Enomoto et al., 2002a) seems to be the human ortholog of murine RST [although, as in the case of murine NKT (Lopez-Nieto et al., 1997) and rat OAT1 (Sekine et al., 1997), the prior identification of RST was not noted in the report on the cloning of URAT1]. URAT1 and RST are 74% identical and 81% similar at the amino acid level, map to syntenic regions of the mouse and human genomes, and have (to the extent characterized) identical tissue distributions (both are found in the proximal tubule of the kidney and are absent from multiple other tissues examined). Interestingly, the multispecificity of the organic anion transport system resides not in the availability of multiple transporters, but is, in fact, a property of individual transporters, which each transport multiple substrates of diverse structures. However, whereas the substrate specificities of individual OATs overlap substantially, there are some distinct differences (reviewed in Dresser et al., 2001; Russel et al., 2002). This suggests the possibility of "affinity maturation" (Sweet et al., 2001), whereby the constitutive expression of multispecific transporters permits an adequate response to short-term exposure to any particular substrate, whereas exposure in the longer term leads to the "induction" (through increased transcription, message, or protein stability, alternative splicing, or covalent modification that affects activity or sorting) of the more selective OAT(s). Now that all (or nearly all) family members have been identified, it should be possible to test this hypothesis.

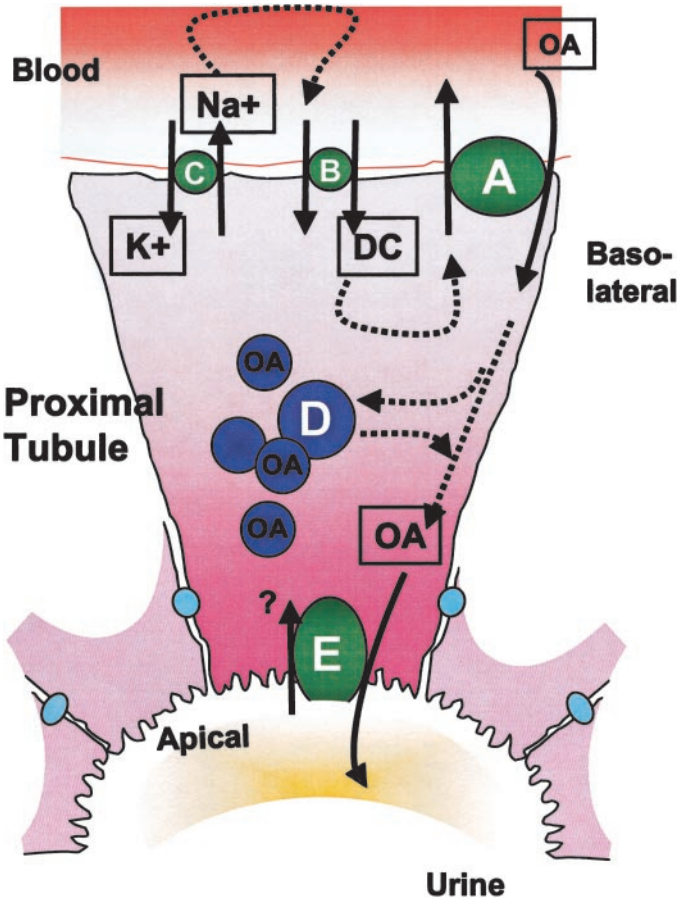

Fig. 1. "Tertiary" active transport of organic anions in the renal proximal tubule. Basolateral entry (A) of organic anions (OA) is coupled to the exit of dicarboxylates (DC) down their concentration gradient. This gradient is maintained through the action of the Na^+/dicarboxylate cotransporter (B), in turn driven by the Na^+ gradient generated by the Na^+/K^+ ATPase (C). Within the cytoplasm, organic anions may be concentrated in vesicles (D). Apical exit (E) of OA is less well understood, but is independent of the Na^+ gradient.

Among the aforementioned transporters, OAT1 and OAT3 have been localized to the basolateral surface of the renal proximal tubule (Kojima et al., 2002; Motohashi et al., 2002), and OAT4 and URAT1 to its apical surface (Babu et al., 2002a; Enomoto et al., 2002a). Furthermore, the functional properties of OAT1 and OAT3 on the one hand and OAT4 and URAT1 on the other are consistent with what is known about the mechanisms of basolateral and apical organic anion transport, respectively, in this structure (Fig. 1): OAT1 couples organic anion influx to dicarboxylate efflux (Sekine et al., 1997; Sweet et al., 1997); OAT3, originally described as a facilitated diffusion carrier (Kusuhara et al., 1999; Cha et al., 2001), has also now been demonstrated to function as an organic anion/dicarboxylate exchanger (Sweet et al., 2003); finally, OAT4 and URAT1 mediate transport in a Na^+-independent manner (Cha et al., 2000; Enomoto et al., 2002a). Therefore, it would seem that OAT1/OAT3 and OAT4/URAT1 underlie, at least in part, the basolateral and apical steps, respectively, of the classic pathway of renal organic anion secretion. However, it should be noted that other (phylogenetically unrelated) organic anion-transporting proteins, such as the OATPs/OAT-Ks, and MDRs (Dean et al., 2001; Russel et al., 2002; Hagenbuch and Meier, 2003), also seem to contribute to the apical secretion of some OAT substrates.

The OATs are listed in Table 1 along with their alternate designations and sequence accession numbers. As can be seen, the rapid proliferation of cloned transporters has resulted in an unsystematic nomenclature, with designations often reflecting historical rather than phylogenetic relationships, and a revised nomenclature may be in order once all the substrate specificities are determined. Interestingly, the nearest phylogenetic relations of the OATs (as revealed by sequence similarity) are transporters with the opposite charge selectivity—organic cation transporters, OCTs (Sweet and Pritchard, 1999; Burckhardt and Wolff, 2000) and OCTNs (Tamai et al., 2000). The other close relations of the OATs are the ORCTLs (Nishiwaki et al., 1998; Eraly et

al., 2003b) and the recently described fly-like putative transporters (Enomoto et al., 2002c; Eraly and Nigam, 2002), which are of unknown specificity. Conversely, despite their functional similarity, there is no significant sequence homology between OATs, OATPs, and MDRs, and there do not seem to be even any common structural motifs among these various protein families, at least at the level of primary structure. For example, the characteristic OAT motifs of STIVTEWD/NLVC and ELYPT (Schomig et al., 1998) are absent from the other anion transporters, which instead possess lineage-specific motifs of their own, such as the nucleotide-binding fold of the MDRs (Dean et al., 2001) and the zinc finger-like motif of the OATPs (Hagenbuch and Meier, 2003). Conceivably, structural similarity might exist between these protein families at the secondary, tertiary, or quaternary levels.

All the OATs are expressed in kidney and/or liver, the two major excretory organs of the body; individual family members are also expressed in diverse barrier epithelia (discussed below), including placenta and choroid plexus. OAT function in the placenta is interesting because of its possible contribution to the excretion of organic anions from the fetal to maternal circulation (Cha et al., 2000). Similarly, OAT function in choroid plexus [which seems to be caused by OAT1 and OAT3 (Pritchard et al., 1999; Nagata et al., 2002; Sweet et al., 2002)] is interesting because of its likely role in regulating the availability of organic anions of physiological or pharmacological importance in the cerebrospinal fluid. Not only would this be important for the pharmacology of such drugs as methotrexate and β-lactam antibiotics, which partition between blood and cerebrospinal fluid, but it might also have significant implications for neuropsychiatric disorders and their treatments.

Structure and Function

Based on sequence/hydropathy analyses, OATs, which are members of the major facilitator superfamily of transmembrane transporters (MFS) (Saier et al., 1999), are thought to comprise 12 membrane-spanning α-helices (Sweet and Pritchard, 1999; Burckhardt and Wolff, 2000; You, 2002) (Fig. 2). The segments connecting these helices are relatively short (around 10 amino acids or fewer) with the exception of the large interconnecting loops between helices 1 and 2 and helices 6 and 7. The first of these loops is extracellular and contains multiple consensus *N*-linked glycosylation sites. Glycosylation has been demonstrated to be necessary for the proper trafficking of OAT1 to the membrane (Kuze et al., 1999), although the role of these particular sites has not been specifically established. The second of the large loops is intracellular and has several canonical protein kinase C (PKC) phosphorylation sites (Sweet et al., 2001; You, 2002). However, although PKC is known to suppress OAT-mediated transport (reviewed in Berkhin and Humphreys, 2001; You, 2002; Terlouw et al., 2003), these canonical PKC sites do not seem to be involved in this process: PKC down-regulation of OAT1 function was not accompanied by OAT1 phosphorylation (You et al., 2000), and, more definitively, down-regulation was unaffected by mutation of the PKC sites (Wolff et al., 2003). It is interesting that the OATs (like other MFS proteins) manifest subtle, but significant, sequence homology (and thus presumed structural similarity) between their N- and C-terminal halves [*trans*-membrane domains (TMD) 1–6 and 7–12] (Saier et al., 1999; Burckhardt and Wolff, 2000). This feature has been interpreted as indicating the origin of MFS proteins from the tandem duplication of an ancestor with six membrane-spanning helices.

OATs exhibit broad specificity for small molecules and interact with a large number of compounds, often with disparate biochemical (and pharmacologic) properties. Numerous studies have investigated the structural requirements for OAT substrates and indicate that substrate recognition is based on physiochemical characteristics such as hydrophobicity, the presence of one or more hydrogen-bonding sites, and an ionized or partial negative charge distribution, rather than on specific atomic details (Ullrich, 1997). By contrast, beyond the general features noted above, little is currently known about the structure-function relationships of the transporters themselves. It is presumed that the various predicted *trans*-membrane helices of the OATs are mutually positioned to form a channel for the permeation of substrates. The amino acids lining such a channel (deriving from the inwardly oriented faces of the various helices) must provide the appropriate physical environment for the substrates, which, as noted above, usually possess anionic, polar, and organic components. However, the exact location and detailed characteristics of the OAT active site are unknown because the tertiary structure of an OAT protein has not been solved; even the proposed secondary structure is speculative at this point. In this regard, the recent elucidation of the crystal structures of two different MFS proteins [the *Esche-*

TABLE 1
Nomenclature and cDNA accession numbers (for all available mouse, rat, and human orthologs) of organic anion transporter genes

Usual Name	Alias/Ortholog	First Cloned as	RefSeq cDNA Accession No.
OAT1	Slc22a6, NKT (mouse)	NKT (Lopez-Nieto et al., 1997)	Mouse: NM_008766 Rat: NM_017224 Human: NM_004790
OAT2	Slc22a7, NLT (rat)	NLT (Simonson et al., 1994)	Mouse: NM_144856 Rat: NM_053537 Human: NM_153320
OAT3	Slc22a8, ROCT (mouse)	ROCT (Brady et al., 1999)	Mouse: NM_031194 Rat: NM_031332 Human: NM_004254
OAT4	Slc22a11	OAT4 (Cha et al., 2000)	Human: NM_018484
OAT5		OAT5 (Sun et al., 2001)	Published, but not reported to the database
URAT1	Slc22a12, RST (mouse)	RST (Mori et al., 1997)	Mouse: NM_009203 Human: NM_144585
UST1		UST1 (Schomig et al., 1998)	Rat: NM_138908
UST3	Slc22a9	UST3 (Accession no. AB062418)	Human: NM_080866

richia coli lactose permease and glycerol-3-phosphate transporter, each of which reveals the expected 12 membrane-spanning helices organized into two symmetric domains; (Abramson et al., 2003; Huang et al., 2003)] is likely to provide significant insight into the probable nature of an OAT structure.

Although a solved structure for an OAT does not exist, the availability of molecular clones has made mutagenesis studies possible, from which functional, and thus, indirectly, structural, inferences can be drawn. Such studies have been initiated (Feng et al., 2001, 2002; Wolff et al., 2001), so far targeting amino acids based upon their degree of evolutionary conservation, on the principle that highly conserved residues are likely to prove critical to protein function. These studies have demonstrated the contribution to OAT activity of conserved aromatic as well as basic residues, which are hypothesized to interact with the organic and anionic moieties, respectively, of the substrates. For example, of nine aromatic residues conserved among the OATs, four located in TMD 7 (two tyrosines, a tryptophan, and a phenylalanine) and one located in TMD 8 (a phenylalanine) were found to be required for optimum transport of the prototypic organic anion substrate *para*-aminohippurate (PAH), as assessed by expression in *Xenopus laevis* oocytes (Feng et al., 2002) (Fig. 2). Similarly, OATs have three conserved basic residues at positions that are occupied by acidic or neutral residues in their cation-transporting homologs, the OCTs: These are a histidine in TMD 1, a lysine in TMD 8, and an arginine in TMD 11 (Fig. 2). Mutation of any of these residues resulted in decreased PAH transport (Feng et al., 2001; Wolff et al., 2001). Remarkably, substitution of the conserved lysine and arginine by one neutral and one acidic residue (the double mutant R454DK370A) resulted in preferential transport of cationic instead of anionic species; in this case, modification of the electrostatic environment was sufficient to effect a switch in charge selectivity (Feng et al., 2001). It is worth emphasizing here that nominally cationic substrates might be uncharged or even anionic in vivo and might therefore serve as substrates for the OATs, OCTs, and OCTNs as multispecific organic ion transporters with overlapping specificities, rather than specifically as anionic or cationic transporters.

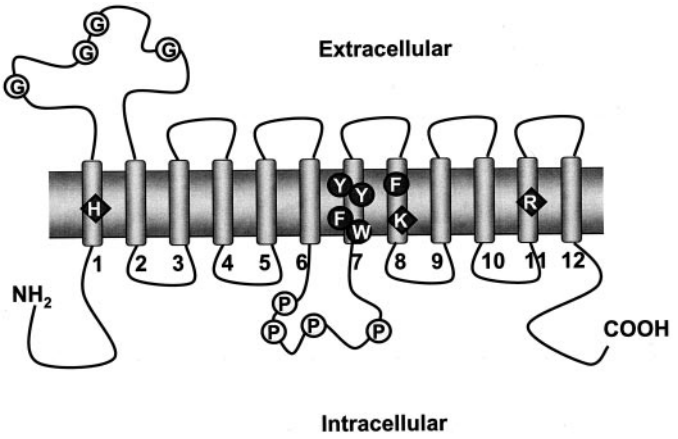

Fig. 2. Schematic illustration of the predicted transmembrane topology of organic anion transporters and other structural features of interest. Hydropathy analyses indicate that the OATs comprise twelve TMDs (numbered in the figure). The large loops between TMD 1 and 2 (extracellular) and TMD 6 and 7 (intracellular) contain several consensus glycosylation (G) and PKC phosphorylation (P) sites, respectively. The locations of the conserved amino acid residues implicated in substrate recognition and transport are indicated (not to scale); aromatic residues are depicted as circles and basic residues as diamonds.

Pairing and Tissue Distribution

The genes that encode OATs (as well as their nearest phylogenetic relations, the OCTs, OCTNs, and ORCTLs) manifest a remarkable trait; they tend to occur in pairs (i.e., as immediate chromosomal neighbors) (Eraly et al., 2003b) (Fig. 3A). Specifically, we found that six of the eight known OATs occur as three pairs in the human genome (located on adjacent segments of chromosome 11q12.3 to 11q13.2); OAT1 and OAT3, OAT4 and URAT1, and UST3 and OAT5. [Similarly, OCT1 and OCT2 are paired (on chromosome 6q26), as are OCTN1 and OCTN2 (chromosome 5q23) and ORCTL3 and ORCTL4 (chromosome 3p22).] These physical pairs are also phylogenetic pairs: pair members fall on neighboring branches of the dendrogram of the OAT family, suggesting that they are one another's nearest relations. We have inspected the human genome to determine how prevalent pairing is among other transporters. We find that less than 10% of the approximately 200 other human genes formally designated as solute carriers (having the prefix "slc") are paired or clustered (J. C. Monte, S. A. Eraly, S. K. Nigam, unpublished observations). Furthermore, although the pairing of OATs and OCTs is preserved in mouse and (to the extent verifiable) in *Fugu* (puffer-fish), it is absent from *Drosophila melanogaster* and *Caenorhabditis elegans* (S. A. Eraly, S. K. Nigam, unpublished observations), suggesting that it might be unique to the vertebrate lineage.

What evolutionary mechanisms could have resulted in the recurrent pairing of OATs (and OCTs and OCTNs)? Successive duplication of an ancestral gene pair seems unlikely, because such a mechanism (unless accompanied by recurrent gene conversion as well) would have produced pair-members that were distant rather than close relations. An alternative explanation is the presence of shared regulatory elements mediating the coordinated expression (coregulation) of pair members (Fig. 3B). This would lead to selective pressure to hold genes together in a pair, after their origin from, say, a tandem duplication event. In support of this interpretation, we have found that pair members do indeed have similar tissue distributions ((Eraly et al., 2003b) (Fig. 3A). OAT1 and OAT3 are found in the kidney and brain (expression in the latter tissue is presumed to be largely in choroid plexus); OAT4 and URAT1 (RST) are also both expressed in the kidney, but are absent from brain, with OAT4 present in placenta as well; and OAT5 and UST3 are liver-specific. Moreover, within the kidney, the tubular and membrane localization of OATs is also concordant with the coexpression of pair members, with OAT1 and OAT3 immunolocalized to the basolateral surface of the proximal tubule (Kojima et al., 2002; Motohashi et al., 2002) and OAT4 and URAT1 to its apical surface (Babu et al., 2002a; Enomoto et al., 2002a), as mentioned above.

If pairing exists to facilitate the coregulation of pair-members through utilization of shared regulatory elements, the further (ultimate rather than proximate) question arises then of the advantage conferred by such coregulation. The fact that individual pair-members have independent trans-

port capacity, and for overlapping rather than complementary classes of substrates, argues against functional interdependence (as exists for instance between OAT1 and the Na^+-dicarboxylate cotransporter) as a rationale for coregulation. The apparent preservation of OAT1-mediated transport in OAT3 knockout mice (discussed below) (Sweet et al., 2002) also argues against functional interdependence of pair members. Similarly, it is unlikely that coexpression is required to accomplish successive steps in vectorial transport of solutes (basolateral uptake and apical efflux), because pair-members (where investigated) have been localized to the same membrane (either apical or basolateral). Conversely, although coordinated expression of functionally similar transporters confers the advantage of redundancy, as well as the potential (as noted above) for affinity maturation, it is not clear why such coregulation should persistently occur in pairs rather than larger groupings.

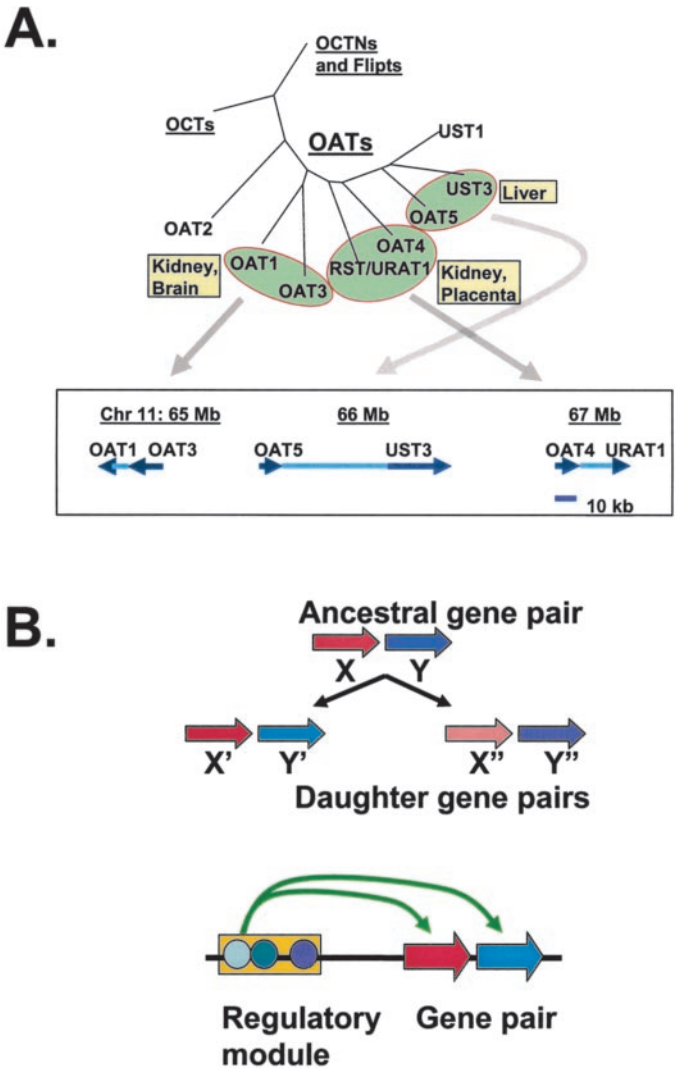

Fig. 3. Pairing, phylogeny, and tissue distribution of the OATs. A, sequences of the indicated OATs, as well as representative OCTs, OCTNs, and fly-like putative transporters (Flipts), were aligned, and the alignment output used to generate a dendrogram. The sequences used were from the human genome, except in the case of UST1 (rat), for which the human ortholog has not been identified. Paired genes are enclosed in ovals, and the adjacent text boxes indicate their tissue distributions (see text for details). The gene pairs are schematically represented in the rectangle below the dendrogram (to the scale in the lower right corner of the figure). Genes are drawn as arrows (with direction indicating orientation) and the intergenic regions between paired genes as line segments. The approximate chromosomal locations of the gene pairs are indicated (in megabases from the p telomere of chromosome 11). B, alternative models to explain pairing of OATs. Top, descent from an ancestral pair. In this case one would expect (absent recurrent gene conversion) that the nearest relation of a particular daughter gene (for example, X′) would not be the paired gene (i.e., Y′) but a gene from another pair (i.e., X″). The opposite however, is the case with the OATs (see Fig. 3A). Bottom, coregulation of pair members by a shared regulatory module. Such an arrangement would result in selective pressure to maintain the proximity of duplicated genes to the regulatory module and thus to each other.

More satisfying teleological explanations for coregulation in pairs might emerge from investigation of the ontogeny and the evolutionary history of the OATs. For example, we have found (unexpectedly) that OAT1–3 manifest transient embryonic expression in a variety of disparate tissues (discussed below), suggesting possible OAT activity in organogenesis (Pavlova et al., 2000). Similarly, characterization of OAT orthologs in evolutionarily distant taxa might suggest additional, possibly nontransport, functions performed by these genes (although, as noted above, pairing might be relatively specific to the vertebrate lineage). We have identified several OAT-like sequences in the genome of *C. elegans* (as well as *D. melanogaster*; S. A. Eraly, S. K. Nigam, unpublished observations); the ready availability of RNA interference-null mutations (Kamath et al., 2003) should facilitate the functional analysis of these putative worm OATs.

Ontogeny and Morphogenesis

It has long been known that renal organic anion (OA) transport undergoes postnatal changes in a number of organisms, including humans. Transport is relatively low at birth, then increases sharply over the first few weeks of neonatal life, before declining to adult levels (Barnett et al., 1949; Horster and Lewy, 1970; Kim et al., 1972; Hook and Hewitt, 1977). This neonatal increase seems to be specifically caused by the maturation of the transport system rather than by the growth and development of the kidney (Hook and Hewitt, 1977). Interestingly, OA transport is inducible by substrate during a limited period that roughly coincides with the phase of postnatal maturation (Hirsch and Hook, 1969, 1970; Stopp et al., 1978), suggesting that similar mechanisms might be involved in both these processes. These increases in transport are prevented when protein synthesis is inhibited by cycloheximide, indicating that they are probably the result of the biosynthesis of OATs or of other proteins involved in OA transport. Because, as described above, renal OA uptake at the basolateral membrane is energized by gradients generated by the Na^+/K^+ ATPase and Na^+-dicarboxylate cotransporter, increased uptake could be indirectly caused by increased expression of these other transporter proteins or indeed by enhanced expression of mitochondrial enzymes or other components of the respiratory cascade that generate the ATP and α-ketoglutarate required for the overall process.

With the identification of specific OAT genes, it has become possible to investigate the maturation of OA transport at the molecular level. In the original description of OAT1 (NKT), it was demonstrated that expression (in mouse) increased as the kidney developed (Lopez-Nieto et al., 1997). After this, another study of the ontogeny of OAT1 (this time in rat) determined that kidney expression increased through birth, peaked postpartum, and then declined to adult levels (Nakajima et al., 2000), approximately correlating with the

changes in OA transport previously observed in physiological studies. More recently, the postnatal expression of OAT1, OAT2, and OAT3 was examined in greater detail in neonatal rat kidneys (Buist et al., 2002). For each of these OATs, expression levels generally increased over the period examined (birth to 45 days postpartum), but there were some salient differences: OAT1 mRNA levels increased progressively in male rats, but seemed to peak at postpartum day 30 in female rats with a subsequent decline, so that by day 45, levels were significantly lower than in male rats. OAT2, manifested minimal expression in either gender for the first 30 days of postnatal development, then increased in female rats only, through day 45. Finally, OAT3 message increased progressively over the observed course of postnatal development in both male and female rats. Taken together, these results suggest that the postnatal maturation of renal OA transport is caused, at least in part, by increased expression of OAT1–3.

We have also investigated the expression of murine OAT1, OAT2, and OAT3 in the entire embryo (rather than just the kidney), and at earlier developmental stages, using in situ hybridization and northern blotting (Pavlova et al., 2000). Within the kidney, we found onset of OAT1–3 expression to be approximately contemporaneous at embryonic days 14–15 (e14–15), suggesting the existence of a common transcriptional regulatory pathway for these genes. Intriguingly, we also detected transient OAT expression in several extrarenal tissues: OAT1 in choroid plexus, dura mater, and spinal cord early in development, disappearing by e16; OAT2 in fetal liver, lung, stomach, intestine, skin, and bone, with expression still detectable in liver, stomach, and skin at 5 days postpartum; OAT3 in fetal liver and brain by e12, in both cases peaking at e14, with only very low expression levels remaining in adult. Expression during embryogenesis in these extrarenal sites suggests the possibility of a developmental role for the OATs. Conceivably, OATs might transport organic anions with morphogenetic activity (such as, for example, folate), requiring their expression during specific developmental periods, with expression down-regulated at the end of those periods. On the other hand, they may serve a transport-independent function.

Knockouts

In theory, OAT function (whether in excretion or in other cellular/organismal processes) might be most directly determined by study of OAT knockout animals. We have recently reported the knockout of OAT3, up till now the only targeted disruption of an OAT gene (Sweet et al., 2002). The resulting mutant mice manifest significantly impaired organic anion transport in kidney and choroid plexus, confirming the in vivo activity of OAT3 in these organs. For example, inhibitor-sensitive uptake of PAH in renal slices from knockout animals was less than half that in slices from wild-type animals, whereas transport of the fluorescent OAT substrate, fluorescein, was approximately three quarters less in knockout than in wild-type choroid plexus as quantified by the fluorescence intensity of confocal microscopic images (Sweet et al., 2002). The residual transporter activity in both cases seems to have been caused largely by OAT1. Besides defective organic anion secretion however, the knockout mice seemed grossly physiologically and morphologically normal, without any discernible developmental phenotype. Nevertheless, given the possibility of functional redundancy conferred by other OATs (particularly by the paired and coexpressed gene, OAT1), this finding does not represent compelling evidence against a role for OAT3 in development. It might be necessary to knock out multiple OATs (and possibly OCTs), or at least both members of an OAT pair, to effectively abrogate broader OAT function.

In this regard, it is worth noting that knocking out both proximal tubular basolateral transporters (OAT1 and OAT3), or both apical transporters (OAT4 and RST/URAT1) would be particularly likely to unmask phenotypes relating to substrate accretion or wasting (Eraly et al., 2003a). Conceivably, for instance, knocking out both apical transporters might result in substrates accumulating in the proximal tubule because of the "unopposed" action of the basolateral transporters. Where substrates are potential toxins (e.g., nephrotoxic drugs, see below), this might result in an animal model for substrate-induced nephrotoxicity. Conversely, knockout of basolateral OATs could lead to delayed clearance, possibly creating a model for any extrarenal toxicity of OAT substrates though protecting against nephrotoxicity. Thus, generation of double knockouts could yield crucial insights into the pharmacology as well as the fundamental biology of OATs.

Transcriptional Regulation

Physiological studies have long established the regulation of organic anion transport by hormones, kinases, growth factors, and by the substrates themselves (for reviews see Berkhin and Humphreys, 2001; Terlouw et al., 2003). More recently, the availability of molecular probes for individual OATs has revealed striking gender differences in the expression of OAT2 (expressed at much greater levels in the kidneys of female rats than in those of male rats) and OAT3 (expressed in liver in male rats only, although expressed in kidney in either gender) (Buist et al., 2002, 2003; Kobayashi et al., 2002a,b; Kudo et al., 2002), findings with potentially highly significant clinical implications for drug efficacy and toxicity in the genders. However, the molecular mechanisms underlying many of these regulatory responses remain unclear. In particular, investigations of the transcriptional regulation of OATs have yet to be described. Although such studies have traditionally been labor-intensive, in the present postgenomic era, computational approaches can be employed to predict likely regulatory elements that can then be prioritized for experimental testing (Frech et al., 1997; Fickett and Wasserman, 2000; Hardison, 2000; Pennacchio and Rubin, 2001). We have adapted such approaches to the analysis of the murine and human OAT1/OAT3 locus (Eraly et al., 2003b), and, promisingly, we have detected binding sites for several factors of demonstrated importance in kidney development, including PAX1, PBX, WT1, TCF, and HNF1 (Pontoglio, 2000; Barasch, 2001; Schnabel et al., 2001). Experimental testing of these predicted regulatory motifs might result in the discovery of the shared control elements hinted at by the pairing of OAT1 and OAT3 (see above).

As discussed above, inception of OAT1–3 expression occurs at e14–15 in mice. This period roughly coincides with the differentiation of the proximal tubule, raising the possibility that OAT gene expression might be under the control of

"master" factors that are key regulators of proximal tubulogenesis. In other words, factors implicated in OAT transcriptional regulation might also be critical developmental regulators. Among the factors listed above, HNF1 is a plausible candidate for such a "master" regulator. HNF1 induces the transcription of other renal transporters—including the Na-phosphate cotransporter (NaP_i; Soumounou et al., 2001) and the type II Na-glucose cotransporter (SGLT2; Pontoglio et al., 2000)—and HNF1 knockout mice are a model of Fanconi syndrome (proximal tubular wasting of glucose, amino acids, and phosphate; Pontoglio et al., 1996). Examination of OAT gene expression (and organic anion secretion) in these mice might prove instructive. Furthermore, comparison of embryonic kidney development in wild-type and HNF1 knockout mice might help dissect the regulatory steps involved in the differentiation and maturation of the proximal tubule.

Pharmacokinetics and Toxicology

As noted earlier, OATs transport a diverse array of clinically important pharmaceuticals. Consequently, OAT activity has been implicated in numerous drug interactions and adverse drug reactions. For example, multiple nephrotoxins have been demonstrated to be OAT substrates; these include ochratoxin A, the putative etiologic agent in Balkan endemic nephropathy (Groves et al., 1998; Tsuda et al., 1999; Jung et al., 2001; Babu et al., 2002a), cephaloridine (Takeda et al., 1999, 2002a; Jung et al., 2002; Khamdang et al., 2003), tetracycline (Babu et al., 2002b), mercuric conjugates (Koh et al., 2002; Zalups and Barfuss, 2002; Aslamkhan et al., 2003), the antivirals adefovir and cidofovir (Cihlar et al., 1999; Ho et al., 2000), nephrotoxic cysteine conjugates (Groves et al., 2003), and uremic toxins, such as indoxyl sulfate (Deguchi et al., 2002; Enomoto et al., 2002b; Motojima et al., 2002). Thus, nephrotoxicity could frequently be caused precisely by the avidity of the basolateral OATs, which leads to accumulation of potential toxins within the proximal tubular cell. In vitro experiments with the cloned OATs support this conclusion. Numerous investigators have found that transfection of proximal tubular as well as heterologous cell cultures with OAT cDNAs confers susceptibility to the toxicity of the substances noted above (as indicated by decreased cell viability or proliferation): ochratoxin A (Tsuda et al., 1999), cephaloridine (Jung et al., 2002; Khamdang et al., 2003), mercuric thiol conjugates (Aslamkhan et al., 2003), adefovir and cidofovir (Ho et al., 2000), and indoxyl sulfate (Enomoto et al., 2002b; Motojima et al., 2002). Such susceptibility was generally reversible with administration of an innocuous competitor substrate (for example, probenecid or PAH), further substantiating the specific role of the OATs in mediating toxicity.

As might be expected, OAT-substrate drugs can compete with one another for the transporter to mutually decrease renal secretion and thus delay the clearance and prolong the duration of action of each compound. Such interactions have the potential to bring about critical adverse outcomes—for example, the severe myelosuppression that can result when the OAT substrate, methotrexate, is coadministered with other substrates, such as NSAIDs, penicillins, or probenecid (reviewed in Takeda et al., 2002b). On the other hand, clinicians sometimes employ competition for OAT transport to their advantage, as exemplified by the common practice of coadministering probenecid to prolong the action of β-lactam antibiotics. Similarly, competitive interactions can also be beneficial when they lead to decreased proximal tubular uptake of nephrotoxins, which is the presumptive explanation for the nephroprotective actions of probenecid and NSAIDs against toxicity from adefovir and cidofovir (Lacy et al., 1998; Mulato et al., 2000), cephaloridine (Tune, 1997), ochratoxin A (Groves et al., 1998), and mercury (Aslamkhan et al., 2003). However, it is clear that the multiplicity of genes in the OAT family and their complex regulation warrant a careful examination of facile explanations of renal drug handling.

Finally, it is noteworthy that the α-ketoglutarate that is discharged to the extracellular space by OAT-mediated exchange for organic anions (Fig. 1) is (as a Krebs cycle intermediate) a key potential energy source for the proximal tubule, because this structure relies almost exclusively on aerobic metabolism in adult life (Ross et al., 1986; Epstein, 1997). Thus OAT uptake of toxins is accompanied by the equimolar loss of a vital nutrient, compromising metabolic reserves when they might be most needed (Eraly et al., 2003a). Such a process could explain some of the vulnerability of the proximal tubule to toxic injury. Indeed, it is conceivable that OAT uptake of even inoffensive substrates could be deleterious during periods of decreased energy availability—which would have important implications for the use of OAT-substrate drugs in the common clinical scenario of renal ischemia. However, whether or not OATs impact renal susceptibility to ischemia remains to be experimentally examined.

Medical Anthropology and Pharmacogenetics

Humans inhabit highly diverse biomes, ranging from tundra to tropical forests. The environmental exposure of various human populations to organic anions is therefore also likely to be highly diverse. Because OATs (as well as other transporters) are among the key genes operating at the organism's interface with new environments potentially filled with toxins, this suggests that there may have been selective pressure favoring different OAT variants (i.e., OAT genes containing different polymorphisms) in these various human populations. One might predict, accordingly, that there exists a relatively high degree of sequence variation (such as single-nucleotide polymorphisms) among the OATs (as well as among other sets of genes mediating communication between humans and their environments). Moreover, such variation might segregate along ethnic lines, because different ethnic groups have, at least for much of human history, resided in different geographical regions. With the continuing cataloging of human polymorphisms (Syvanen, 2001) such predictions could soon be tested and the exact nature of sequence variation among OAT genes clarified.

Given the importance of OAT activity to drug excretion, interactions, and toxicity, any degree of genetic variation in OAT genes might be a significant determinant of interindividual differences in drug-response. This suggests the possibility of determining the "OAT-genotype" of a patient to individualize his or her pharmacologic therapy. However, although the potential of such genetic profiling is intriguing, it is unclear, a priori, whether it will prove useful and cost-effective. After all, drug response is determined by multiple genes (and polymorphisms in those genes) coding for the transcription, regulation, or structure of proteins that are

involved in drug metabolism and excretion (pharmacokinetics), as well as those involved in the action of drugs at their target sites (pharmacodynamics). Furthermore, the use of genetic tests will also be limited if the polymorphisms and/or the outcomes in question are rare (Johnson and Evans, 2002). Nevertheless, the pharmacological importance of OATs would seem to merit association studies that explore correlations between OAT variants (Iida et al., 2001; Nishizato et al., 2003) and particular drug-response phenotypes. Variants/polymorphisms can be prioritized for such analyses based on whether or not they alter critical residues in OAT proteins. These, in turn, can be identified through mutational studies, such as those described above (Feng et al., 2001, 2002; Wolff et al., 2001), or by structural modeling. Alternatively, the importance of particular residues to OAT function can reliably be estimated from their degree of evolutionary conservation (Simon et al., 2002; Blouin et al., 2003). The applicability of this principle has recently been demonstrated by a study of polymorphisms in OCT2 [a member of the OAT-related family of organic cation transporters (Sweet and Pritchard, 1999; Burckhardt and Wolff, 2000)], wherein the only variants that resulted in decreased function were those that altered conserved residues (Shu et al., 2003).

Conclusion

With the identification and characterization of genes encoding organic anion transporters, the study of renal organic anion secretion has moved into the molecular age. The mechanisms, substrate-preferences, regulation (transcriptional and post-transcriptional), ontogeny, and genetic variability of the OATs can now be investigated in rigorous detail, with potentially profound implications for drug disposition and excretion in humans. In due course, study of the OATs will contribute toward the development of therapeutics that are more rational and therefore presumably more beneficial.

Acknowledgments

We thank the anonymous reviewers of the manuscript for their insightful comments and helpful suggestions.

References

Abramson J, Smirnova I, Kasho V, Verner G, Kaback HR, and Iwata S (2003) Structure and mechanism of the lactose permease of Escherichia coli. *Science (Wash DC)* **301:**610–615.

Aslamkhan AG, Han Y-H, Yang X-P, Zalups RK, and Pritchard JB (2003) Human renal organic anion transporter 1-dependent uptake and toxicity of mercuric-thiol conjugates in Madin-Darby canine kidney cells. *Mol Pharmacol* **63:**590–596.

Babu E, Takeda M, Narikawa S, Kobayashi Y, Enomoto A, Tojo A, Cha SH, Sekine T, Sakthisekaran D, and Endou H (2002a) Role of human organic anion transporter 4 in the transport of ochratoxin A. *Biochim Biophys Acta* **1590:**64–75.

Babu E, Takeda M, Narikawa S, Kobayashi Y, Yamamoto T, Cha SH, Sekine T, Sakthisekaran D, and Endou H (2002b) Human organic anion transporters mediate the transport of tetracycline. *Jpn J Pharmacol* **88:**69–76.

Barasch J (2001) Genes and proteins involved in mesenchymal to epithelial transition. *Curr Opin Nephrol Hypertens* **10:**429–436.

Barnett HL, McNamara H, and Schultz S (1949) Renal clearances of sodium penicillin G, procaine penicillin G and inulin in infants and children. *Pediatrics* **3:**418.

Berkhin EB and Humphreys MH (2001) Regulation of renal tubular secretion of organic compounds. *Kidney Int* **59:**17–30.

Blouin C, Boucher Y, and Roger AJ (2003) Inferring functional constraints and divergence in protein families using 3D mapping of phylogenetic information. *Nucleic Acids Res* **31:**790–797.

Brady KP, Dushkin H, Fornzler D, Koike T, Magner F, Her H, Gullans S, Segre GV, Green RM, and Beier DR (1999) A novel putative transporter maps to the osteosclerosis (oc) mutation and is not expressed in the oc mutant mouse. *Genomics* **56:**254–261.

Buist SC, Cherrington NJ, Choudhuri S, Hartley DP, and Klaassen CD (2002) Gender-specific and developmental influences on the expression of rat organic anion transporters. *J Pharmacol Exp Ther* **301:**145–151.

Buist SC, Cherrington NJ, and Klaassen CD (2003) Endocrine regulation of rat organic anion transporters. *Drug Metab Dispos* **31:**559–564.

Burckhardt G and Wolff NA (2000) Structure of renal organic anion and cation transporters. *Am J Physiol* **278:**F853–F866.

Cha SH, Sekine T, Fukushima J-I, Kanai Y, Kobayashi Y, Goya T, and Endou H (2001) Identification and characterization of human organic anion transporter 3 expressing predominantly in the kidney. *Mol Pharmacol* **59:**1277–1286.

Cha SH, Sekine T, Kusuhara H, Yu E, Kim JY, Kim DK, Sugiyama Y, Kanai Y, and Endou H (2000) Molecular cloning and characterization of multispecific organic anion transporter 4 expressed in the placenta. *J Biol Chem* **275:**4507–4512.

Cihlar T, Lin DC, Pritchard JB, Fuller MD, Mendel DB, and Sweet DH (1999) The antiviral nucleotide analogs cidofovir and adefovir are novel substrates for human and rat renal organic anion transporter 1. *Mol Pharmacol* **56:**570–580.

Dantzler WH (2002) Renal organic anion transport: a comparative and cellular perspective. *Biochim Biophys Acta* **1566:**169–181.

Dean M, Rzhetsky A, and Allikmets R (2001) The human ATP-binding cassette (ABC) transporter superfamily. *Genome Res* **11:**1156–1166.

Deguchi T, Ohtsuki S, Otagiri M, Takanaga H, Asaba H, Mori S, and Terasaki T (2002) Major role of organic anion transporter 3 in the transport of indoxyl sulfate in the kidney. *Kidney Int* **61:**1760–1768.

Dresser MJ, Leabman MK, and Giacomini KM (2001) Transporters involved in the elimination of drugs in the kidney: organic anion transporters and organic cation transporters. *J Pharm Sci* **90:**397–421.

Enomoto A, Kimura H, Chairoungdua A, Shigeta Y, Jutabha P, Cha SH, Hosoyamada M, Takeda M, Sekine T, Igarashi T, et al. (2002a) Molecular identification of a renal urate anion exchanger that regulates blood urate levels. *Nature (Lond)* **417:**447–452.

Enomoto A, Takeda M, Tojo A, Sekine T, Cha SH, Khamdang S, Takayama F, Aoyama I, Nakamura S, Endou H, et al. (2002b) Role of organic anion transporters in the tubular transport of indoxyl sulfate and the induction of its nephrotoxicity. *J Am Soc Nephrol* **13:**1711–1720.

Enomoto A, Wempe MF, Tsuchida H, Shin HJ, Cha SH, Anzai N, Goto A, Sakamoto A, Niwa T, Kanai Y, et al. (2002c) Molecular identification of a novel carnitine transporter specific to human testis: insights into the mechanism of carnitine recognition. *J Biol Chem* **277:**36262–36271.

Epstein FH (1997) Oxygen and renal metabolism. *Kidney Int* **51:**381–385.

Eraly SA, Blantz RC, Bhatnagar V, and Nigam SK (2003a) Novel aspects of renal organic anion transporters. *Curr Opin Nephrol Hypertens* **12:**551–558.

Eraly SA, Hamilton BA, and Nigam SK (2003b) Organic anion and cation transporters occur in pairs of similar and similarly expressed genes. *Biochem Biophys Res Commun* **300:**333–342.

Eraly SA and Nigam SK (2002) Novel human cDNAs homologous to *Drosophila* Orct and mammalian carnitine transporters. *Biochem Biophys Res Commun* **297:**1159–1166.

Feng B, Dresser MJ, Shu Y, Johns SJ, and Giacomini KM (2001) Arginine 454 and lysine 370 are essential for the anion specificity of the organic anion transporter, rOAT3. *Biochemistry* **40:**5511–5520.

Feng B, Shu Y, and Giacomini KM (2002) Role of aromatic transmembrane residues of the organic anion transporter, rOAT3, in substrate recognition. *Biochemistry* **41:**8941–8947.

Fickett JW and Wasserman WW (2000) Discovery and modeling of transcriptional regulatory regions. *Curr Opin Biotechnol* **11:**19–24.

Frech K, Quandt K, and Werner T (1997) Finding protein-binding sites in DNA sequences: the next generation. *Trends Biochem Sci* **22:**103–104.

Groves CE, Morales M, and Wright SH (1998) Peritubular transport of ochratoxin a in rabbit renal proximal tubules. *J Pharmacol Exp Ther* **284:**943–948.

Groves CE, Munoz L, Bahn A, Burckhardt G, and Wright SH (2003) Interaction of cysteine conjugates with human and rabbit organic anion transporter 1. *J Pharmacol Exp Ther* **304:**560–566.

Hagenbuch B and Meier PJ (2003) The superfamily of organic anion transporting polypeptides. *Biochim Biophys Acta* **1609:**1–18.

Hardison RC (2000) Conserved noncoding sequences are reliable guides to regulatory elements. *Trends Genet* **16:**369–372.

Hirsch GH and Hook JB (1969) Maturation of renal organic acid transport: substrate stimulation by penicillin. *Science (Wash DC)* **165:**909–910.

Hirsch GH and Hook JB (1970) Maturation of renal organic acid transport: substrate stimulation by penicillin and p-aminohippurate (PAH). *J Pharmacol Exp Ther* **171:**103–108.

Ho ES, Lin DC, Mendel DB, and Cihlar T (2000) Cytotoxicity of antiviral nucleotides adefovir and cidofovir is induced by the expression of human renal organic anion transporter 1. *J Am Soc Nephrol* **11:**383–393.

Hook JB and Hewitt WR (1977) Development of mechanisms for drug excretion. *Am J Med* **62:**497–506.

Horster M and Lewy JE (1970) Filtration fraction and extraction of PAH during neonatal period in the rat. *Am J Physiol* **219:**106–119.

Huang Y, Lemieux MJ, Song J, Auer M, and Wang D-N (2003) Structure and mechanism of the glycerol-3-phosphate transporter from *Escherichia coli*. *Science (Wash DC)* **301:**616–620.

Iida A, Saito S, Sekine A, Mishima C, Kondo K, Kitamura Y, Harigae S, Osawa S, and Nakamura Y (2001) Catalog of 258 single-nucleotide polymorphisms (SNPs) in genes encoding three organic anion transporters, three organic anion-transporting polypeptides and three NADH:ubiquinone oxidoreductase flavoproteins. *J Hum Genet* **46:**668–683.

Johnson JA and Evans WE (2002) Molecular diagnostics as a predictive tool: genetics of drug efficacy and toxicity. *Trends Mol Med* **8:**300–305.

Jung KY, Takeda M, Kim DK, Tojo A, Narikawa S, Yoo BS, Hosoyamada M, Cha SH, Sekine T, and Endou H (2001) Characterization of ochratoxin A transport by human organic anion transporters. *Life Sci* **69:**2123–2135.

Jung KY, Takeda M, Shimoda M, Narikawa S, Tojo A, Kim do K, Chairoungdua A, Choi BK, Kusuhara H, Sugiyama Y, et al. (2002) Involvement of rat organic anion transporter 3 (rOAT3) in cephaloridine-induced nephrotoxicity: in comparison with rOAT1. *Life Sci* **70:**1861–1874.

Kamath RS, Fraser AG, Dong Y, Poulin G, Durbin R, Gotta M, Kanapin A, Le Bot N, Moreno S, Sohrmann M, et al. (2003) Systematic functional analysis of the Caenorhabditis elegans genome using RNAi. *Nature (Lond)* **421:**231–237.
Khamdang S, Takeda M, Babu E, Noshiro R, Onozato ML, Tojo A, Enomoto A, Huang XL, Narikawa S, Anzai N, et al. (2003) Interaction of human and rat organic anion transporter 2 with various cephalosporin antibiotics. *Eur J Pharmacol* **465:**1–7.
Kim JK, Hirsch GH, and Hook JB (1972) In vitro analysis of organic ion transport in renal cortex of the newborn rat. *Pediatr Res* **6:**600–605.
Kobayashi Y, Hirokawa N, Ohshiro N, Sekine T, Sasaki T, Tokuyama S, Endou H, and Yamamoto T (2002a) Differential gene expression of organic anion transporters in male and female rats. *Biochem Biophys Res Commun* **290:**482–487.
Kobayashi Y, Ohshiro N, Shibusawa A, Sasaki T, Tokuyama S, Sekine T, Endou H, and Yamamoto T (2002b) Isolation, characterization and differential gene expression of multispecific organic anion transporter 2 in mice. *Mol Pharmacol* **62:**7–14.
Koh AS, Simmons-Willis TA, Pritchard JB, Grassl SM, and Ballatori N (2002) Identification of a mechanism by which the methylmercury antidotes *N*-acetylcysteine and dimercaptopropanesulfonate enhance urinary metal excretion: transport by the renal organic anion transporter-1. *Mol Pharmacol* **62:**921–926.
Kojima R, Sekine T, Kawachi M, Cha SH, Suzuki Y, and Endou H (2002) Immunolocalization of multispecific organic anion transporters, OAT1, OAT2 and OAT3, in rat kidney. *J Am Soc Nephrol* **13:**848–857.
Kudo N, Katakura M, Sato Y, and Kawashima Y (2002) Sex hormone-regulated renal transport of perfluorooctanoic acid. *Chem Biol Interact* **139:**301–316.
Kusuhara H, Sekine T, Utsunomiya-Tate N, Tsuda M, Kojima R, Cha SH, Sugiyama Y, Kanai Y, and Endou H (1999) Molecular cloning and characterization of a new multispecific organic anion transporter from rat brain. *J Biol Chem* **274:**13675–13680.
Kuze K, Graves P, Leahy A, Wilson P, Stuhlmann H, and You G (1999) Heterologous expression and functional characterization of a mouse renal organic anion transporter in mammalian cells. *J Biol Chem* **274:**1519–1524.
Lacy SA, Hitchcock MJM, Lee WA, Tellier P, and Cundy KC (1998) Effect of oral probenecid coadministration on the chronic toxicity and pharmacokinetics of intravenous cidofovir in cynomolgus monkeys. *Toxicol Sci* **44:**97–106.
Lopez-Nieto CE, You G, Barros EJG, Beier DR, and Nigam SK (1996) Molecular cloning and characterization of a novel transport protein with very high expression in the kidney (Abstract). *J Am Soc Nephrol* **7:**130119.
Lopez-Nieto CE, You G, Bush KT, Barros EJG, Beier DR, and Nigam SK (1997) Molecular cloning and characterization of NKT, a gene product related to the organic cation transporter family that is almost exclusively expressed in the kidney. *J Biol Chem* **272:**6471–6478.
Miller DS and Pritchard JB (1994) Nocodazole inhibition of organic anion secretion in teleost renal proximal tubules. *Am J Physiol* **267:**R695–R704.
Miller DS, Stewart DE, and Pritchard JB (1993) Intracellular compartmentation of organic anions within renal cells. *Am J Physiol* **264:**R882–R890.
Mori K, Ogawa Y, Ebihara K, Aoki T, Tamura N, Sugawara A, Kuwahara T, Ozaki S, Mukoyama M, and Tashiro K (1997) Kidney-specific expression of a novel mouse organic cation transporter-like protein. *FEBS Lett* **417:**371–374.
Motohashi H, Sakurai Y, Saito H, Masuda S, Urakami Y, Goto M, Fukatsu A, Ogawa O, and Inui K (2002) Gene expression levels and immunolocalization of organic ion transporters in the human kidney. *J Am Soc Nephrol* **13:**866–874.
Motojima M, Hosokawa A, Yamato H, Muraki T, and Yoshioka T (2002) Uraemic toxins induce proximal tubular injury via organic anion transporter 1-mediated uptake. *Br J Pharmacol* **135:**555–563.
Mulato AS, Ho ES, and Cihlar T (2000) Nonsteroidal anti-inflammatory drugs efficiently reduce the transport and cytotoxicity of adefovir mediated by the human renal organic anion transporter 1. *J Pharmacol Exp Ther* **295:**10–15.
Nagata Y, Kusuhara H, Endou H, and Sugiyama Y (2002) Expression and functional characterization of rat organic anion transporter 3 (rOat3) in the choroid plexus. *Mol Pharmacol* **61:**982–988.
Nakajima N, Sekine T, Cha SH, Tojo A, Hosoyamada M, Kanai Y, Yan K, Awa S, and Endou H (2000) Developmental changes in multispecific organic anion transporter 1 expression in the rat kidney. *Kidney Int* **57:**1608–1616.
Nishiwaki T, Daigo Y, Tamari M, Fujii Y, and Nakamura Y (1998) Molecular cloning, mapping and characterization of two novel human genes, ORCTL3 and ORCTL4, bearing homology to organic-cation transporters. *Cytogenet Cell Genet* **83:**251–255.
Nishizato Y, Ieiri I, Suzuki H, Kimura M, Kawabata K, Hirota T, Takane H, Irie S, Kusuhara H, and Urasaki Y (2003) Polymorphisms of OATP-C (SLC21A6) and OAT3 (SLC22A8) genes: consequences for pravastatin pharmacokinetics. *Clin Pharmacol Ther* **73:**554–565.
Pavlova A, Sakurai H, Leclercq B, Beier DR, Yu ASL, and Nigam SK (2000) Developmentally regulated expression of organic ion transporters NKT (OAT1), OCT1, NLT (OAT2) and Roct. *Am J Physiol* **278:**F635–F643.
Pennacchio LA and Rubin EM (2001) Genomic strategies to identify mammalian regulatory sequences. *Nat Rev Genet* **2:**100–109.
Pontoglio M (2000) Hepatocyte Nuclear factor 1, a transcription factor at the crossroads of glucose homeostasis. *J Am Soc Nephrol* **11:**S140–S143.
Pontoglio M, Barra J, Hadchouel M, Doyen A, Kress C, Bach JP, Babinet C, and Yaniv M (1996) Hepatocyte nuclear factor 1 inactivation results in hepatic dysfunction, phenylketonuria and renal Fanconi syndrome. *Cell* **84:**575–585.
Pontoglio M, Prie D, Cheret C, Doyen A, Leroy C, Froguel P, Velho G, Yaniv M, and Friedlander G (2000) HNF1α controls renal glucose reabsorption in mouse and man. *EMBO (Eur Mol Biol Organ) Reports* **1:**359–365.
Pritchard JB, Sweet DH, Miller DS, and Walden R (1999) Mechanism of organic anion transport across the apical membrane of choroid plexus. *J Biol Chem* **274:**33382–33387.
Ross BD, Espinal J, and Silva P (1986) Glucose metabolism in renal tubular function. *Kidney Int* **29:**54–67.
Russel FGM, Masereeuw R, and van Aubel RAMH (2002) Molecular aspects of renal anionic drug transport. *Annu Rev Physiol* **64:**563–594.
Saier MH Jr, Beatty JT, Goffeau A, Harley KT, Heijne WH, Huang SC, Jack DL, Jahn PS, Lew K, Liu J, et al. (1999) The major facilitator superfamily. *J Mol Microbiol Biotechnol* **1:**257–279.
Schnabel CA, Selleri L, Jacobs Y, Warnke R, and Cleary ML (2001) Expression of Pbx1b during mammalian organogenesis. *Mech Dev* **100:**131–135.
Schomig E, Spitzenberger F, Engelhardt M, Martel F, Ording N, and Grundemann D (1998) Molecular cloning and characterization of two novel transport proteins from rat kidney. *FEBS Lett* **425:**79–86.
Sekine T, Watanabe N, Hosoyamada M, Kanai Y, and Endou H (1997) Expression cloning and characterization of a novel multispecific organic anion transporter. *J Biol Chem* **272:**18526–18529.
Shu Y, Leabman MK, Feng B, Mangravite LM, Huang CC, Stryke D, Kawamoto M, Johns SJ, DeYoung J, Carlson E, et al. (2003) Evolutionary conservation predicts function of variants of the human organic cation transporter, OCT1. *Proc Natl Acad Sci USA* **100:**5902–5907.
Simon AL, Stone EA, and Sidow A (2002) Inference of functional regions in proteins by quantification of evolutionary constraints. *Proc Natl Acad Sci USA* **99:**2912–2917.
Simonson G, Vincent A, Roberg K, Huang Y, and Iwanij V (1994) Molecular cloning and characterization of a novel liver-specific transport protein. *J Cell Sci* **107:**1065–1072.
Soumounou Y, Gauthier C, and Tenenhouse HS (2001) Murine and human type I Na-phosphate cotransporter genes: structure and promoter activity. *Am J Physiol* **281:**F1082–F1091.
Stopp M, Hartwich R, and Braunlich H (1978) Enhancement of p-aminohippurate accumulation in renal cortical slices after repeated administrations of various organic anionic drugs to rats of different ages. *Experientia* **34:**1493–1494.
Sun W, Wu RR, van Poelje PD, and Erion MD (2001) Isolation of a family of organic anion transporters from human liver and kidney. *Biochem Biophys Res Commun* **283:**417–422.
Sweet DH, Bush KT, and Nigam SK (2001) The organic anion transporter family: from physiology to ontogeny and the clinic. *Am J Physiol* **281:**F197–F205.
Sweet DH, Chan LM, Walden R, Yang XP, Miller DS, and Pritchard JB (2003) Organic anion transporter 3 (Slc22a8) is a dicarboxylate exchanger indirectly coupled to the Na^+ gradient. *Am J Physiol* **284:**F763–F769.
Sweet DH, Miller DS, Pritchard JB, Fujiwara Y, Beier DR, and Nigam SK (2002) Impaired organic anion transport in kidney and choroid plexus of organic anion transporter 3 (Oat3 [Slc22a8]) knockout mice. *J Biol Chem* **277:**26934–26943.
Sweet DH and Pritchard JB (1999) The molecular biology of renal organic anion and organic cation transporters. *Cell Biochem Biophys* **31:**89–118.
Sweet DH, Wolff NA, and Pritchard JB (1997) Expression cloning and characterization of ROAT1. The basolateral organic anion transporter in rat kidney. *J Biol Chem* **272:**30088–30095.
Syvanen AC (2001) Accessing genetic variation: genotyping single nucleotide polymorphisms. *Nat Rev Genet* **2:**930–942.
Takeda M, Tojo A, Sekine T, Hosoyamada M, Kanai Y, and Endou H (1999) Role of organic anion transporter 1 (OAT1) in cephaloridine (CER)-induced nephrotoxicity. *Kidney Int* **56:**2128–2136.
Takeda M, Babu E, Narikawa S, and Endou H (2002a) Interaction of human organic anion transporters with various cephalosporin antibiotics. *Eur J Pharmacol* **438:**137–142.
Takeda M, Khamdang S, Narikawa S, Kimura H, Hosoyamada M, Cha SH, Sekine T, and Endou H (2002b) Characterization of methotrexate transport and its drug interactions with human organic anion transporters. *J Pharmacol Exp Ther* **302:**666–671.
Tamai I, Ohashi R, Nezu J-I, Sai Y, Kobayashi D, Oku A, Shimane M, and Tsuji A (2000) Molecular and functional characterization of organic cation/carnitine transporter family in mice. *J Biol Chem* **275:**40064–40072.
Terlouw SA, Masereeuw R, and Russel FGM (2003) Modulatory effects of hormones, drugs and toxic events on renal organic anion transport. *Biochem Pharmacol* **65:**1393–1405.
Tsuda M, Sekine T, Takeda M, Cha SH, Kanai Y, Kimura M, and Endou H (1999) Transport of ochratoxin A by renal multispecific organic anion transporter 1. *J Pharmacol Exp Ther* **289:**1301–1305.
Tune BM (1997) Nephrotoxicity of beta-lactam antibiotics: mechanisms and strategies for prevention. *Pediatr Nephrol* **11:**768–772.
Ullrich KJ (1997) Renal transporters for organic anions and organic cations. Structural requirements for substrates. *J Membr Biol* **158:**95–107.
Wolff NA, Grunwald B, Friedrich B, Lang F, Godehardt S, and Burckhardt G (2001) Cationic amino acids involved in dicarboxylate binding of the flounder renal organic anion transporter. *J Am Soc Nephrol* **12:**2012–2018.
Wolff NA, Thies K, Kuhnke N, Reid G, Friedrich B, Lang F, and Burckhardt G (2003) Protein kinase C activation downregulates human organic anion transporter 1-mediated transport through carrier internalization. *J Am Soc Nephrol* **14:**1959–1968.
You G, Kuze K, Kohanski RA, Amsler K, and Henderson S (2000) Regulation of mOAT-mediated organic anion transport by okadaic acid and protein kinase C in LLC-PK1 cells. *J Biol Chem* **275:**10278–10284.
You G (2002) Structure, function and regulation of renal organic anion transporters. *Med Res Rev* **22:**602–616.
Zalups RK and Barfuss DW (2002) Renal organic anion transport system: a mechanism for the basolateral uptake of mercury-thiol conjugates along the pars recta of the proximal tubule. *Toxicol Appl Pharmacol* **182:**234–243.

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