Basolateral localization of organic cation transporter 2 in intact renal proximal tubules

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Sweet, Douglas H., David S. Miller, and John B. Pritchard. Basolateral localization of organic cation transporter 2 in intact renal proximal tubules. Am J Physiol Renal Physiol 279: F826–F834, 2000.—The localization of organic cation transporter 2 (OCT2) within renal cells is the subject of considerable controversy, resulting in marked uncertainty as to its function. To resolve this issue, we made an OCT2/green fluorescent protein (GFP) fusion construct (rOCT2-GFP) and determined its localization within Xenopus laevis oocytes and renal cells using confocal microscopy. Oocytes expressing rOCT2-GFP exhibited plasma membrane fluorescence as well as greatly increased specific, potential-driven uptake of [14C]tetraethylammonium (TEA). Polarized monolayers of renal epithelial cell lines [LLC-PK1 and Madin-Darby canine kidney (MDCK)] transiently transfected with pEGFP-C3, which codes for a cytoplasmic GFP, showed a diffuse, evenly distributed cytoplasmic signal with no plasma membrane fluorescence. In contrast, cells transiently transfected with pEGFP-C3/rOCT2 (the vector coding for rOCT2-GFP) showed predominantly plasma membrane fluorescence, which was most prominent in the lateral membrane. MDCK cells stably expressing rOCT2-GFP (MDCK/rOCT2-GFP) maintained in long-term culture showed a greatly increased basal and lateral membrane fluorescence. When grown on porous supports, MDCK/rOCT2-GFP monolayers showed specific, potential-driven TEA uptake from the basal side. Finally, expression and distribution of rOCT2-GFP were investigated in isolated killifish (Fundulus heteroclitus) renal proximal tubules. On expression of rOCT2-GFP, transfected tubules showed markedly basal and lateral membrane fluorescence, with no detectable signal at the apical membrane. In contrast, tubules expressing a luminal sodium-dicarboxylate cotransporter (rbNaDC-1)-GFP construct showed apical membrane fluorescence, and tubules expressing cytoplasmic GFP had a diffuse cytoplasmic fluorescence. These results indicate that rOCT2 is basolateral in renal proximal tubule cells.

green fluorescent protein; kidney; killifish; transfection; Madin-Darby canine kidney cells; LLC-PK1 cells

THE RENAL ORGANIC CATION TRANSPORT system is responsible for the excretion of potentially toxic waste products of metabolism and of xenobiotics including drugs, pesticides, and herbicides. Transit of organic cations across the renal proximal tubule epithelium is an energetically uphill, multistep process that utilizes different driving forces for each step (for review, see Refs. 12 and 17). Basolateral entry of organic cations into the cell is driven by the electrical potential difference (inside negative) across the plasma membrane. Once inside the cell, a significant fraction of organic cations may be sequestered in acidic endosomal compartments driven by organic cation/H+ exchange (14). Luminal exit of organic cations is accomplished via one-for-one exchange for H+ or by the ATP-powered multidrug resistance transporter. Because of their very different driving forces, it was assumed that when the individual transporters were cloned, their basolateral or luminal localization would be readily discerned from their physiological properties. However, this has not proven to be the case.

Several members of the organic cation transporter (OCT) family have been cloned from mammalian kidney. When expressed in heterologous systems, these proteins exhibit specific transport of the model organic cation, tetraethylammonium (TEA). Of the OCTs, OCT2 is strongly expressed in the kidneys of rats, mice, pigs, and humans. For all species, there is a high level of homology at the amino acid level. Nevertheless, there is considerable controversy over the localization, and thus the function, of OCT2. When first cloned from rat and expressed in Xenopus laevis oocytes, rOCT2 mediated pH-independent TEA uptake, indicating that it is a basolateral transporter (9). However, Grunemann et al. (4) found that when the porcine OCT2 homolog cloned from LLC-PK1 cells (pOCT2) was expressed in cultured cells, TEA uptake decreased as the pH of the external buffer was lowered. In addition, the inhibitor specificity of pOCT2 matched the profile for apical organic cation transport in LLC-PK1 cells. From these findings, Grunemann et al. concluded that pOCT2 is located in the apical membrane and is an organic cation/H+ exchanger. On the basis of specificity, the aforementioned group has now reached the same conclusion for rOCT2 (6). They found that the affinity of rOCT2, expressed in cultured cells, for various substrates coincides with the affinities measured for the luminal organic cation/H+ exchanger in per-

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fused mammalian renal tubules and kidney brush-border membrane vesicle preparations. However, no direct evidence for organic cation/H+ exchange was presented (6).

In contrast, two recent studies examining rOCT2-mediated transport in cultured cells and X. laevis oocytes concluded that rOCT2 is a basolateral transporter but that rOCT2-mediated TEA uptake was modulated by pH (18, 20). Indeed, one of the studies showed that external protons inhibit rOCT2-mediated TEA efflux and that a large inward current was induced on application of TEA, providing functional evidence that rOCT2 does not operate as an electroneutral, organic cation/H+ exchanger (18). Finally, TEA uptake by the human OCT2 homolog (hOCT2) was found to be pH independent, and electrophysiological measurements detected a TEA-induced inward current, arguing that hOCT2 does not function as an organic cation/H+ exchanger (3).

Clearly, on the basis of the function of the cloned transporter, the subcellular localization of OCT2 is uncertain. Although this issue could be resolved through the use of OCT2-specific antibodies, none are available. In the present report we have used a different approach to the problem, making a fusion construct of rOCT2 and green fluorescent protein (GFP), which allows direct visual observation of the subcellular localization of the fusion protein (rOCT2-GFP). Plasma membrane insertion and specific transport function of rOCT2-GFP were confirmed in X. laevis oocytes and transfected renal cells. In cultured renal cell monolayers, rOCT2-GFP was strongly expressed in the basal and lateral plasma membrane. Additionally, when intact killifish renal proximal tubules were transfected, rOCT2-GFP expression was again clearly detected in the basolateral membrane but not the apical membrane.

**MATERIALS AND METHODS**

**Plasmid construction.** To make the rOCT2-GFP fusion construct, the fragment containing the full-length rOCT2 cDNA was removed from the isolated library clone (21), pSPORT1/rOCT2, with the restriction enzymes BamHI and KpnI. The fragment was gel isolated and ligated into pEGFP-C3 (Clontech, Palo Alto, CA), resulting in the plasmid pEGFP-C3/rbNaDC-1, which has the ER retention signal KDEL fused to the COOH terminus of GFP (rbNaDC-GFP). The reengineered clone, which now contained the rbNaDC-1 cDNA with a KpnI site 3’ to the poly(A) tail, was confirmed to the poly(A) tail, was confirmed as described previously (2). Briefly, adult female X. laevis (Xenopus One, Ann Arbor, MI) were anesthetized by hypothermia and decapitated. Follicle-free stage V and stage VI oocytes were isolated by treatment with collagenase A and maintained at 18°C in Barth’s buffer containing 0.05 mg/ml gentamycin sulfate, 2.5 mM sodium pyruvate, and 5% heat-inactivated horse serum. Oocytes were allowed to recover overnight before injection.

Capped cRNA for microinjection was synthesized from linearized plasmid DNA by using Ambion’s mMessage mMachine in vitro transcription kit (Ambion, Austin, TX), and oocytes were injected as described previously (16). Uptake assay was performed as described previously (18). Briefly, 3 days after injection, oocytes were divided into experimental groups of 10 each and incubated at 22°C for 60 min in oocyte Ringer 2 containing 200 μM [3H]TEA (4 Ci/ml) in the absence or presence of inhibitor. For uptake studies done under short-circuiting conditions (external K+ concentration raised to 102.5 mM), the osmotic pressure of the medium was maintained by a corresponding drop in Na+ concentration from 102.5 to 2.5 mM. After uptake, oocytes were rapidly rinsed three times with ice-cold oocyte Ringer 2 and placed into individual scintillation vials containing 0.5 ml 1 M NaOH, incubated at 65°C for 20 min, and neutralized with 0.5 ml 1 M HCl. Oocyte radioactivity was measured in disintegrations per minute in a Packard 1600TR liquid scintillation counter with external quench correction. TEA uptake was calculated in picromoles per oocyte, i.e., from disintegrations per minute per oocyte and medium specific activity.

**Tissue culture.** MDCK (established cell line derived from distal renal tubules of an adult female cocker spaniel) and LLC-PK1 (established cell line derived from renal proximal tubules of a 3- to 4-wk-old male pig) cell lines were obtained from the American Type Culture Collection (Manassas, VA) and were negative for mycoplasma on receipt from the supplier. Cell lines were retested before publication and found to be negative for mycoplasma. Cells were maintained in Eagle’s modified essential medium supplemented with 10% fetal bovine serum in a humidified incubator at 37°C with 5% CO2. Cultures were split 1:20 every 3–4 days.

Killifish (Fundulus heteroclitus) were collected near the Duke University Marine Laboratory (Beaufort, NC) and maintained in tanks with recirculating artificial seawater at the National Institute of Environmental Health Sciences. After decapsulation of the killifish, renal tubular masses were...
isolated, adherent hematopoietic tissue was removed, and individual proximal tubules were dissected.

**Transfection.** One day before transfection, $1.5 \times 10^5$ cells were plated into poly-D-lysine-treated, glass-bottom confocal chambers ($\sim 4.9 \text{ cm}^2$). Cells were transfected with 0.5–1 $\mu$g plasmid DNA by using Effectene reagent (Qiagen, Chatsworth, CA) and maintained at 37°C with 5% CO$_2$ for the remainder of the experiment. Confluent monolayers were examined by confocal fluorescence microscopy 24 and 48 h after transfection.

Groups of killifish tubules were transfected with 0.5–1 $\mu$g plasmid DNA by using Effectene reagent and maintained at 12°C in a confocal chamber containing 2 ml of 199 or Ham’s F-12 medium. Tubules were examined by confocal fluorescence microscopy 24 h after transfection.

To make the stably transfected MDCK/rOCT2-GFP cell line, 1 day before transfection $2 \times 10^5$ MDCK Type II cells were plated into individual wells of a 12-well culture plate (3.5 cm$^2$). The MDCK type II cells were provided by Dr. Daniel Balkovetz (University of Alabama at Birmingham) and were originally subclone in the laboratory of Dr. Kai Simons (EMBL, Heidelberg, Germany). Cells were transfected with 0.5 $\mu$g of linearized pEGFP-C3/rOCT2 plasmid DNA by using Effectene reagent and maintained as described above. Stably transfected cells were selected by addition of 1 mg/ml G418 (Mediatech, Herndon, VA) to the culture medium. After 1 mo, fluorescence-activated cell sorting was performed to ensure a pure culture of rOCT2-GFP-expressing cells. The new cell line was maintained in $200 \mu$g/ml G418. An MDCK cell line stably transfected with the vector pCDNA3.1 (MDCK/pCDNA3.1, previously established in our laboratory) and similarly maintained in 200 $\mu$g/ml G418, was used as a control (18).

For molecular mass determination of the rOCT2-GFP fusion construct, plasma membranes were isolated from MDCK/rOCT2-GFP cells by centrifugation. Cells were grown
to confluence in 175-cm$^2$ tissue culture flasks, removed by scraping, and suspended in ice-cold homogenizing buffer [(in mM) 300 mannitol, 12 Tris/HEPES, 0.1 phenylmethylsulfonyl fluoride at pH 7.4]. The cells were homogenized, and the homogenate was spun at 500 $g$ for 5 min. The supernatant was decanted into a fresh tube and centrifuged at 20,500 $g$ for 20 min. The supernatant was discarded, and the pellet was resuspended in fresh homogenizing buffer and centrifuged at 20,500 $g$. The supernatant was again discarded, and the pellet was resuspended in vesicle buffer [(in mM) 100 mannitol, 100 KCl, 1 MgSO$_4$, and 20 Tris/HEPES at pH 7.4], followed by a final spin at 20,500 $g$ for 20 min. The plasma membrane pellet was resuspended in vesicle buffer at a final protein concentration of 2–5 mg/ml and stored in liquid nitrogen. Approximately 300 µg of isolated membranes and 50 ng of purified recombinant EGFP protein (Clontech) were separated on a 12% SDS-polyacrylamide gel and transferred overnight onto Hybond P polyvinylidene difluoride membrane (Amersham Pharmacia Biotech, Piscataway, NJ). The blot was then examined by confocal microscopy. A fluorescent band was detected at ~27 kDa in the EGFP lane (EGFP-predicted molecular mass 29.9 kDa) and at ~85–95 kDa in the lane containing the isolated MDCK/rOCT2-GFP plasma membranes (rOCT2-GFP-predicted molecular mass 98.5 kDa). The native rOCT2 peptide has a predicted molecular mass of 66 kDa.

**Cell culture transport assay.** For uptake experiments, 1 × 10$^6$ cells were plated onto 24-mm (4.5 cm$^2$, 0.4-µm pores) clear polyester Transwells (Corning, Acton, MA). Cell monolayers were cultured for 3 days in Eagle’s modified essential medium supplemented with 10% fetal bovine serum and 200 µg/ml G418 in a humidified incubator at 37°C with 5% CO$_2$. The culture medium was changed daily. Before transport experiments, the culture medium was removed from both sides of the monolayers and the cells were washed twice with Hank’s balanced salt solution (HBSS; Sigma, St. Louis, MO), with a final application of HBSS to each side (2.6 ml basolateral and 1.5 ml apical). The HBSS (pH 7.4) was then...
removed from the basolateral or apical side and replaced with HBSS containing 100 μM \[^{14}C\]TEA (2 μCi/ml) in the absence or presence of 200 μM quinine. For the membrane potential experiments, the K\(^+\) concentration was raised from 2.5 to 102.5 mM and the osmolality was adjusted by lowering the Na\(^+\) concentration from 102.5 to 2.5 mM. The experiment was repeated with an n of 3 with and without the addition of 10 μM valinomycin in the 102.5 mM K\(^+\) treatment. After incubation at 37°C, the medium was removed from both sides of the monolayer and the cells were rapidly rinsed three times with ice-cold 0.1 M MgCl\(_2\). The cells were dissolved in 2 ml 1 M NaOH and neutralized with 2 ml 1 M HCl. Aliquots were removed for protein assay (1) with a Bio-Rad Protein Assay kit (Bio-Rad Laboratories, Hercules, CA), with bovine serum albumin used as a standard, and for liquid scintillation counting. Uptake was expressed as picomoles of substrate per milligram protein.

**Confocal fluorescence microscopy.** Cultured cells and tubules were imaged by using a Zeiss model 410 inverted laser scanning confocal microscope fitted with a ×40 water-immersion objective (NA 1.2). Fluorescent images were collected by illuminating samples with an Ar-Kr laser at 488 nm. A 510-nm dichroic filter was positioned in the light path, and a 512-nm-long pass emission filter was positioned in front of the detector. Confocal images (512 × 512 × 8 bits) were acquired as single 8- or 16-s scans, saved to a Jaz disk or CD-ROM, and analyzed on a Power Macintosh G3 computer by using National Institutes of Health Image 1.61 software.

**Chemicals.** \[^{14}C\]TEA (53 mCi/mmol) was obtained from American Radiolabeled Chemicals. Unlabeled TEA and quinine were obtained from Sigma. The fluorescent plasma membrane-specific marker N-(3-triethylammoniumpropyl)-4-[6-(4-diethylamino)phenyl]hexatrienyl)pyridinium dibromide (FM 4-64) was purchased from Molecular Probes (Eugene, OR). All other chemicals were obtained from commercial sources and were of the highest grade available.

**Statistics.** Uptake data are presented as means ± SE. Differences in mean values were considered to be significant when P < 0.05 as determined by unpaired Student's t-test.

**RESULTS**

**Functional characterization of rOCT2-GFP.** The ability of the rOCT2-GFP fusion construct to mediate uptake of TEA was demonstrated by X. laevis oocyte expression assay (Fig. 1A). Three days after injection with rOCT2-GFP cRNA, oocytes supported quinine-sensitive TEA uptake 15-fold greater than in water-injected control oocytes (P < 0.0001). Furthermore, as previously demonstrated for rOCT2 (18), rOCT2-GFP-mediated TEA uptake was largely potential driven, as indicated by the threefold reduction in uptake when the membrane potential was short-circuited by raising the external potassium ion concentration from 2.5 to 102.5 mM (+10 μM valinomycin) (Fig. 1B). When examined by confocal microscopy, rOCT2-GFP-expressing oocytes had marked plasma membrane fluorescence, indicating proper targeting of the fusion protein (Fig. 2). No such fluorescence was detected in water-injected oocytes.

**Expression of rOCT2-GFP in renal cell monolayers.** The subcellular localization of several GFP constructs was examined in confluent monolayers of two polarized renal epithelial cell lines (LLC-PK\(_1\), and MDCK). Cells were transiently transfected with pEGFP-C3 (expresses cytoplasmic GFP), pcDNA3/ER-GFP (expresses ER-targeted GFP), or pEGFP-C3/rOCT2-GFP (expresses rOCT2-GFP). Confluent monolayers were examined by confocal microscopy 24 and 48 h after transfection. It is important to note that only transfected cells within the monolayers are visible in the micrographs (16). Both renal cell types yielded identical results with all of the constructs tested. Cells expressing cytoplasmic GFP showed diffuse fluorescence that freely penetrated the nucleus and was not concentrated at the plasma membrane (Fig. 3, A and B). Those expressing ER-GFP showed a pattern similar to that which was previously demonstrated for rOCT2 (18), with rOCT2-GFP fluorescent in the nucleus and at the plasma membrane. The plasma membrane fluorescence was restricted to the basolateral membrane, as indicated by the lack of fluorescence in the apical membrane. The subcellular localization of rOCT2-GFP in renal cell monolayers suggests that rOCT2-GFP is an effective tool for studying the localization of OCT2 in renal epithelial cells.

Fig. 4. Cross-sectional image analysis of rOCT2-GFP-expressing LLC-PK\(_1\) and MDCK cells in culture. Transfection was carried out as described in MATERIALS AND METHODS, and confluent monolayers were examined by confocal microscopy. The microscope was focused on the bottom of the chamber, and 8-s scans were collected 0.5 μm apart (i.e., up through the cell). The individual images were then compiled to render a 3-dimensional reconstruction of the tissue. Top: fluorescence images (single plane) of pEGFP-C3/rOCT2-transfected LLC-PK\(_1\), (left) or MDCK cells (right). The diagonal lines designate the axes used for cross sections through the image stacks. Bottom: cross-sectional projections corresponding to the lines at the top. Note the strong labeling of the lateral plasma membranes and absence of signal in the nucleus. Bars: 20 μm.
showed a reticular pattern of fluorescence within the cytoplasm but no nuclear or plasma membrane-associated signal (Fig. 3, C and D). In contrast, pEGFP-C3/rOCT2-GFP-transfected cells had strong lateral membrane fluorescence, with no detectable signal in the nucleus (Fig. 3, E and F).

Image stacks through rOCT2-GFP-expressing cells were collected and reconstructed in cross section for evidence of apical and basal membrane targeting (Fig. 4). In transiently transfected cells, extensive labeling of the lateral membranes was clearly evident, as was the absence of signal in the nucleus; however, targeting to the basal membrane and exclusion from the apical membrane could not be conclusively discerned. A weak reticular pattern of cytoplasmic fluorescence that resembled the pattern seen in ER-GFP-transfected cells was observed as well (16).

To determine whether longer time in culture would provide a more conclusive basal or lateral localization, a stably transfected rOCT2-GFP MDCK cell line was established and observed for up to 7 days at confluent culture. In marked contrast to the transiently transfected cells, intense labeling of the basal membrane could be seen (Fig. 5). To determine whether the MDCK/rOCT2-GFP cell line was expressing the transporter in a polarized manner, [14C]TEA was applied either basally or apically to cells grown on Transwell filter inserts. After 60 min, mediated TEA uptake in the MDCK/rOCT2-GFP cells was significantly greater than in the MDCK/pcDNA3.1 control cells (Fig. 6A). Importantly, TEA uptake from the basal side more than doubled, whereas uptake from the apical side was unchanged. Thus expression of the GFP-linked transporter only increased transport from the basal pole of the cell. When the buffer K+ concentration was raised to membrane-depolarizing levels (102.5 mM), basal TEA uptake in MDCK/rOCT2-GFP cells was reduced significantly, indicating a large potential-driven component to rOCT2-GFP function (Fig. 6B).

Expression of rOCT2-GFP in killifish renal proximal tubules. Because of extended viability in vitro and well-established epithelial polarity (8, 11), the killifish tubule is an ideal model for the expression and localization of GFP constructs in an intact renal tubular epithelium. Indeed, we recently used this preparation to demonstrate basolateral localization of a cloned organic anion transporter, rROAT1 (16).

In initial experiments, we used a fluorescent plasma membrane marker, FM 4-64, to reveal the spatial relationships among the plasma membrane, the cell interior, and the luminal compartment. Figure 7A shows that after a 30-min incubation with 1 μM FM 4-64, each cell within the tubular epithelium was surrounded by a wall of fluorescence. The extensive infoldings of the basolateral membrane can be seen in some cells as alternating lines of fluorescence extending into the interior of the cell. The luminal (brush-border) membrane appears as a broad band lining the lumen of the tubule. The cell interior and the luminal space were unlabeled (Fig. 7A). Tubules transfected with cytoplasmic GFP exhibited a diffuse fluorescence that extended over the cytoplasm and nucleus (Fig. 7B). In contrast, rOCT2-GFP was clearly targeted to the basal and lateral plasma membranes and was excluded from the nucleus and apical region of epithelial cells (Fig. 7C). For comparison, the apical membrane localization marker, rbNaDC-GFP, localized to the apical region of killifish tubule cells and was not observed in the nucleus or basal region of the cells (Fig. 7, D and E). Cross sections taken through image stacks of FM 4-64-stained and rOCT2-GFP-expressing tubules are shown in Fig. 8. The labeling of the basal, lateral, and apical regions of the plasma membrane is readily apparent in the FM 4-64-treated tubule, but only the basal and lateral membranes of the pEGFP-C3/rOCT2-transfected tubule are fluorescent.
DISCUSSION

To support vectorial movement of solutes, specialized transporting epithelia exhibit polarity of structure and function. In the renal proximal tubule, the OCT system utilizes carriers with very different driving forces at the basolateral and luminal membranes, giving uptake into the cell and secretion into the lumen physiologically distinct properties. Because of this polarity of function, it was believed that, once cloned, the individual OCT proteins would be readily assigned to the basolateral or luminal membranes on the basis of their physiology. However, as described at the beginning of this study, this has not been the case for OCT2, and difficulties in obtaining specific antibodies to this transporter have left the issue of cellular localization unresolved. We recently developed an alternative approach to transporter localization using GFP fusion constructs, cultured renal cell monolayers, and isolated killifish proximal tubules. With this system, we have observed the proper targeting of cytoplasmic, ER, and mitochondrial GFP variants (present study and 16). Furthermore, we have previously used the killifish tubule system to conclusively demonstrate the basolateral subcellular localization of the organic anion transporter rROAT1 in the kidney (16). In contrast, the same rROAT1-GFP construct was localized at the apical membrane of the rat choroid plexus, as predicted by functional data in this epithelium (13). Thus these GFP constructs appear to accurately reflect the subcellular localization of these carrier proteins.

The functional characteristics of OCT2 are well studied, yet there is no consensus of opinion as to whether it is a basolateral or an apical transporter (3–5, 9, 18–20). Here, we have taken an alternative approach to this issue and assembled a rOCT2-GFP construct. When mRNA for rOCT2-GFP was injected into Xenopus oocytes, fluorescent product was inserted into the plasma membrane and shown to possess specific, potential-driven organic cation transport as previously demonstrated for rOCT2 (Figs. 1 and 2; 18). Furthermore, rOCT2-GFP transiently expressed in confluent renal cell monolayers (LLC-PK1 and MDCK) showed strong lateral membrane localization but no convincing basal membrane signal (Figs. 3 and 4). When localization was examined in stably transfected MDCK cells maintained at confluence for 1 wk, strong basal and lateral membrane localization was detected (Fig. 5). Increased basal membrane fluorescence correlated with increased specific, potential-driven, basal TEA uptake (Fig. 6), again as previously demonstrated for rOCT2 (18). Finally, transfection experiments with renal proximal tubules provided direct evidence of rOCT2 trafficking to the basal and lateral membranes of an intact, polarized renal epithelium, with no evidence for luminal membrane localization (Figs. 7 and 8).

In addition to surface membrane-based fluorescence, there appeared to be some internal fluorescence in the cells of rOCT2-GFP- and rbNaDC-GFP-expressing tubules. However, as evidenced by cytoplasmic GFP, if
this were truly a free, diffuse cytoplasmic fluorescence, then at some point it would have been visible within the nucleus and in the region above it for rOCT2-GFP or within and below the nucleus for rbNaDC-GFP. Clearly, this was not the case. These patterns are consistent with the labeling of basolateral membrane infoldings (7) that extend into the interior of the cell (for rOCT2-GFP) and with labeling of the fingerlike
projections of the apical brush-border membrane (for rbNaDC-GFP). These patterns were confirmed in tubules labeled with the plasma membrane-specific marker FM 4-64 (Figs. 7A and 8). The basolateral membrane infoldings are clearly visible in many cells as “stripes” of fluorescent signal reaching into the cytoplasm at the basal pole of the cell, and the brush border does not appear as a distinct, thin line but rather as a broad, fuzzy band that separates the apical pole of the cell from the lumen.

A basolateral localization for OCT2 (present study) is consistent with previous findings of potential-dependent uptake and H⁺-inhibited efflux of TEA in rOCT2-expressing oocytes and cultured cells (18, 19). Obviously, these findings do not support the suggestion of Grundemann et al. (6) that rOCT2 is apical. However, the argument presented was largely circumstantial (based on substrate specificity and interaction), and no direct evidence for organic cation/H⁺ exchange was given (6). Grundemann et al. have generated similar specificity data for pOCT2, but again, without evidence demonstrating organic cation/H⁺ exchange; these data are also inconclusive (4). Clearly, the significance of the differences in substrate affinity observed by these authors for the OCT family isoforms (OCT1, OCT2, and OCT3) is yet to be understood, but the differences do not appear to define transporter localization.

A large number of studies with renal brush-border membranes from a wide variety of species uniformly show organic cation/H⁺ exchange (12). Yet, with the exception of hOCTN1 (22), none of the OCTs cloned so far are organic cation/H⁺ exchangers. At present, we do not know whether OCTN1 localizes to the luminal membrane of proximal tubule cells. However, if it is not a luminal transporter, other possibilities must be pursued. Perhaps luminal organic cation/H⁺ exchange is mediated by a different (sub)family of proteins yet to be identified.

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