ACCELERATED PUBLICATION

Polarized expression of members of the solute carrier *SLC19A* gene family of water-soluble multivitamin transporters: implications for physiological function

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Humans lack biochemical pathways for the synthesis of the micronutrients thiamine and folate. Cellular requirements are met through membrane transport activity, which is mediated by proteins of the *SLC19A* gene family. By using live-cell confocal imaging methods to resolve the localization of all *SLC19A* family members, we show that the two human thiamine transporters are differentially targeted in polarized cells, establishing a vectorial transport system. Such polarization decreases functional redundancy between transporter isoforms and allows for independent regulation of thiamine import and export pathways in cells.

Key words: folate, polarity, thiamine, transport, vitamin.

INTRODUCTION

The water-soluble micronutrients folate and thiamine (vitamin B1) are critical co-factors in many cellular reactions. Foliates are required for the synthesis of nucleic acids and certain amino-acid precursors, and thiamine is essential for carbohydrate metabolism and energy production. The importance of both micronutrients for metabolic function is underscored by the spectrum of pathological disorders associated with abnormalities in folate [1,2] and thiamine homeostasis [3,4]. Since humans lack enzymic pathways for synthesis of both compounds *de novo*, cellular requirements are met by uptake from the extracellular milieu. Therefore regulation of the plasma concentrations of both micronutrients is critical to prevent metabolic insufficiencies, and this is primarily accomplished by intestinal and renal mechanisms that transport micronutrients transcellularly from intestine to bloodstream or from glomerular filtrate to bloodstream respectively. Understanding how such vectorial transport occurs requires knowledge of how micronutrient transporters are distributed within epithelia and how their activity is regulated.

Molecular identification of the relevant transporters has been aided by the recent cloning of the *SLC* (solute carrier) *19A* gene family. To date three members of this family have been identified, *SLC19A1* [5,6], *SLC19A2* [7–10] and *SLC19A3* [11], each encoding proteins with approx. 25 % overall amino-acid identity. Hydropathy analyses predict topologies of 12 transmembrane domains between cytoplasmic N- and C-termini [7,11,12]. Despite this structural similarity, the substrate specificities of these transporters are distinct: *SLC19A1* encodes a reduced folate carrier (RFC [13]), *SLC19A2* and *SLC19A3* encode thiamine transporters (THTR1 [7] and THTR2 [11,14]). Functionally, RFC constitutes the major pathway for membrane transport of reduced folates and a route for uptake of 4-amino antifolate chemotherapeutics, e.g. methotrexate [13,15].

The transporters THTR1 and THTR2 both translocate thiamine with similar pH sensitivities and a shared insensitivity to organic cations [7,14]. The extent of functional redundancy between these two transporters is unknown: they share identical substrate specificities and are co-expressed in several tissues [7,11,14]. There is little known about their cellular localizations, which is unfortunate because their distribution in polarized epithelia will reflect their physiological roles.

To address this issue, we used live-cell confocal imaging methods in the Madin–Darby canine kidney (MDCK) cell line. This is a widely used model system for studying membrane protein targeting in culture, as these cells form fully polarized monolayers with characteristics mimicking the organization of tight epithelia in the renal collecting duct *in vivo* [16]. For the first time, we have demonstrated that the two human (h)THTRs are differentially targeted in polarized cells: hTHTR2 is expressed apically, whereas hTHTR1 distribution is biased more basolaterally. Furthermore, hRFC targets basolaterally, which is a distribution distinct from the known apical localization of the folate receptor [17]. The likely consequence of this differential distribution of both pairs of micronutrient transporters is the creation of a transcellular pathway that mediates vectorial transport of micronutrients across renal epithelia, using a system where influx and efflux steps may be controlled independently through protein-specific regulation.

EXPERIMENTAL

Reagents

[³H]Thiamine (555 GBq/mmol) and [3',5',7,9-³H(N)]folate (12.7 Ci/mmol) were from ARC (St. Louis, MO, U.S.A.) and Moravsek Biochemicals respectively (Brea, CA, U.S.A.). Vectors for enhanced green fluorescent protein (EGFP-N3), red fluorescent protein (pDsRed2-N1) and a plasma membrane-

Abbreviations used: EGFP, enhanced green fluorescent protein; h, human; MDCK, Madin–Darby canine kidney; RFC, reduced folate carrier; SLC, solute carrier; THTR, thiamine transporter; V_{1a}R, vasopressin 1a receptor; YFP, yellow fluorescent protein.

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targeted yellow fluorescent protein (pEYFP-Mem) were from BD Biosciences (Palo Alto, CA, U.S.A.). MDCK cells (NBL-2) were from the A.T.C.C. (Manassas, VA, U.S.A.). Polyclonal anti-hTHTR2 antibodies were raised against a synthetic peptide (CENPDVSHPEESNI, amino acid residues 477–490), prepared as a keyhole-limpet hydrolysate-conjugate (Sigma Genesys, TX, U.S.A.) for immunization in rabbits. Antibodies specifically against hRFC have been described previously [18]. Anti-Xpress tag antibody that recognizes epitope-tagged (His-) hTHTR1 was from Invitrogen (Carlsbad, CA, U.S.A.). Secondary antibodies and Citifluor antifade reagent were from Jackson ImmunoResearch (West Grove, PA, U.S.A.) and Ted Pella (Redding, CA, U.S.A.).

cDNA constructs

Construction of SLC19A1-EGFP (hRFC-EGFP) and SLC19A2-EGFP (hTHTR1-EGFP) have previously been described [19,20]. The cDNA for SLC19A3 was amplified by RT (reverse transcriptase)-PCR from the total RNA from HEK-293 cells using specific primers, 5'-CCGCTCGAGATGGATTGTTACAGAACTTCACTAAG-3' and 5'-CGGGATCCTTAGAGTTTGTGACATGATGATATTAC-3', and subcloned into pGEM-T vector (Promega, WI, U.S.A.). The SLC19A3-EGFP fusion protein (hTHTR2-EGFP) was generated under similar PCR conditions with the following primers: 5'-CCGCTCGAGATGGATTGTTACAGAACTTCACTAAG-3' and 5'-CGGGATCCGAGTTTGTGACATGATGATATTAC-3', [19]. PCR products and the EGFP-N3 vector were digested with the restriction enzymes *Bam*HI and *Xho*I, and the products were isolated from the gel and ligated to generate an in-frame fusion protein (hTHTR2-EGFP), with green fluorescent protein fused to the C-terminus of hTHTR2. Nucleotide sequences of all constructs were confirmed by sequencing. V_{1a}R-EGFP is a fusion of the rat vasopressin 1a receptor (V_{1a}R) with a C-terminal EGFP tag and was a gift from Dr C. B. González (Department of Physiology, Universidad Austral de Chile, Valdivia, Chile) [21]. A fusion protein of the neurotrophin receptor to a C-terminal EGFP tag (p75-EGFP) was a gift from Dr E. Rodríguez-Bolan (Dyson Vision Research Institute, Cornell University, New York, U.S.A.) [22]. YFP_{MEMB} is a fusion of the N-terminal 20 amino acids of GAP-43 (GTPase-activating protein) to the YFP (BD Biosciences, CA, U.S.A.).

Cell culture and generation of stable cell lines

MDCK cells were maintained in MEM (minimal essential medium) supplemented with 10 % fetal bovine serum, NaHCO₂ (2.2 g/l), glutamine (0.29 g/l), penicillin (50 000 units/l) and streptomycin (50 mg/l). For transient transfections on cover-glasses, cells were grown to 90 % confluency on sterile glass-bottomed Petri dishes (MatTek, MA, U.S.A.) and incubated with 1 µg of plasmid DNA and LIPOFECTAMINETM 2000 in OPTIMEM (Invitrogen). For transient transfections on filters, MDCK cells were seeded on to collagen-coated filters (Corning Costar, Cambridge, MA, U.S.A.) and grown to confluence. Individual filter dishes were transfected approx. 5 days post confluence in OPTIMEM containing approx. 2 µg of plasmid DNA. In both cases, cells were imaged approx. 36–48 h after transfection. Stable MDCK cell lines were generated by G418 selection (0.9 mg/ml). Semi-quantitative RT-PCR analysis of stable clones was used to quantify expression of constructs. Total RNA (5 µg) was isolated from stably transfected and non-transfected MDCK cells and was used to generate first-stranded cDNA with gene-specific primers. Following PCR, the product intensity on the gel was quantified by densitometry.

Confocal microscopy

Cells were imaged using a BioRad MRC1024 confocal scanner attached to an Olympus AX70 microscope equipped with a 60 × oil- and a 60 × water-immersion objective for imaging coverslips and filters respectively. Fluorophores were excited with the 488 nm line of an argon ion laser, and emitted fluorescence was monitored with 530 ± 20 nm band pass (EGFP) or 620 nm long-pass filters (DsRed, Rhodamine Red). Fluorescence distribution was quantified using the IDL analysis package (Research Systems, CO, U.S.A.). Resulting measurements of transporter distribution were analysed using one-way ANOVA and subsequent Dunnett's tests to compare experimental data with controls [23]. Data were considered significant at *P* < 0.05.

Immunofluorescence

MDCK cells transfected with constructs were grown to confluence on coverslips, fixed for 10 min with a 4 % paraformaldehyde solution, permeabilized with 2 % Triton X-100 for 15 min and then incubated in PBS containing 1 % BSA ('blocking solution') for 30 min. Cells were incubated with primary antibodies in blocking solution for 2 h at 21 °C and then with Rhodamine Red-conjugated secondary antibodies in blocking solution for 1 h. Samples were mounted in Citifluor medium before imaging. Owing to lack of a native antibody against hTHTR1, His-tagged hTHTR1 was used for epitope marking in experiments with this construct.

Uptake assays

The distribution of functional transporters in the apical and basolateral domains of stable cell lines was assessed via radiolabel uptake using a filter-dish assay, as described in detail previously [16,24]. In brief, stable cell lines were seeded on to collagen-coated filters and grown past confluence (transepithelial resistance, approx. 300–500 Ω/cm²) with regular changes of medium. Thereafter, the culture medium was replaced with folate- or thiamine-deficient medium for 48 h. To measure radiolabel uptake, cells were washed three times and then incubated (37 °C) with Krebs–Ringer buffer (pH 5.5 for folinic acid; pH 7.4 for thiamine) supplemented with either [³H]folinic acid (24 nM) or [³H]thiamine (30 nM), then cells were added to either the apical or basolateral chamber contacting the filter support (see Figure 3C). After 3 min, the filter insert was removed, washed twice in ice-cold stopping solution, solubilized and accumulated radiolabel was quantified by scintillation counting [19,20]. Radiolabel accumulation across the apical and basolateral domains was compared relative to measurements made in parallel with a control MDCK stable cell line.

RESULTS

To investigate the cellular localization of human RFC, THTR1 and THTR2, cDNA for each transporter was fused to EGFP and the resulting constructs (hRFC-EGFP, hTHTR1-EGFP and hTHTR2-EGFP) were transiently transfected into MDCK cells. Confocal imaging of cross-sections of live cells (lateral 'x-y' planes) confirmed that each construct was targeted to the cell surface, since transporter localization ('green' channel) exhibited a more peripheral distribution than that of a co-transfected red fluorescent protein that served as a convenient cytosolic marker to visualize cellular dimensions ('red' channel, Figure 1A). However, the cell-surface distribution of hRFC-EGFP, hTHTR1-EGFP and

Figure 1 Localization of hRFC-EGFP, hTHTR1-EGFP and hTHTR2-EGFP

(A) Confocal images of MDCK cells transiently co-transfected with DsRed and hRFC-EGFP, hTHTR1-EGFP or hTHTR2-EGFP. Images were taken in lateral (x - y) section across the centre of each cell, and superficially for hTHTR2-EGFP. (B) Method for quantification of polarized expression using an axial (z) image of an MDCK cell expressing YFP_{MEMB}. Fluorescence values were resolved from the apical (yellow box), basal and both lateral membranes (blue boxes) using the intensity profiles (right-hand side). Basal and lateral fluorescence values were averaged to yield a basolateral value, which was compared with apical membrane fluorescence. (C, D) Axial scans of representative cells grown on filter supports transfected with (C) V1aR-EGFP, YFP_{MEMB} and p75-EGFP, and (D) hRFC-EGFP, hTHTR1-EGFP and hTHTR2-EGFP. (E) Collated measurements from entire data set.

hTHTR2-EGFP was clearly different: hTHTR2 was predominantly expressed in a focal plane superficial from the bulk cytoplasm (diagnostic of apical targeting), whereas both hRFC-EGFP and hTHTR1-EGFP were expressed in the lateral domains of the same focal section as the bulk of the cytoplasm (Figure 1A).

To define the distribution of each transporter more clearly, measurements of fluorescence intensities were recorded in axial (x - z) sections from MDCK cells grown on filter supports [16], which were transfected with EGFP-tagged constructs alone. Figure 1(B) shows an MDCK cell expressing a plasma membrane-targeted fluorescent protein (YFP_{MEMB}) to illustrate the method

used for quantification. Fluorescence intensities were averaged across the lateral and vertical axis of each cell, encompassing the 'top' (apical), 'bottom' (basal) and 'side' (lateral) membranes, and the peak fluorescence values recorded. Histograms were plotted of the apical fluorescence (yellow box, Figure 1B) versus the basolateral fluorescence, defined as the average of the two lateral and basal measurements (blue boxes, Figure 1B). For example, the YFP_{MEMB} construct was evenly distributed across the surface of the cell shown in Figure 1(B), reflected by cumulative measurements of $51.9 \pm 4.5\%$ apical and $48.1 \pm 4.5\%$ basolateral ($n = 12$ cells; three independent transfections).

Figure 2 Immunocytochemistry of hRFC, hTHTR1 and hTHTR2

(A) Immunological localization of hRFC (top panels), hTHTR1 (middle panels) and hTHTR2 (lower panels) in MDCK cells. Left-hand panels: EGFP fluorescence of EGFP-tagged constructs from fixed cells. Middle panels: antibody staining using primary antibodies against each construct (anti-hRFC, anti-Xpress tag hTHTR1 and anti-hTHTR2 antibodies) with Rhodamine Red-tagged secondary antibodies. Right-hand panels, composite overlay from both channels. (B) Immunological probing of non-EGFP-tagged hRFC, hTHTR1 and hTHTR2 expressed in MDCK cells. Left-hand panels: lateral (*x-y*) section showing fluorescence of Rhodamine Red secondary antibodies. Right-hand panels: axial (*z*) section of the same cells. (C) Quantitative analysis of untagged construct polarity measured from cells as shown in (B).

Figure 1(C) shows measurements from cells expressing constructs with known targeting polarity: an apical marker (p75-EGFP, [22]), a basolateral marker ($V_{1a}R$ -EGFP, [21]) and YFP_{MEMB} , whereas the distributions of hRFC-EGFP, hTHTR1-EGFP and hTHTR2-EGFP are shown in Figure 1(D). Comparison of results from all six constructs showed that each vitamin transporter possessed a different distribution: hRFC-EGFP was targeted basolaterally, hTHTR2-EGFP was targeted apically and hTHTR1-EGFP was distributed more uniformly across the cell (Figure 1E; $n \geq 9$ cells for 3 or more independent transfections). Statistical comparisons via one-way ANOVA and subsequent Dunnett's tests demonstrated that hRFC-EGFP distribution ($81.3 \pm 6.5\%$ basolateral) was not significantly different from that of the known basolateral marker $V_{1a}R$ -EGFP ($82.8 \pm 3.6\%$ basolateral), whereas hTHTR2-EGFP distribution ($93.0 \pm 2.8\%$ apical) paralleled that of the known apical marker p75-EGFP ($86.4 \pm 3.4\%$ apical). The distribution of hTHTR1-EGFP ($61.3 \pm 3.7\%$ basolateral) was intermediate between that of YFP_{MEMB} ($48.1 \pm 4.5\%$ basolateral) and $V_{1a}R$ -EGFP ($82.8 \pm 3.6\%$ basolateral). Similar results were obtained with MDCK cells grown on coverglasses (hRFC-EGFP, $81.1 \pm 6.0\%$

basolateral; hTHTR1-EGFP, $63.1 \pm 5.5\%$ basolateral; hTHTR2-EGFP, $88.7 \pm 2.5\%$ apical; $V_{1a}R$ -EGFP, $79.3 \pm 3.1\%$ basolateral; YFP_{MEMB} , $50.9 \pm 5.3\%$ basolateral; p75-EGFP, $86.4 \pm 3.4\%$ apical; $n \geq 10$ cells for three or more independent transfections).

To substantiate the validity of construct targeting, we were concerned to exclude the possibility that ligation of EGFP (approx. 27 kDa) perturbed the normal cellular targeting of the transporters. Therefore we performed immunocytochemical analyses on MDCK cells transfected with the non-EGFP-tagged constructs, hRFC, hTHTR1 and hTHTR2. Initially, the specificity of antibodies for individual transporters was confirmed by examining the degree of co-localization of Rhodamine Red immunostaining with EGFP fluorescence in MDCK cells transfected with hRFC-EGFP, hTHTR1-EGFP and hTHTR2-EGFP. Figure 2(A) confirms the high degree of fluorescence co-localization between 'green' (EGFP) and 'red' channels (Rhodamine Red immunocytochemistry) in lateral confocal sections overlaid from fixed MDCK cells. Subsequent experiments with the non-EGFP-tagged transporters (Figure 2B), captured in both lateral *x-y* and axial *x-z* section, confirmed that these constructs exhibited a similar polarized distribution to that seen with the EGFP-fusion proteins (compare Figure 2C with Figure 1E), demonstrating that the observed polarity is not an artifact of EGFP fusion.

More importantly, the asymmetric distribution of these transporters is expected to produce a distinct polarization of nutrient transport activity. To perform functional assays of micronutrient accumulation, we generated stable MDCK cell lines expressing hRFC-EGFP, hTHTR1-EGFP and hTHTR2-EGFP. Figure 3(A) represents a characterization of these stable cell lines after approx. 8 weeks of antibiotic selection. Confocal (*x-y* images) of each stable line demonstrated that a high proportion of cells (approx. 30–80%) exhibited EGFP fluorescence with the expected cell-surface distribution. RT-PCR analysis confirmed the up-regulated expression of each transporter relative to mock-transfected cells (approx. 2–7.5-fold; Figure 3A). To assess the distribution of functional transporters in these cell lines, we performed radiolabel uptake assays using stable cells grown on permeable filter supports, using established methods [16,24] where radiolabel can be selectively introduced to media bathing either the apical or basolateral domains of the confluent monolayer (Figure 3C). Compared with a control MDCK stable line, basolateral uptake increased ($215 \pm 18\%$) with the hRFC-EGFP stable cell line, apical uptake increased ($280 \pm 65\%$) with the hTHTR2-EGFP stable cell line, and both apical ($156 \pm 189\%$) and basolateral uptake ($202 \pm 21\%$) increased with the hTHTR1-EGFP stable cell line. This asymmetry in radiolabel uptake observed in these assays was consistent with the morphological distribution of each *SLC19A* transporter (compare Figures 3C and 1E).

DISCUSSION

In the present study, we have resolved the polarized targeting of each member of the *SLC19A* gene family of micronutrient transporters in MDCK cells. This is a suitable system for two reasons. Firstly, cultured MDCK monolayers exhibit fully polarized apical and basolateral domains that provide an experimentally tractable system for understanding protein targeting and vectorial transport [16]. Secondly, the presence of each *SLC19A* isoform in human kidney [7,8,11,15] highlights the relevance of defining renal mechanisms governing thiamine and folate transport.

Figure 3 Functional analysis of micronutrient uptake in stable MDCK cell lines

(A) Confocal images of stable MDCK cell lines expressing hRFC-EGFP (left-hand panel), hTHTR1-EGFP (middle panel) and hTHTR2-EGFP (right-hand panel). Insets: RT-PCR analysis of untransfected MDCK cells (left lanes) and stable cell lines (right lanes) probed with primers specific for hRFC, hTHTR1 or hTHTR2. (B) Measurements of radiolabel accumulation by stable cell lines grown on filter supports. Radiolabel was introduced to either the apical (AP) or the basolateral (BL) chamber, as shown in (C), which were separated by a confluent monolayer of stable cells. The polarity of transporter expression was quantified through measurements of [³H]folinic acid (open bars) or [³H]thiamine (closed bars) accumulation after apical or basolateral addition (see the Experimental section). Results are expressed as the means $\pm$ S.E.M of uptake values obtained from multiple inserts from more than three independent experiments.

Targeting of SLC19A2 and SLC19A3

The major result of the present study is the resolution of discrete cellular targeting of hTHTR1 and hTHTR2. These results provide the first demonstration of the differential localization of hTHTR1 and hTHTR2 in polarized epithelia: hTHTR2 targets to the apical domain and hTHTR1 is biased more, but not exclusively, basolaterally. This is an important observation, since their similar substrate specificity and tissue localization previously implied significant functional overlap. In contrast, the distinct cellular distribution of hTHTR1 and hTHTR2 is immediately suggestive of their likely physiological roles in establishing vectorial transport: hTHTR2 is positioned to play a crucial role in the accumulation of thiamine into the cell, whereas hTHTR1 is appropriately positioned to contribute to thiamine export/accumulation from the bloodstream. Additionally, given the potential for bi-directional transport activity of these proteins [13], their co-ordinated action may also provide a secretory route in the kidney under certain conditions (for example, following pharmacological dosing of these micronutrients) [24]. Finally,

given that each thiamine transporter contains several known and unique regulatory motifs [7,11], isoform-specific regulation may permit independent control of thiamine influx and efflux steps to allow fine tuning of *trans*-cellular thiamine fluxes.

Targeting of SLC19A1

Although the molecular localization of hRFC has not previously been defined in renal epithelia, our results are consistent with the biochemical signature of hRFC activity that has been previously demonstrated within the basolateral domain of human proximal tubule cells [24]. Similarly, murine RFC has been localized by immunocytochemistry to the basolateral domain of mouse renal cortical and medullary sections [25]. How then does hRFC contribute to transcellular transport of reduced folate compounds? This is probably in concert with apically localized proteins, including the classic folate receptors (e.g. FR α [26]), as well as other anion transporters which translocate folate {e.g. OAT-K2 (organic anion transporter K2) [27]}. The relative contribution of such proteins to physiological folate fluxes is probably complex, but, simplistically, the cellular segregation of folate receptors and hRFC resembles the discrete localizations of the thiamine transporters in the same cells. Precedent for such discrete roles of the folate transporters in establishing *trans*-epithelial fluxes has been elegantly demonstrated in retinal pigmented epithelium [28,29]. In native tissue and a retinal cell line, folate receptor α functioned to accumulate folates, whereas RFC contributed to folate efflux, thereby completing a vectorial pathway for folate translocation [28], where the bi-directional transport capacity of RFC allows it to function as an export carrier under physiological conditions.

In conclusion, we have shown that the cellular targeting of hTHTR1 and hTHTR2 is divergent, serving to establish a pathway for vectorial transport of thiamine across epithelia. The dual-transporter system for thiamine has obvious similarity to the differential cell-surface targeting of hRFC and the folate receptor, implying that both pairs of transport proteins may function in concert to effect micronutrient transport across polarized cells. Such segregation of micronutrient transport activities as discrete molecular species will permit physiological fine-tuning of cellular fluxes via protein-specific regulation.

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REFERENCES

- Kim, Y. I. J. (1999) Folate and carcinogenesis: evidence, mechanisms and implications. *J. Nutr. Biochem.* **10**, 66–88
- Stanger, O. (2002) Physiology of folic acid in health and disease. *Curr. Drug Metab.* **3**, 211–223
- Rogers, L. E., Porter, F. S. and Sidbury, J. B. (1969) Thiamine-responsive megaloblastic anemia. *J. Pediatr.* **74**, 494–504
- Singleton, C. K. and Martin, P. R. (2001) Molecular mechanisms of thiamine utilization. *Curr. Mol. Med.* **1**, 197–207
- Dixon, K. H., Lanpher, B. C., Chiu, J., Kelley, K. and Cowan, K. H. (1994) A novel cDNA restores reduced folate carrier activity and methotrexate sensitivity to transport deficient cells. *J. Biol. Chem.* **269**, 17–20
- Williams, F. M. R., Murray, R. C., Underhill, T. M. and Flintoff, W. F. (1994) Isolation of a hamster cDNA clone coding for a function involved in methotrexate uptake. *J. Biol. Chem.* **269**, 5810–5816
- Dutta, B., Huang, W., Molero, M., Kekuda, R., Leibach, F. H., Devoe, L. D., Ganapathy, V. and Prasad, P. D. (1999) Cloning of the human thiamine transporter, a member of the folate transporter family. *J. Biol. Chem.* **274**, 31925–31929

- 8 Diaz, G. A., Banikazemi, M., Oishi, K., Desnick, R. J. and Gelb, B. D. (1999) Mutations in a new gene encoding a thiamine transporter cause thiamine-responsive megaloblastic anaemia syndrome. *Nat. Genet.* **22**, 309–312
- 9 Fleming, J. C., Tartaglioni, E., Steinkamp, M., Schorderit, D. F., Cohen, N. and Neufeld, E. J. (1999) The gene mutated in thiamine responsive anaemia with diabetes and deafness (TRMA) encodes a functional thiamine transporter. *Nat. Genet.* **22**, 305–308
- 10 Labay, V., Raz, T., Baron, D., Mandel, H., Williams, H., Barrett, T., Szargel, R., McDonald, L., Shalata, A., Nosaka, K. et al. (1999) Mutations in SLC19A2 cause thiamine-responsive megaloblastic anaemia associated with diabetes mellitus and deafness. *Nat. Genet.* **22**, 300–304
- 11 Eudy, J. D., Spiegel, O., Barber, R. C., Wlodarczyk, B. J., Talbot, J. and Finnell, R. H. (2000) Identification and characterization of the human and mouse *SLC19A3* gene: a novel member of the reduced folate family of micronutrient transporter genes. *Mol. Genet. Metab.* **71**, 581–590
- 12 Ferguson, P. L. and Flintoff, W. F. (1999) Topological and functional analysis of the human reduced folate carrier by hemagglutinin epitope insertion. *J. Biol. Chem.* **273**, 16269–16278
- 13 Matherly, L. H. (2001) Molecular and cellular biology of the human reduced folate carrier. *Prog. Nucleic Acid Res. Mol. Biol.* **67**, 131–162
- 14 Rajgopal, A., Edmondson, A., Goldman, D. and Zhao, R. (2001) *SLC19A3* encodes a second thiamine transporter ThTr2. *Biochim. Biophys. Acta* **1537**, 175–178
- 15 Whetstone, J. R., Flateley, R. M. and Matherly, L. H. (2002) The human reduced folate carrier gene is ubiquitously and differentially expressed in normal human tissues: identification of seven non-coding exons and characterization of a novel promoter. *Biochem. J.* **367**, 629–640
- 16 Balcarova-Stander, J., Pfeiffer, S. E., Fuller, S. D. and Simons, K. (1984) Development of cell surface polarity in the epithelial Madin–Darby canine kidney (MDCK) cell line. *EMBO J.* **3**, 2687–2694
- 17 Antony, A. (1996) Folate receptors. *Annu. Rev. Nutr.* **16**, 501–521
- 18 Dudeja, P. K., Kode, A., Alnounou, M., Tyagi, S., Torania, S., Subramanian, V. S. and Said, H. M. (2001) Mechanism of folate transport across the human colonic basolateral membrane. *Am. J. Physiol.* **281**, G54–G60
- 19 Subramanian, V. S., Marchant, J. S., Parker, I. and Said, H. M. (2001) Intracellular trafficking/membrane targeting of human reduced folate carrier expressed in *Xenopus* oocytes. *Am. J. Physiol.* **281**, G1477–G1486
- 20 Subramanian, V. S., Marchant, J. S., Parker, I. and Said, H. M. (2003) Cell biology of the human thiamine transporter-1 (hTHTR1): intracellular trafficking and membrane targeting mechanisms. *J. Biol. Chem.* **278**, 3976–3984
- 21 Campos, D. M., Reyes, C. E., Sarmiento, J., Navarro, J. and Gonzalez, C. B. (2001) Polarized expression of the GFP-tagged rat V_{1a} vasopressin receptor. *Biochem. Biophys. Res. Commun.* **289**, 325–328
- 22 Kreitzer, G., Marmorstein, A., Okamoto, P., Vallee, R. and Rodriguez-Bolan, E. (2000) Kinesin and dynamin are required for post-Golgi transport of a plasma membrane protein. *Nat. Cell Biol.* **2**, 125–127
- 23 Loffing-Cueni, D., Loffing, L., Shaw, C., Taplin, A. M., Govindan, M., Stanton, C. R. and Stanton, B. A. (2001) Trafficking of GFP-tagged $\Delta F508$ -CFTR to the plasma membrane in a polarized epithelial cell line. *Am. J. Physiol. Cell Physiol.* **281**, C1889–C1897
- 24 Morshed, K. M., Ross, D. M. and McMartin, K. E. (1997) Folate transport proteins mediate the bidirectional transport of 5-methyltetrahydrofolate in cultured human proximal tubule cells. *J. Nutr.* **127**, 1137–1147
- 25 Wang, Y., Zhao, R., Russell, R. G. and Goldman, D. I. (2001) Localization of the murine reduced folate carrier as assessed by immunohistochemical analysis. *Biochim. Biophys. Acta* **1513**, 49–54
- 26 Birn, H., Nielsen, S. and Christensen, E. I. (1997) Internalization and apical-to-basolateral transport of folate in rat kidney proximal tubule. *Am. J. Physiol.* **272**, F70–F78
- 27 Masuda, S., Ibamoto, K., Takeuchi, A., Saito, H., Hashimoto, Y. and Inui, K.-I. (1999) Cloning and functional characterization of a new multispecific organic anion transporter, OAT-K2, in rat kidney. *Mol. Pharmacol.* **55**, 743–752
- 28 Chancy, C. D., Kekuda, R., Huang, W., Prasad, P. D., Kuhnel, J. M., Sirotnak, F. M., Roon, P., Ganapathy, V. and Smith, S. B. (2000) Expression and differential polarization of the reduced-folate transporter-1 and the folate receptor alpha in mammalian retinal pigment epithelium. *J. Biol. Chem.* **275**, 20676–20684
- 29 Bridges, C., El-Sherbeny, A., Ola, S., Ganapathy, V. and Smith, S. (2002) Transcellular transfer of folate across the retinal pigment epithelium. *Curr. Eye Res.* **24**, 129–138

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