LAT2, a New Basolateral 4F2hc/CD98-associated Amino Acid Transporter of Kidney and Intestine*

Grégoire Rossiër‡§, Christian Meier§§, Christian Bauch‡, Vanessa Summa, Bernard Sordat‡, François Verrey¶, and Lukas C. Kühn‡‡

From the ‡Swiss Institute for Experimental Cancer Research, CH-1066 Epalinges s/Lausanne, Switzerland and the ¶Institute of Physiology, University of Zürich, CH-8057 Zürich, Switzerland

Glycoprotein-associated amino acid transporters (gpaAT) are permease-related proteins that require heterodimerization to express their function. So far, four vertebrate gpaATs have been shown to associate with 4F2hc/CD98 for functional expression, whereas one gpaAT specifically associates with rBAT. In this study, we characterized a novel gpaAT, LAT2, for which mouse and human cDNAs were identified by expressed sequence tag data base searches. The encoded ortholog proteins are 531 and 535 amino acids long and 92% identical. They share 52 and 48% residues with the gpaATs LAT1 and y’LAT1, respectively. When mouse LAT2 and human 4F2hc cRNAs were co-injected into Xenopus oocytes, disulfide-linked heterodimers were formed, and an L-type amino acid uptake was induced, which differed slightly from that produced by LAT1–4F2hc: the apparent affinity for L-phenylalanine was higher, and the apparent molecular mass of 85–93 kDa due to four extracellular domains require heterodimerization with a type II glycoprotein to express their function. As yet, four of these transporters, LAT1 (1–4), y’LAT1 (5, 6), y’LAT2 (5, 6), and xCT (7), associate with the same glycoprotein 4F2hc (4F2 heavy chain)/CD98, whereas another member, b0,+ AT, associates with the 4F2hc-related protein rBAT.2 According to the nomenclature of Christensen et al. (8), the first identified member of the gpaAT family was named LAT1 (E16, TA1, AmAT-l-1c, ASUR4) (1–4) to indicate its l-leucine-prefering (versus l-alanine) amino acid transport range. Screening of EST data bases resulted in the discovery of two related y’-type transporters, y’LAT1 and y’LAT2 (HA7016 cDNA, product of the KIATA0245 gene; see Ref. 9) (5, 6), which accept basic amino acids and require Na+ for the transport of neutral amino acids. Recently, a 4F2hc-associated cystine/glutamate transporter, xCT (7), and the partner of rBAT, with specificity for b0,+ -type amino acid transport at the apical surface of epithelial cells,2 were identified.

The association of gpaATs with 4F2hc or possibly rBAT is a prerequisite for the transporters to reach the cell surface (2). Thus, the glycoproteins play probably a role in the polarized expression of transport functions. In epithelial cells, 4F2hc is localized at the basolateral membrane (10), whereas rBAT is apical (11, 12). Moreover, the glycoproteins may regulate the surface expression of the transporters. 4F2hc mRNA is strongly induced in cell proliferation (13–15) and expressed in numerous tissues (16). 4F2 antigen was originally described in dividing T cells as an activation antigen composed of a heavy chain and a disulfide-linked light chain (17, 18). Human 4F2hc comprises 529 amino acids with an intracellular NH2 terminus and a single transmembrane region, but migrates with an apparent molecular mass of 85–93 kDa due to four extracellular glycosylation sites (19–22). rBAT has a similar membrane topology and is ~30% identical to 4F2hc. Both proteins activate amino acid transport when expressed alone in Xenopus oocytes, presumably due to their association with endogenous light chains of the gpaAT family (23–28). When 4F2hc is coexpressed with a gpaAT, this leads to a much earlier appearance of amino acid transport, which is light chain-specific.

The actual vectorial transport of amino acids is thought to be mediated by cotransporter(s) and/or uniporter(s), which have a limited range of amino acid specificity. Therefore, the gpaAT family members, which function as amino acid exchangers for a broad range of amino acids, are required to extend the transport specificity range to amino acids not accepted by the

The transport of amino acids across cellular membranes is adapted to the needs of specific cells as well as to local and systemic requirements. For instance, active amino acid uptake is a necessity for growing cells. On the other hand, transepithelial transport of amino acids across (re)absorptive epithelia requires, besides an active amino acid uptake on the luminal side, an extrusion mechanism on the basolateral side.

Recently, various members of the novel family of glycoprotein-associated amino acid transporters (gpaATs) have been identified and shown to play a role in cellular uptake and/or basolateral extrusion of basic and neutral amino acids. These permease-related proteins with 12 putative transmembrane domains require heterodimerization with a type II glycoprotein to express their function. As yet, four of these transporters, LAT1 (1–4), y’LAT1 (5, 6), y’LAT2 (5, 6), and xCT (7), associate with the same glycoprotein 4F2hc (4F2 heavy chain)/CD98, whereas another member, b0,+ AT, associates with the 4F2hc-related protein rBAT.2 According to the nomenclature of Christensen et al. (8), the first identified member of the gpaAT family was named LAT1 (E16, TA1, AmAT-l-1c, ASUR4) (1–4) to indicate its l-leucine-prefering (versus l-alanine) amino acid transport range. Screening of EST data bases resulted in the discovery of two related y’-type transporters, y’LAT1 and y’LAT2 (HA7016 cDNA, product of the KIATA0245 gene; see Ref. 9) (5, 6), which accept basic amino acids and require Na+ for the transport of neutral amino acids. Recently, a 4F2hc-associated cystine/glutamate transporter, xCT (7), and the partner of rBAT, with specificity for b0,+ -type amino acid transport at the apical surface of epithelial cells,2 were identified.

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mLAT, mouse LAT; rLAT, rat LAT; h4F2hc, human 4F2hc; PBS, phosphate-buffered saline.

1 Pfeiffer, R., Loffing, J., Rossier, G., Meier, C., Eggerman, T., Bauch, C., Loffing, D., Kühn, L. C., and Verrey, F. (1999) Mol. Biol. Cell, in press.

From the §§Swiss Institute for Experimental Cancer Research, CH-1066 Epalinges s/Lausanne, Switzerland and the ¶Institute of Physiology, University of Zürich, CH-8057 Zürich, Switzerland

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Amino Acid Transporter LAT2

Experimental Procedures

Construct of hLAT2—An EST with the cDNA for mLAT2 (GenBankTM/EBI accession number AA885581) was identified by database searches (BLAST programs of NCBI and EMNet-CH) with the sequences of ASUR4 and E16 as query. Based on the sequence of mLAT2, several overlapping ESTs of the human cDNA were found. The clones were obtained from the IMAGE consortium. Two of them (GenBankTM/EBI accession numbers H99270 and N32639) were digested with AIII and NoI to generate fragments of 1371 and 2357 base pairs, respectively, which were ligated into pGEM-1Zf(+) (Promega) previously linearized with NotI and dephosphorylated with alkaline phosphatase (Roche Molecular Biochemicals).

Sequence Analysis and cRNA Synthesis—The mLAT2 and hLAT2 ESTs were sequenced on both strands by automated sequencing (LIFE TECHNOLOGIES Corporation) with the sequences of ASUR4 and E16 as query. The sequences of hLAT2 and mLAT2 cDNAs are available under GenBankTM/EBI accession numbers AF171669 and AF171668, respectively.

The open reading frames of mLAT2 and hLAT2 were amplified by polymerase chain reaction using Vent® polymerase (New England BioLabs Inc.) and transferred to the pSD5eas vector (31). mLAT2 was flanked at its 5′-end by 35 untranslated nucleotides with an XhoI site and at its 3′-end by a 32-nucleotide HindIII site, whereas hLAT2 had 26 5′-nucleotides and a HindIII site and 26 3′-nucleotides with a BamHI site. For cRNA synthesis, plasmids containing the cDNAs of hLAT2 (vector pSPort) (19) and mLAT2 and hLAT2 (pSD5eas) were linearized using the restriction sites HindIII and BglII, respectively. cRNA was synthesized with T7 and SP6 RNA polymerases (Promega), respectively, according to standard protocols.

Xenopus laevis Oocytes—Oocytes were treated with collagenase A for 2–3 h at room temperature in Ca2+-free buffer containing 82.5 mM NaCl, 2 mM KCl, 1 mM MgCl2, and 10 mM HEPES (pH 7.4) to remove follicular cells and then kept at 16 °C in ND96 buffer containing 96 mM NaCl, 2 mM KCl, 1 mM MgCl2, 1.8 mM CaCl2, and 5 mM HEPES (pH 7.4). The oocytes were washed twice in ND96 buffer and lysed in oocyte lysis buffer (20 mM Tris-HCl (pH 8.0), 1 mM EDTA, 0.5% Nonidet P-40, and 500 mM LiCl and four times in the same buffer without LiCl. The beads were resuspended in SDS-polyacylamide gel electrophoresis loading buffer and incubated at 95 °C for 5 min. β-Mercaptoethanol (2%) was added for reduced samples. After migration, the 8% SDS-polyacrylamide gels were fixed in 30% methanol and 10% glacial acetic acid for 1 h, treated with Amplify (Amersham Pharmacia Biotech) for 15 min, and rinsed in H2O for 5 min. The gels were dried and exposed to Eastman Kodak BiomaxTM films at −75 °C.

Amino Acid Uptake and Efflux in Xenopus Oocytes—Xenopus oocytes were injected with 5 ng of cRNA dissolved in 33 nl of water and incubated for 24 h at 16 °C in ND96 buffer. Before the experiment, they were washed six times with (+Na)- or (−Na)-buffer containing 100 mM NaCl ((+Na)-buffer) or choline Cl ((−Na)-buffer), 2 mM KCl, 1 mM MgCl2, 1 mM CaCl2, and 10 mM HEPES (pH 7.5). Six oocytes/experiment were preincubated for 2 min at 22 °C. The buffer was replaced with (+Na)- or (−Na)-buffer, respectively, with a 100 μM concentration of the indicated L-amino acid and the corresponding L-α-hydroxy acid. Uptakes were determined by liquid scintillation. Uptakes for dose-response curves were performed at five different amino acid concentrations. Sigmoid curves corresponding to Michaelis-Menten kinetics were fitted to the experimental data, and amino acid concentrations for half-maximal activation (apparent Kₐ) were derived.

For efflux studies, oocytes were injected 18 h after injection of the corresponding cRNA with 1 nmol of the indicated amino acid and 10 pmol of 3H-labeled amino acid as a tracer (except for L-[14C]glutamine). Uptakes were performed for 3 min because pilot experiments had shown linear amino acid uptake during this period. The oocytes were washed five times with 3 nmol of (+Na)- or (−Na)-buffer, respectively, and distributed to individual vials. After oocyte lysis in 2% SDS, the radioactivity was determined by liquid scintillation. Uptakes for dose-response curves were performed at five different amino acid concentrations. Sigmoid curves corresponding to Michaelis-Menten kinetics were fitted to the experimental data, and amino acid concentrations for half-maximal activation (apparent Kₐ) were derived.

Northern Blot Analysis—Total RNA was isolated from various tissues of the mouse (B10.D2), rat, hamster, and human by a modification of the method of Chirgwin et al. (33). Nitrogen-frozen tissues were homogenized in RNA lysis buffer (3 mM sodium acetate (pH 6), 4 mM guanidinium thiocyanate, and 8% β-Mercaptoethanol) using a homogenizer. The lysates were spun at 9700 × g for 10 min, and the supernatants were layered on top of 1.4 ml of a 5.7 M cesium chloride cushion in Ultra-Clear tubes (Beckman Instruments Inc.). The tubes were centrifuged at 100,000 × g for 18 h at 20 °C. The pellet was dissolved in H2O, extracted with phenol/chloroform/isomyl alcohol, and precipitated with ethanol. The pellet was dissolved in H2O.

Fifteen micrograms of total RNA were migrated on formaldehyde-containing 1.2% agarose gels, transferred onto GeneScreen Plus membrane (NEN Life Science Products), and UV-cross-linked. The membranes were fixed in 30% methanol and 10% glacial acetic acid for 1 h, treated with Amplify (Amersham Pharmacia Biotech) for 15 min, and rinsed in H2O for 5 min. The gels were dried and exposed to Eastman Kodak BiomaxTM films at −75 °C.

Immunofluorescence—Kidneys from a nu/nu mouse were sectioned in halves through the papilla, immediately snap-frozen in 2-methylbutane cooled to keyhole limpet hemocyanin (Eurogentec). The anti-human rBAT polyclonal antibody was raised similarly using a synthetic peptide with the 18 NH₂-terminal amino acids as antigen.

Antibodies were bound to 30 μl of Affi-Gel-protein A-agarose (Bio-Rad) in oocyte lysis buffer for 2 h at room temperature using a shaker at low speed. Equal volumes (2 × 10⁶ oocytes) were mixed with the coupled beads and shaken slowly overnight at 4 °C. The beads were washed four times in buffer containing 100 mM NaCl, 20 mM Tris-HCl (pH 8.0), 1 mM EDTA, 0.5% Nonidet P-40, and 500 mM LiCl and four times in the same buffer without LiCl. The beads were resuspended in SDS-polyacrylamide gel electrophoresis loading buffer and incubated at 95 °C for 5 min. β-Mercaptoethanol (2%) was added for reduced samples. After migration, the 8% SDS-polyacrylamide gels were fixed in 30% methanol and 10% glacial acetic acid for 1 h, treated with Amplify (Amersham Pharmacia Biotech) for 15 min, and rinsed in H2O for 5 min. The gels were dried and exposed to Eastman Kodak BiomaxTM films at −75 °C.

Y1/y1 system transporters best exemplifies such duplication: whereas y¹ LAT2 has a broad tissue expression (9, 3) y¹ LAT1 is expressed mainly in intestine and kidney epithelia. The actual function of y¹ LAT1 is the exchange of intracellular cationic amino acids against extracellular neutral amino acids cotransported with a Na⁺ ion (5, 6). Mutations in y¹ LAT1 cause the impressive phenotype of lysinuric protein intolerance (29, 30), indicating the central role of this gpaAT in the basolateral export of cationic amino acids from epithelial cells of the small intestine and kidney proximal tubule (5, 6). They also highlight the crucial role of gpaATs for the extension of the specificity range of amino acid transport.

In this study, we characterize a new gpaAT, LAT2, which is structurally and functionally most related to LAT1. In contrast to LAT1, which is expressed widely, LAT2 is prominently expressed in the basolateral membrane of (re)absorbing epithelia and is thus suggested to play a role in amino acid (re)absorption.

3 F. Verrey, unpublished results.
precooled with liquid nitrogen, and then stored at −75 °C until sectioning. The small intestine from a B10D2 mouse was directly nitrogen-frozen and stored at −75 °C until sectioning. Sections of 7 μm were made with a cryomicrotome (Jung CM 1800, Leica Inc.), placed on gelatin-coated glass slides, fixed for 90 s in methanol, rinsed in PBS, and incubated in PBS and 1% bovine serum albumin for 30 min. Primary antibodies (rabbit anti-mouse LAT2 or rat anti-mouse 4F2hc/RL388 (22, 35), kindly provided by Dr. Claude Bron) were added at several dilutions for 1 h. The slides were extensively washed in PBS, and secondary antibodies (Cy3-conjugated donkey anti-rabbit IgG and fluorescein isothiocyanate-conjugated rabbit anti-rat IgG) were added for 1 h in the dark. As a specificity control for the anti-LAT2 antibody, preimmune serum of the same rabbit was used. No staining was observed. Furthermore, the filters for the detection of green or red fluorescence were highly specific, and no spillover was observed. For double-staining experiments, primary antibodies were added simultaneously, but secondary antibodies were added sequentially: first, the Cy3-conjugated donkey anti-rabbit IgG for 45 min, and after extensive washing with PBS, the fluorescein isothiocyanate-conjugated rabbit anti-rat IgG for 45 min. The slides were extensively washed in PBS, mounted, and observed with a fluorescence microscope (Axioskop, Carl Zeiss, Inc.) equipped with a CCD camera.

RESULTS

Mouse and human ESTs encoding a new member of the gpαAT family were identified by data base searches with BLAST programs at NCBI and EMNet-CH using the LAT1 and y-LAT1 protein sequence as queries. The mouse full-length protein is encoded by a single 3717-base pair cDNA (GenBank™/EBI accession number AF171668). The human ortholog cDNA (3728 base pairs; GenBank™/EBI accession number AF171669) was obtained by fusing two partial ESTs (GenBank™/EBI accession numbers H99270 and N32639). These new transporter cDNAs and their protein products were subsequently named mLAT2 and hLAT2 based on their structural and functional relationship with LAT1 (see below). The mouse and human proteins encoded by the LAT2 cDNAs are composed of 531 and 535 amino acids, respectively, and their sequences are 91.9% identical to each other (data not shown). The highest level of identity to other members of the gpαAT family is obtained for LAT1 (52% identity between hLAT1 and hLAT2). The identity level is slightly lower for human y-LAT2 (46.6%). Human hLAT1 AT, the light chain associated with rBAT, is 40.7% identical. The primary structure of mLAT2 and hLAT2 suggests, as for LAT1 and y-LAT1 (1, 2, 5, 6), a protein with 12 transmembrane domains with intracellular NH2 and COOH termini (TMpred (36), Swiss Institute of Bioinformatics). A conserved cysteine involved in disulfide bridging with 4F2hc is located in the extracellular loop between putative transmembrane domains 3 and 4 in X. laevis LAT1 (ASUR4; X. laevis AmAt-Lc) and SPRM1 (Schistosoma mansoni homolog) (37). It is also conserved in LAT2 (residues 153 and 154 in mLAT2 and hLAT2, respectively).

LAT2 Is Disulfide-linked to h4F2hc—Using immunoprecipitation of [35S]methionine-labeled proteins expressed in X. laevis oocytes, we tested whether LAT2 is disulfide-linked to h4F2hc or rBAT. In Fig. 1, we show that h4F2hc expressed alone, independently of sample reduction, migrated at ~80 kDa when immunoprecipitated with anti-4F2hc antibody (lanes 5 and 16), whereas no band appeared when anti-LAT2 antibody was used (lanes 2 and 15). Correspondingly, LAT2 expressed alone appeared as a band of ~42 kDa when immunoprecipitated with anti-LAT2 antibody (lanes 1 and 12), but was absent when immunoprecipitated with anti-4F2hc antibody (lanes 4 and 15). When both h4F2hc and LAT2 were coexpressed in oocytes, they were coprecipitated with anti-4F2hc (lanes 6 and 17) or anti-LAT2 (lanes 3 and 14) antibody. Under nonreducing conditions, a LAT2–4F2hc complex that migrated at ~120 kDa was precipitated by either antibody (lanes 3 and 6), together with some uncoupled 4F2hc precipitated with anti-4F2hc antibody (lane 6; 80 kDa) and some uncoupled LAT2 precipitated with anti-LAT2 antibody (lane 3; 42 kDa). Under reducing conditions, the complex was almost completely reduced to an ~80-kDa band (4F2hc) and an ~42-kDa band (LAT2) (lanes 14 and 17).

We then tested whether LAT2 possibly also associates with rBAT. Immunoprecipitation of rBAT expressed alone, independently of sample reduction, displayed two bands at ~75 and 85 kDa (Fig. 1, lanes 9 and 20), representing most probably core- and terminally glycosylated forms of the protein. LAT2 expressed alone was not precipitated by anti-rBAT antibody (lanes 11 and 22). Surprisingly, when rBAT and LAT2 were coexpressed, rBAT was coprecipitated by anti-LAT2 antibody, as seen under reducing conditions (lane 19). Under nonreducing conditions, however, the complex was not detected at the level of a heterodimer (~130 kDa), but migrated in a high molecular mass complex, the composition of which is not known (lane 8). After immunoprecipitation with anti-rBAT antibody, only a weak band was detected near 40 kDa under reducing conditions, which could correspond to LAT2 (lane 18).

We conclude that LAT2 binds to 4F2hc via a disulfide bridge with high efficiency when expressed in Xenopus oocytes and that a fraction of LAT2 can be disulfide-linked to rBAT as well. However, immunofluorescence results shown below, together with the known apical expression of rBAT (11, 12), indicate that the interaction of LAT2 with rBAT does not take place or is unstable in kidney tubule and intestine, despite their being coexpressed in the same epithelial cells. Thus, this interaction can be considered as a peculiarity of (over)expression in the Xenopus oocyte system.

Differential Tissue Distribution of LAT2 and LAT1—Northern blot analysis of mouse tissue RNA with the mLAT2 probe revealed a band at ~4 kilobases (Fig. 2). This mRNA was strongly expressed in small intestine and kidney and moder-
rates induced by the expressed hetero-oligomers. Because of the similarity of LAT2 to LAT1, we first tested whether LAT2-h4F2hc transports L-leucine and whether this transport is inhibitable by the system L-specific substrate 2-amino-2-norbornanecarboxylic acid. At 100 μM L-leucine, a transport of 50–70 pmol/h was observed in microinjected oocytes with h4F2hc and mLAT2, hLAT2, or hLAT1. This transport was >90% inhibited by 10 μM 2-amino-2-norbornanecarboxylic acid, whereas α-(methylamino)isobutyrate, an A-type transporter substrate, did not interfere with L-leucine transport. The specificity and sodium dependence of the amino acid uptake by LAT2-h4F2hc were further tested using 100 μM concentrations of various amino acids in the presence or absence of sodium (Fig. 4). The results were similar to those obtained for hLAT1, some of which have been determined earlier (2) and are shown for comparison. Both light chains induced a sodium-independent transport of the large neutral amino acids L-phenylalanine, L-tyrosine, L-tryptophan, L-leucine, and L-histidine. We observed transport of L-isoleucine and L-valine, indicating that these transporters also accept amino acids with differentially branched side chains. At the amino acid concentration used (100 μM), transport of L-methionine, the negatively charged amino acid L-glutamic acid, and the positively charged amino acids L-arginine and L-lysine was not observed. Interestingly, mLAT2 induced the uptake of L-alanine, whereas L-glycine, with only one methyl group less than L-alanine, was not transported. Besides all similarities between LAT1- and LAT2-induced transport, there are two clear differences: L-alanine and L-glutamate uptake (at 100 μM) were much higher with LAT2 than with LAT1. Uptake experiments with hLAT2 using a selection of amino acids (L-phenylalanine, L-methionine, L-glutamine uptake (at 100 μM), transport of L-methionine, the negatively charged amino acid L-glutamic acid, and the positively charged amino acids L-arginine and L-lysine was not observed. Interestingly, mLAT2 induced the uptake of L-alanine, whereas L-glycine, with only one methyl group less than L-alanine, was not transported. Besides all similarities between LAT1- and LAT2-induced transport, there are two clear differences: L-alanine and L-glutamate uptake (at 100 μM) were much higher with LAT2 than with LAT1. Uptake experiments with hLAT2 using a selection of amino acids (L-phenylalanine, L-methionine, L-glutamic acid, and L-arginine), in the presence of sodium, showed the same specificity range as with mLAT2. The apparent K_m values for the uptake of various amino acids by mLAT2-h4F2hc were determined (Fig. 5). L-Phenylalanine showed the highest apparent affinity (apparent K_m = 12.2 μM), followed by L-leucine (48 μM), L-alanine (167 μM), L-glutamic acid (275 μM), and L-histidine (294 μM). This contrasts with results obtained previously with X. laevis LAT1 (ASUR4), which showed the highest apparent affinity for L-leucine (32 μM) and L-histidine (35 μM), followed by L-phenylalanine (740 μM), L-glutamic acid (2.2 mM), and L-alanine (>10 mM). In conclusion, these uptake experiments show that LAT2 has many properties similar to LAT1, justifying its name. However, there are important differences in the apparent affinity for some amino acids, and in particular, it is interesting that LAT2 also accepts L-alanine.

We tested whether LAT2 functions as an exchanger by performing efflux experiments (data not shown). One nanomole of L-phenylalanine together with 10 pmol of L-[3H]phenylalanine were injected into oocytes expressing h4F2hc alone or h4F2hc and mLAT2. Four hours later, the efflux of labeled L-phenylalanine was measured in the presence of 1 mM unlabeled L-phenylalanine in the incubation buffer. The efflux was 9 pmol/mrnin/oocyte and depended on the expression of mLAT2–4F2hc complexes since oocytes injected with 4F2hc alone did not show an efflux significantly higher than that of uninjected oocytes (<1 pmol/min/oocyte). As the labeled amino acid virtually did not leak out of the oocytes during the 4-h preincubation in amino acid-free buffer, and measurements of efflux in the absence of extracellular amino acids did not show a consistent efflux, the results suggest that LAT2 functions as an exchanger. This is analogous to γ-LAT1, which also associates with mLAT2.

* The lack of L-tryptophan uptake by El6 (hLAT1) published in an earlier study (2) was incorrect and due to the use of a deficient vial of [3H]tryptophan.
Association of LAT2 with 4F2hc—LAT2 belongs to the gpaAT family, the members of which require association with a glycoprotein (4F2hc or rBAT) for functional surface expression. The protein sequence identity of LAT2 to the other members of this family (see the Introduction) varies from 40 to 53%, and LAT2 shares with them a predicted membrane topology of 12 transmembrane segments with intracellular COOH and NH2 termini. Moreover, the extracellular cysteine residue localized between putative transmembrane segments 3 and 4 is conserved. We have previously shown for other gpaATs, *X. laevis* LAT1 and *S. mansoni* SPRM1, that this residue forms the intermolecular disulfide bridge with h4F2hc (37). This disulfide bond formation is per se not required to enable 4F2hc to induce functional gpaAT surface expression in the *Xenopus* expression system. However, it has to be mentioned that expression levels obtained in oocytes with transporters devoid of the bond-forming cysteine residues were lower than with wild-type polypeptides (37). Thus, association of the gpaATs with a heavy chain and the formation of an interchain disulfide link are highly conserved features, which are suggested to play, in vivo, an important role for the control of transporter cell-surface expression.

In this study, we show in *Xenopus* oocytes that LAT2 associates not only with 4F2hc via a disulfide bridge, but also to a lesser extent with rBAT. This 4F2hc-related protein is expressed in the same epithelial cells of the small intestine and

**FIG. 3.** Immunolocalization of LAT2 and 4F2hc in mouse kidney and small intestine. Mice kidneys (panels a, b, d, and e) were sectioned in halves through the papilla and immediately snap-frozen in 2-methylbutane. Small intestine was directly nitrogen-frozen. Immunohistochemistry was performed on cryosections using a 1:200 dilution of a rabbit anti-mouse LAT2 antiserum (panels a–c), revealed with a Cy3-conjugated donkey anti-rabbit IgG. 4F2 antigen was revealed with a rat anti-mouse 4F2hc antibody (RL388) (panels d–e), followed by fluorescein isothiocyanate-conjugated rabbit anti-rat IgG. Double stainings are shown as separate figures. Panels b and e are an 8-fold magnification of panels a and d, respectively. The bars represent 200 μm (panels a and d), 25 μm (panels b and e), and 100 μm (panels c and f).

**FIG. 4.** Specificity and sodium dependence of L-amino acid uptake by h4F2hc and mLAT2 or by h4F2hc and hLAT1. Uptake experiments were performed in a 100 μM concentration of the tested amino acid in the presence or absence of sodium. Background uptake by oocytes injected with h4F2hc alone was subtracted. The means of 12 oocytes ± S.E. pooled from two independent experiments and normalized to L-phenylalanine uptake (+Na) are shown. With L-phenylalanine (+Na), the mean uptakes were 75.0 ± 12.8 pmol/h for mLAT2 and 139.4 ± 14.6 pmol/h for hLAT1. L-AA, L-amino acid.

**FIG. 5.** Concentration dependence of L-amino acid uptake by oocytes coexpressing h4F2hc and mLAT2. Uptake experiments were performed at five different amino acid concentrations. Background uptake by oocytes injected with h4F2hc alone was subtracted. The means of 12 oocytes ± S.E. pooled from two independent experiments are shown, except for L-leucine, where one representative experiment is shown. Sigmoid curves corresponding to Michaelis-Menten kinetics were fitted to the experimental data. The derived apparent $K_m$ values are given under “Results.”
kidney proximal tubule, where it is localized at the apical brush-border membrane (11, 12). The strict basolateral colocalization of LAT2 with 4F2hc (Fig. 3) indicates that the interaction of rBAT and LAT2 observed in oocytes does not reflect the in vivo situation in epithelial cells. Thus, either this interaction is an artifact of the oocyte expression system, possibly favored by overexpression and/or lower incubation temperature (16 °C instead of 37 °C), or it does not lead to stable surface expression of the transporter in vivo. In contrast to this cross-interaction, the recently identified rBAT-associated light chain, b0, AT, which shows an apical localization in epithelial cells, does not associate with 4F2hc in Xenopus oocytes.2 The unexpected interaction of LAT2 with rBAT in oocytes is probably not significant physiologically. It is reminiscent of the “illicit” but functional association of the β-subunit of the apical gastric H/K-ATPase with the Na,K-ATPase α1-subunit that was observed in Xenopus oocytes (38). It will be of interest to study how specific light chains discriminate between rBAT and 4F2hc in epithelial cells and whether the information leading to apical or basolateral expression of the hetero-oligomeric transporters is encoded entirely in the heavy chains or whether the light chains also influence the polarity of surface expression.

**Differential Localization and Function of LAT1 and LAT2**—LAT1 is so far the gpaAT with the highest degree of identity to LAT2, and correspondingly, their functions are related. However, their tissue distribution pattern is very different: whereas LAT1 mRNA expression is broad, LAT2 mRNA is restricted to tissues rich in (re)absorbing epithelia (Fig. 2). Studies on hLAT1 and rLAT1 (originally termed E16 and TA1, respectively) revealed, besides an expression in many differentiated tissues, a strong induction of their mRNAs upon activation of peripheral blood lymphocytes or in hepatoma (34, 39, 40). These observations suggest that LAT1 plays a role in the uptake of amino acids required for growth and proliferation. Correspondingly, 4F2hc has also been shown to be strongly induced at the mRNA and protein levels in cell proliferation (13–15). Furthermore, 4F2hc mRNA accumulation observed in activated lymphocytes results from a post-transcriptional regulation (15). It will now be of interest to test how and to what extent the induction of heavy and light chains is mechanistically similar or different and how it is coordinated.

In contrast to LAT1, LAT2 is apparently restricted to the basolateral membrane of (re)absorbing epithelia (Figs. 2 and 3), where it colocalizes with 4F2hc. It is interesting to note that 4F2hc has a wide expression range that cumulates the characteristics of the expression of LAT1 and LAT2: wide expression in most differentiated tissues and induction in some proliferating cells, as for LAT1, and strong expression in the basolateral membrane of (re)absorbing epithelia, as for LAT2. The differential localization of LAT1 and LAT2 is reminiscent of that of y+LAT1 and y+LAT2 (6).3 In this gpaAT subfamily, y+LAT1 has a predominantly epithelial distribution and thus might compete with LAT2 for association with 4F2hc in epithelial cells of the small intestine and kidney proximal tubule.

Coexpressed with 4F2hc in Xenopus oocytes, LAT2 functionally resembles LAT1, but shows some clear differences. Similar to LAT1, it fits the definition of an γ-type amino acid transporter since it transports large neutral amino acids (Fig. 4), “prefers” l-leucine over l-alanine (higher apparent affinity) (Fig. 5), functions independently of Na+ (Fig. 4), and accepts 2-(-endo)-endoamino-bicycloheptane-2-carboxylic acid (BCH). The apparent affinities for the uptake of several amino acid substrates are, however, quite different between the two LAT proteins. Compared with LAT1, LAT2 shows ~50- and 10-fold higher apparent affinities for the uptake of L-phenylalanine and L-glutamine, respectively, and an ~10-fold lower affinity for histidine. Another striking difference is that LAT2 accepts l-alanine much more efficiently than LAT1. This variability among closely related gpaATs might reflect specific requirements of transport at different membranes.

**Putative Role of LAT2 in Basolateral Amino Acid Extrusion**—The results of the efflux experiment suggest that LAT2, similar to LAT1, functions as an exchanger. LAT2 is expressed at the basolateral membrane of amino acid-transporting epithelia. Thus, its main function is expected to be the extrusion of amino acids from the epithelial cell to the extracellular space, as for y+LAT1, another gpaAT that mediates the efflux of cationic amino acids from the same epithelial cells (see the Introduction). We propose that LAT2 mediates the efflux of a different set of amino acids, presumably neutral ones, that are not (efficiently) transported by a unidirectional export system located in the same membrane. Indeed, a parallel functioning transport system, which translocates amino acids unidirectionally across the basolateral membrane, has to be postulated to explain the net transepithelial transport. We expect that amino acids taken up by LAT2 in exchange for exported ones would be good substrates for this postulated unidirectional export system. Thus, it will be of interest to characterize the transport kinetics of LAT2 for the export of amino acids and to test whether this exchanger shows, in the context of a cell, an asymmetric amino acid exchange function, as previously shown for y+LAT1 (6). The identification of transport system(s) of the basolateral membrane that mediate a unidirectional amino acid export will be an important step toward understanding the interplay of the different transporters involved in transepithelial (re)absorption of amino acids.

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Grégoire Rossier, Christian Meier, Christian Bauch, Vanessa Summa, Bernard Sordat, François Verrey and Lukas C. Kühn

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