J. Membrane Biol. 172, 181–192 (1999)

The Journal of
Membrane
Biology

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Topical Review

New Glycoprotein-Associated Amino Acid Transporters

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Received: 20 July 1999/Revised: 7 September 1999

Abstract. The L-type amino acid transporter LAT1 has recently been identified as being a disulfide-linked "light chain" of the ubiquitously expressed glycoprotein 4F2hc/CD98. Several LAT1-related transporters have been identified, which share the same putative 12-transmembrane segment topology and also associate with the single transmembrane domain 4F2hc protein. They display differing amino acid substrate specificities, transport kinetics and localizations such as, for instance, y^+LAT1 which is localized at the basolateral membrane of transporting epithelia, and the defect of which causes lysinuric protein intolerance. The $b^{0,+}$AT transporter which associates with the 4F2hc-related rBAT protein to form the luminal high-affinity diamino acid transporter defective in cystinuria, belongs to the same family of glycoprotein-associated amino acid transporters (gpaATs). These glycoprotein-associated transporters function as amino acid exchangers. They extend the specificity range of vectorial amino acid transport when located in the same membrane as carriers that unidirectionally transport one of the exchanged substrates. gpaATs belong to a phylogenetic cluster within the amino acid/polyamine/choline (APC) superfamily of transporters. This cluster, which we designate the LAT family (named after its first vertebrate member), includes some members from nematodes, yeast and bacteria. The latter of these proteins presumably lack association with a second subunit. In this review, we focus on the animal members of the LAT cluster that form, together with some of the nematode members, the family of glycoprotein-associated amino acid transporters (gpaAT family).

Key words: Cystinuria — Lysinuric protein intolerance — Proximal kidney tubule — Membrane carrier — Permease

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The LAT Cluster of the APC Family

The amino acid/polyamine/choline (APC) superfamily of transporters is one of the largest families of transporters known in nature with approximately 300 sequenced members currently in the protein databases. Only the ATP-binding cassette (ABC) and major facilitator (MFS) superfamilies have significantly more members identified [38] (http://www-biology.ucsd.edu/~ipaulsen/transport/). The APC superfamily includes members that function as solute:cation symporters (importers) and solute:solute antiporters (exchangers) [25, 28]. These homologous integral membrane transport proteins appear to exhibit uniform topology, generally with twelve transmembrane α-helical spanners (TMDs) in a single polypeptide which varies in size from about 400 amino acid residues to about 800 residues [12, 22–24, 55]. Some of the larger APC family members of animals have been shown to serve as viral receptors [47].

The substrate specificities of some APC superfamily transporters have been carefully studied revealing that while some have exceptionally broad specificity for amino acids, others are restricted to just one or a few amino acids [4, 25, 28, 51]. The APC superfamily is represented in each of the major phylogenetic kingdoms (plants, animals, yeast, bacteria and archaea) (http://www-biology.ucsd.edu/msaier/transport/). A recent

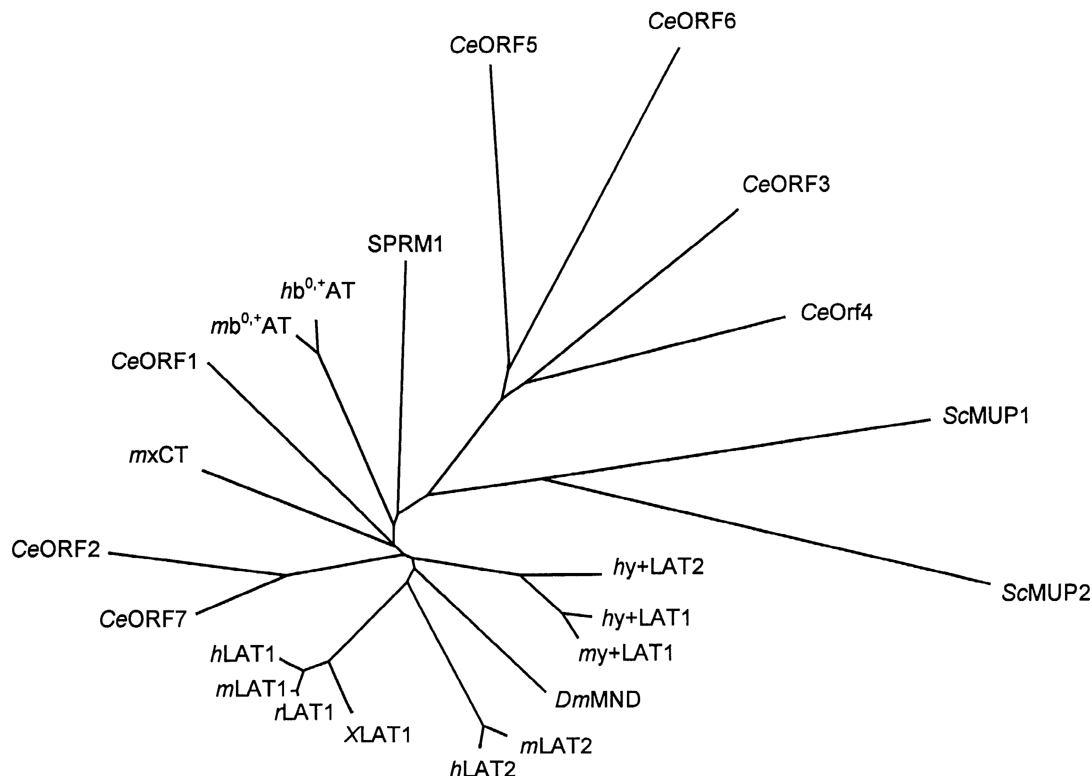

Fig. 1. Phylogenetic tree of the proteins of the LAT family. The protein abbreviations are provided in Table 1 and the species are indicated by the prefix (*italic*). The branch lengths are a measure of the sequence divergence of the proteins and are approximately proportional to phylogenetic distance. The tree displayed was prepared from a complete multiple sequence alignment of the proteins of the LAT cluster using the program TREE (Feng & Doolittle, 1996). The accuracy of this tree was evaluated by bootstrap analysis and confirmed by the preparation of similar trees using the programs ClustalX (Higgins et al., 1996) and Phylip (Felsenstein, 1989).

study has led to the suggestion that this superfamily is related to other currently recognized families of amino acid transporters, but this has yet to be rigorously established [67]. Phylogenetic characterization of the superfamily has revealed that it can be divided into ten distinct families or phylogenetic clusters [D.L. Jack, I.T. Paulsen and M.H. Saier, *unpublished data*].

One of these clusters within the APC superfamily, which we designate the LAT family, consists primarily of amino acid exchangers from animals, which transport neutral, cationic or anionic amino acids (Fig. 1, Table 1). All characterized vertebrate members of the LAT cluster show identity levels higher than 40% with each other and have been shown to require association with an auxiliary type II transmembrane glycoprotein of the 4F2hc family for functional surface expression [2, 16, 31, 37, 40–42, 45, 51, 53, 57–59, 64]. This fact forms the basis for calling them the glycoprotein-associated amino acid transporters (gpaATs). The names used for the newly cloned members of this family were generally based on the nomenclature proposed by Christensen et al., [10] and dependent on functional expression experiments that permitted association of observed transport function with established systems.

Transmembrane Amino Acid Transport Requirements in Animal Cells

Transport of amino acids across cellular membranes is adapted to the needs of each specific cell as well as to local and systemic requirements. For instance, unidirectional active transport of amino acids occurs across (re) absorptive epithelia such as the small intestine and the kidney proximal tubule, and across cell layers separating body compartments such as the vascular endothelium and the choroid plexus epithelium of the blood brain barrier. Such vectorial transcellular transport require active amino acid uptake on one side of the polarized cells and an extrusion mechanism on the contralateral side. Another situation in which a cell requires specific amino acid transport is during growth and proliferation when amino acids are taken up as building blocks for the synthesis of proteins or as energy sources. Specific tasks of specialized cells, such as reuptake of amino acid neurotransmitters, nitric oxide synthesis, nitrogen metabolism and glutathione synthesis, also require the presence of specific active amino acid transporters. To fulfill these numerous tasks, various dissimilar amino acid transporters are required. Functional studies using membrane

vesicles, cells, tissues, organs etc., have allowed the characterization of a large number of transport systems [9, 14, 54]. In the last decade, the availability of molecular biological methods has permitted the identification of a growing number of amino acid transporters at the mRNA level [36]. The functions of the encoded transporters could often be tested outside their complex natural context by expression in heterologous systems such as *Xenopus* oocytes. Of the 20 currently recognized families of amino acid transporters, only four families have had members identified in mammals (APC, AAAP, NSS and DAACS families; Table. 2). Two of the ten families or clusters within the APC superfamily are represented in mammals: the LAT family (2.3.8.) and the cationic amino acid transporter (CAT) family (2.3.3). Members of the CAT family have a COOH-terminal extension approximately 100 amino acids in length which includes two additional putative α-helical transmembrane segments lacking in the LAT family transporters (TMpred server, ISREC). The remainder of the CAT family proteins share approximately 25% sequence identity with the vertebrate LAT family members (gpaATs). The first CAT transporter was originally identified because of its function as a retrovirus receptor. It was subsequently shown to be a y^+-type importer of cationic amino acids (L-arginine, L-lysine) [29, 62]. The reader is referred to other reviews for descriptions of excitatory and inhibitory neurotransmitter amino acid transporter families (DAACS, NSS) [26, 34, 36, 52] as well as the amino acid/auxin transporter family (AAAP) [67].

Glycoprotein-Associated Amino Acid Transporters of the LAT Family

These permeases are approximately 500 amino acids in length with 12 putative transmembrane domains (TMpred sever, ISREC) (*see* model in Fig. 2). Expression of amino acid transport by these gpaATs has been shown to require heterodimerization with a type II glycoprotein (*see below*). Using the first identified members of the family (*X*LAT1 and SPRM1), Pfeiffer et al., demonstrated that the cysteine residue forming the disulfide bond with the glycoprotein is located in the loop between putative TMD3 and TMD4 [41]. This places the NH_2- and COOH-termini on the cytoplasmic side of the membrane as expected from the transmembrane probability plots. Intracellular localization of the COOH-terminus and an even number of TMDs is further supported by the fact that a fusion protein joining the COOH-terminus of $b^{0,+}$AT to the intracellular NH_2-terminus of the type II glycoprotein rBAT is functional [39] (*see below*).

Five vertebrate gpaATs, LAT1, LAT2, y^+LAT1, y^+LAT2 and xCT, have been shown to associate with the same glycoprotein 4F2hc/CD98 [27, 31, 40, 42, 51, 53, 59]. Interestingly, the plathyhelminth transporter SPRM1 was also shown to functionally associate with human 4F2hc, demonstrating a high level of conservation for this interaction [31]. Another gpaAT, $b^{0,+}$AT, has been shown to associate with the 4F2hc-related protein rBAT which is expressed at the luminal side of epithelial cells in both the small intestine and the proximal kidney tubule [39] (Table 1).

Associated Glycoprotein "Heavy Chains"

The associated "heavy chain" glycoproteins were recognized before the discovery of their so called "light chains" which are in fact the catalytic subunits of these heterodimeric or heterooligomeric amino acid transporters. The human surface glycoprotein *h*4F2hc and its murine homologue CD98 had indeed been detected in the early eighties in activated lymphocytes [20] and subsequently shown to be expressed widely [37], with an exclusively basolateral localization in intestine and kidney epithelia [45] [B. Sordat, *personal communication*]. It was known that they associate covalently with so-called light chains that are not glycosylated and migrate on SDS-PAGE as proteins of ~40 kDa [20, 21]. However, serious attempts to obtain sequence information of these light chains following biochemical purification had failed [C. Bron, *personal communication*].

The notion that 4F2/CD98 could be involved in amino acid transport was inferred by conducting amino acid transporter expression cloning experiments with the *Xenopus* oocyte expression system. The 4F2hc-related surface glycoprotein rBAT was cloned because it induced a high level of $b^{0,+}$-type amino acid transport activity (Na^+-independent uptake of dibasic and neutral amino acids, *see below*) [2, 57, 64]. Thus, by analogy, the possibility that amino acid transport is induced by 4F2hc was tested, and a weaker but clear induction of y^+L-type transport (Na^+-independent uptake of cationic amino acids and Na^+- dependent uptake of large neutral amino acids, *see below*) and/or L-type transport (Na^+-independent uptake of large neutral amino acids, *see below*) was described [1, 5, 65]. However, rBAT and 4F2hc are type II membrane glycoproteins related to glycosidases and do not resemble transport proteins. Both have been shown to covalently associate with light chains [21, 63]. Thus, it was hypothesized that they are not themselves transporters but rather "activate" endogenous systems of the oocytes which could be light chains [35].

The rBAT protein (685 amino acid residues) contains approximately 150 residues more than 4F2hc (529 amino acid residues), and these two proteins are about 28% identical. The gaps are distributed over the entire length of the alignment suggesting that they share a common structure with an intracellular NH_2-terminus and an extracellular glycosidase-like domain [64]. The specific

Table 1. The LAT family

Acronym	Name/functional description	Associated protein	Localization	Influx	Efflux	Cotransported ion	Organism	AA #	GI Acc # (Acc #)	Ref # (original name)
LAT1	L-type [1]AAT 1 (Na^+- independent, prefers L over A, accepts BCH, functions as exchanger)	4F2hc/ CD98	-Ovary, placenta, brain > spleen, testis > ubiquitous; -activated lymphocytes; -some tumor cells	[2]L,H,I,F,Y, W,V,(M,Q)	L, other nd	none	*H. sapiens* *M. musculus* *R. norvegicus* *X. laevis*	512	4507051 4519256 3582136 3114983	[31] (E16) [33] [27] (TA1) [31](ASUR4)
LAT2	L-type AAT 2 (higher affinity for small nAA (e.g. A) than LAT1, functions as exchanger (+facilitated diffusion?), mostly at bl membrane of transporting epithelia)	4F2hc/ CD98	-Kidney PT, s. intestine ≫ ovary, placenta, brain	F,Y,W > T,I,C,S,V,L, C,Q,A,H, N,M > (G)	F,I,(L), other nd	none	*H. sapiens* *M. musculus* *R. norvegicus*	534 531	(AF171669) (AF171668) (5545342)	[42] [49] [49] [53]
y^+LAT1	y^+LAT-type AAT 1 (exchanges extracellular nAAs cotransported with Na^+ against intracellular cAAs, mostly at bl membrane of transporting epithelia	4F2hc/ CD98	-S. intestine, kidney ≫ epididyme > many other tissues	Na^+-dep: L,Q,M,H^0, (A,F) Na^+-indep: (R,K,H^+)	R, other nd	Na^+ influx with nAA only	*H. sapiens* *M. musculus*	511 510	4507055 3970725 3970791	[59] [40] [40]
y^+LAT2	y^+LAT-type AAT 2 (transport function similar to y^+LAT1 but in nonepithelial tissues (asymmetry?)	4F2hc/ CD98	Not enriched in epithelial tissues	similar to y^+LAT1	nd	Na^+ influx with nAA only	*H. sapiens*	515	4507053	[40]
$b^{0,+}$AT1	$b^{0,+}$-type AAT (Na^+- independent exchanger of diAAs (high affinity L-cystine transporter) at apical membrane of transporting epithelia	rBAT	S. intestine, kidney PT > lung, placenta, brain, thymus > many other tissues	C-C,R,K, > L,Y,A	[3]L,R,A other nd	none	*H. sapiens* *M. musculus*	487	(5823977) (5824164)	[39] [39]
xCT	Cystine/Glutamate exchange transporter corresponding to system x_c^-, mainly Na^+-independent	4F2hc/ CD98	Activated macrophages, cultured cells, brain	C-C > E	E	none	*M. musculus* *H. sapiens*	502	4689081 5668544	[51] [51]
SPRM1	Schistosoma AA permease 1 (transport characteristics between L and y^+L-type)	4F2hc/CD98 (in *Xenopus* oocytes)		Na^+-indep: R,K partial Na^+-dep.: L,F,H,Q,M	nd	(Na^+ influx with nAA only)	*S. mansoni*	502	407047	[31]
ORF1 to ORF7	Caenorhabditis elegans reading frames deduced from genomic sequences, function not characterized. Some lacking C*	?		nd	nd	?	*C. elegans*	440 –662	3876345, 1226279 2435610, 2088757 1293821, 3879689 2746835	

Continued on next page

Table 1. *Continued*

Acronym	Name/functional description	Associated protein	Localization	Influx	Efflux	Cotransported ion	Organism	AA #	GI Acc # (Acc #)	Ref # (original name)
MUP1	Yeast high affinity methionine permease	(no C*)		M	nd	?	*S. cerevisiae*	574	1709181	[25]
MUP3	Yeast low affinity methionine permease	(no C*)		(M)	nd	?	*S. cerevisiae*	546	731613	[25] (Yhd6)

[1] *Abbreviations:* C* stands for the L-cysteine residue situated in the second putative extracellular loop which is involved in intermolecular disulfide bond formation of gpaATs. The single letter amino acid code is used; C-C stands for L-cystine; H^+ is the protonated and H^0 the zwitterionic form of L-histidine. AA = amino acid, AAT = amino acid transporter, nAA = neutral-, cAA = cationic-, diAA = diamino acid, bl = basolateral, n/a = not available, nd = not determined.

[2] The amino acids are indicated in an order corresponding approximately to that of their apparent affinity, where known. Amino acids in brackets indicate low apparent affinity.

[3] [R. Pfeiffer and F. Verrey, *unpublished results*].

role of the extracellular glycosidase-like moiety remains elusive, and no catalytic activity has yet been attributed to it. An alternative topological model has been proposed for rBAT but has not been confirmed [32]. Both rBAT and 4F2hc are glycoproteins and thus migrate on SDS-PAGE with apparent molecular weights higher than expected from their primary sequences. This contrasts with the light chains which have calculated molecular weights similar to 4F2hc, but migrate much faster on SDS-PAGE due to their hydrophobic nature and the lack of glycosylation (apparent MW ~40 kD *vs.* ~90 kD for the fully glycosylated 4F2hc). Both heavy chains have an extracellular cysteine residue near the transmembrane domain. In the case of 4F2hc, it was shown that this is the residue which makes the intermolecular link with the light chain [16, 41].

4F2hc is known to reach the plasma membrane in the absence of light chain as has been shown in L-cells and *Xenopus* oocytes [31, 58]. Using the SPRM1 light chain, Mastroberardino et al., demonstrated in oocytes that this light chain, in contrast to 4F2hc, remained intracellular when expressed alone: only when co-expressed with 4F2hc did it reach the plasma membrane [31]. Since co-expressed SPRM1 devoid of the cysteine residue forming the intermolecular link was also expressed at the surface [41], cell surface expression was independent of disulfide bond formation.

The heterodimeric structure and the requirement of 4F2hc for surface expression are reminiscent of the situation for the Na,K-ATPase. This ion-exchange pump is composed of a catalytic multitransmembrane subunit (α-subunit) and a type II glycoprotein (β-subunit). The β-subunit is required for stabilization, functional maturation and trafficking of the α-subunit from the endoplasmic reticulum to the plasma membrane [19]. We hypothesize that the 4F2hc type II glycoprotein serves a similar function. It is not yet clear whether rBAT plays the same role. One important difference in terms of cell surface expression between 4F2hc and rBAT is their differential localization at the basolateral and apical membrane of amino acid transporting epithelia, respectively [18, 45] [B. Sordat, *personal communication*]. It is an open question as to whether the heavy chain has an impact on the amino acid transport specificity and/or kinetics of the heterooligomer transporters or whether it only fulfills a “chaperone” function.

The L-type Transporters (LAT)

The transport of large neutral amino acids, in particular those with branched and aromatic side chains, has been attributed to system L [9]. Even before the various L-type transporters were cloned, it was apparent that “system L” was not a single transporter. For example, depending on the tissue tested, differences in substrate

Table 2. Mammalian families of amino acid transporters

Family name 1	Family name 2	Evolutionary family (http://www-biology.ucsd.edu/~msaier/transport/)	Transporter acronyms	Structure	Amino acid uptake	Amino acid efflux	Cotransported or exchanged ions	Induced conductance	Associated subunit
Na^+-dependent transporters of anionic and zwitterionic AAs	Excitatory amino acid transporters, glutamate transporters	2.23 (DAACS) Dicarboxylate/amino acid:cation (Na^+ or H^+) symporter family	EAAT1 (GLAST) EAAT2 (GLT1) EAAT3 (EAAC1) EAAT4, EAAT5 ASCT1, ASCT2 (ATB^0)	~550 aa 10 put. TMD	E,D Small neutral AAs	—	influx of Na^+ (and/or H^+) efflux of K^+	Cl^- (molecularly and biophysically not clear)	
Na^+ and Cl^--dependent AA transporters within family of neurotransmitter transporters	Neurotransmitter transporters	2.22 (NSS) Neurotransmitter: sodium symporter family	GAT-1-GAT-3, BGT-1, TAUT, GLYT1-GLYT2, PROT (+transporters of biogenic amines)	~590–800 aa 12 put. TMD	GABA, betaine, GABA taurine G P (NA, DA, serotonine)	—	influx of Na^+ and Cl^-	—	
Cationic AA transporters	CAT family	2.3.3 (CAT) family within 2.3 (APC) Amino acid-polyamine-choline superfamily	CAT1-CAT4	~610–660 aa 14 put. TMD	R,K	—	—	—	
Glycoprotein-associated AA transporters (gpaATs)	gpaAT family (within LAT family)	2.3.8 (LAT) family within 2.3 (APC) amino acid-polyamine-choline superfamily	LAT1, LAT2 y^+LAT1, y^+LAT2 xCT $b^{0,+}$AT	~480–550 aa 12 put. TMD	Zwitterionic and/or cationic or anionic AA (*see* Table 1)	Similar to influx	No or cotransport of Na^+ with zwitterionic AA (y^+LAT)	—	4F2hc/CD98 (S-S) 4F2hc/CD98 (S-S) 4F2hc/CD98 (S-S) rBAT/NBAT/D2 (S-S)
—	—	—2.18 (AAAP) The amino acid/auxin permease family	Vesicular GABA transporter	525 aa 10 put. TMD	GABA	—	H^+ efflux	—	

[1] *Abbreviations:* The single letter amino acid code is used. AA = amino acid; S-S = intermolecular disulfide bridge; put. TMD = putative transmembrane domain.

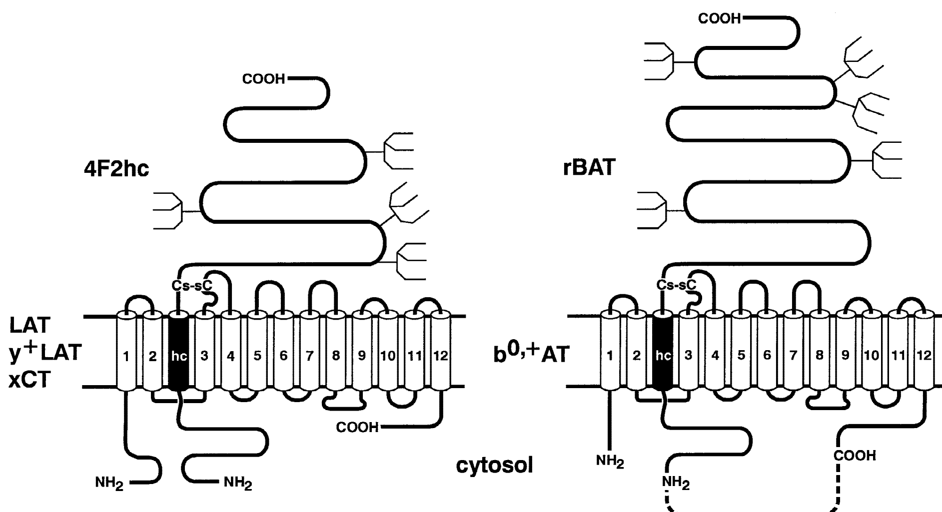

Fig. 2. Schematic representation of the heterodimers formed by the heavy chains 4F2hc and rBAT with their respective light chains. The single evident transmembrane domain of the heavy chains is labeled hc and the 12 putative transmembrane domains of the permease light chains are numbered 1–12. The dotted line between the COOH-terminus of $b^{0,+}$AT and the NH_2-terminus of rBAT represents the linker sequence introduced to form a functional fusion protein. The localization of the conserved Cys residues forming the intermolecular disulfide bond (Cs-sC) and the potential N-gylcosylation sites (forks) are indicated.

specificity and transport kinetics had been noted [9, 56 and references in 53].

Two members of the gpaAT family that associate with 4F2hc have been identified as L-type transporters [27, 31, 42, 49, 53] (*see* Table 1). Interestingly, they have a complementary tissue distribution, one (LAT1) being expressed quite widely, in particular in testis, ovary, placenta and tumor cells, and the other (LAT2) being expressed at the basolateral membrane of transporting epithelia [27, 33, 49, 66].

Xenopus and human LAT1 (ASUR4 and E16) as well as *Schistosoma mansoni* SPRM1 were first presumed to be amino acid transporters based on the similarity of their sequences with yeast and prokaryotic amino acid transporters. However, they alone did not induce amino acid transport activity when expressed in *Xenopus* oocytes. The possibility that they were light chains of 4F2hc was therefore tested and this led to their identification as such by co-immunoprecipitation and functional analysis [31]. Rat LAT1 was independently identified by expression cloning in *Xenopus* oocytes using a complementation strategy [27]. Subsequently, the identification of orthologues by different approaches, including purification followed by microsequencing or antibody production, was reported [30, 33, 44, 61].

LAT1 appears to function as an obligatory exchanger of amino acids since efflux of L-leucine from oocytes expressing LAT1 and 4F2hc strictly depends on the presence of an extracellular substrate amino acid [31]. Thus, if the exchange stoichiometry proves to be 1:1, the function of the broad specificity LAT1 exchanger could be viewed as equilibration of the various substrate amino acids. However, in the presence of a vectorial uptake mechanism functioning in parallel, such an exchanger would function secondarily as an active importer for amino acids not transported otherwise. For instance, the driving force could be supplied by a secondary active Na^+/neutral amino acid co-transporter importing amino acids which could then be used by LAT1 as substrates for exchange against other extracellular amino acids. Such Na^+-dependent transporters are for instance system ASC, for which ASCT1 and ASCT2 cDNAs have been cloned (DAACS family, *see* Table 2) and system A, the molecular nature of which is not yet known [9]. It will be of considerable interest to study the substrate-specificity overlap and thus the probable cooperation of these transporters with LAT1 for amino acid uptake.

LAT2 was identified in EST sequence databases because of its similarity to LAT1 [42, 49, 53]. Interestingly, it was shown to be expressed only in tissues containing amino acid transporting cell layers, especially in the small intestine and kidney where it colocalizes with 4F2hc at the basolateral membrane of epithelial cells [49]. Thus, LAT2 is expected to play a role in transepithelial amino acid transport, in particular in the basolateral extrusion step. Sequence comparisons show that it has a higher degree of identity with LAT1 (52%) than with the other members of the gpaAT family (see also tree in Fig. 1), and when expressed in *Xenopus* oocytes, it shows a pattern of amino acid uptake which resembles that of LAT1 and thus justifying the name LAT2. Indeed, it mediates the uptake of large neutral amino acids, prefers L-leucine to L-alanine, and transports the

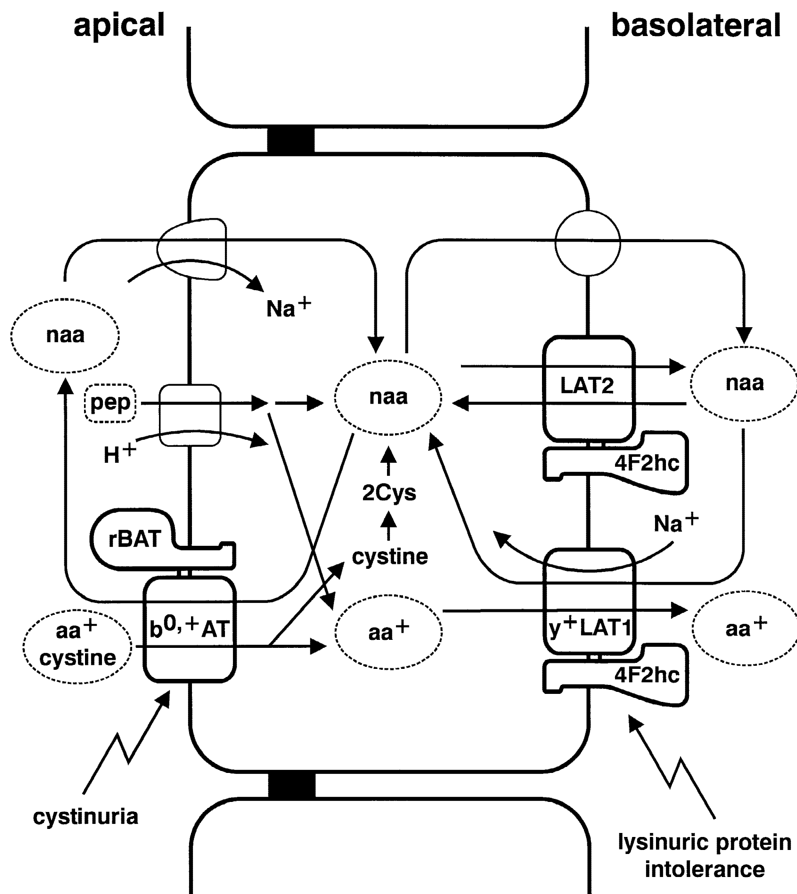

Fig. 3. Model for the transcellular transport of cationic and neutral amino acids. On the apical side of the cell, vectorial influx of neutral amino acids (naa) is mediated by a B^0-type Na^+/amino acid co-transporter which is not yet molecularly defined [36, 54]. Small peptides entering the cell via the peptide/H^+ cotransporter [17], after their hydrolysis, contribute to the intracellular amino acid pool. Dibasic amino acids enter the cell via the $b^{0,+}$AT complex. Mutations in either of the subunits ($b^{0,+}$AT or rBAT) of this transporter lead to cystinuria [7, 13]. For the basolateral amino acid extrusion step, we postulate the presence of an exporter. A broad specificity range that includes cationic amino acids is provided by at least two amino acid exchangers of the gpaAT family which are associated with 4F2hc. Mutations in y^+LAT1 lead to the severe recessive genetic disease lysinuric protein intolerance [3, 60].

typical substrate of L-type transporters 2-(–)-endoamino-bicycloheptane-2-carboxylic acid (BCH). However, there are some significant differences in the relative affinities for different substrates, for instance a higher affinity for L-phenylalanine. It also imports L-alanine at physiological concentrations. The question of whether LAT2 mediates an obligatory amino acid exchange, or whether it might also function in unidirectional facilitated diffusion, is not resolved. Rossier et al., and Pineda et al., described a highly trans-stimulated efflux of L-phenylanine and L-isoleucine, respectively [42, 49] and L-leucine did not appear to be transported efficiently outward [C. Meier and F. Verrey, *unpublished results*]. In contrast, Segawa et al., described an efflux of L-leucine via LAT2 that could occur by facilitated diffusion without exchange [53]. If LAT2 turns out to function under physiological conditions as an exchanger, this would mean that, analogous to the situation for amino acid import by LAT1, net basolateral export of zwitterionic (and cationic) amino acids from epithelial cells would require, in addition to LAT2 (and y^+LAT1, *see below*), a unidirectional amino acid exporter with overlapping specificity. A model for transepithelial transport of neutral and cationic amino acids is shown in Fig. 3.

The y^+L-type Transporters (y^+LAT)

Another subgroup of 4F2hc-associated amino acid transporters is defined by both a higher pairwise degree of identity than with the other gpaAT family members (*see* tree, Fig. 1) and by common y^+L-type amino acid transport properties. y^+L-type transport of amino acids was first described by Déves et al., [15] in human erythrocytes as a broad scope exchange system which, in the absence of Na^+, mediates the exchange of cationic amino acids and, in the presence of Na^+, mediates the exchange of intracellular cationic amino acids for extracellular neutral amino acids.

y^+LAT1 was identified on the basis of its identity with LAT1 [40, 59] and was shown like LAT2, to be highly expressed in small intestine and proximal kidney tubules. Its basolateral localization has not been formally demonstrated with antibodies but is clear (G. Rossier et al., *unpublished data*), also because of its association with the basolaterally restricted 4F2hc and the pathophysiological findings observed in patients in which this transporter is mutated [46]. Functional experiments performed with oocytes coexpressing y^+LAT1 and 4F2hc have shown that these two chains form a

disulfide-linked heterodimer and that this transporter imports neutral amino acids plus Na^+ with a higher affinity than cationic amino acids. L-leucine was not exported at all, and cationic amino acid export was dependent on the presence of an external substrate amino acid (e.g. L-leucine plus Na^+) [40]. Thus, the localization and transport pattern observed for y^+LAT1 rendered it a highly probable candidate for the autosomal recessive disease lysinuric protein intolerance. Linkage analysis and the finding of mutations within the corresponding gene (*SLC7A7*) confirmed that y^+LAT1 was the defective transporter in lysinuric protein intolerance [3, 60].

The second member of this subgroup, human y^+LAT2, was originally cloned in the context of a genome sequencing project (product of *KIAA0245* gene). It is 72% identical with y^+LAT1 [40] and is not highly expressed in tissues with transporting epithelia [B. Spindler and F. Verrey, *unpublished results*]. The amino acid specificity profile of its uptake activity, expressed in *Xenopus* oocytes, is similar to that of y^+LAT1 [R. Pfeiffer et al., *unpublished results*].

The Cystine-Glutamate Exchanger xCT

The Transport System x_c^- consists of an obligatory exchange (molar ratio 1:1) of extracellular L-cystine in its anionic form for intracellular L-glutamate and is expressed in some cultured mammalian cells as well as activated macrophages. Sato et al., succeeded in identifying both subunits of this transporter by expresssion cloning in *Xenopus* oocytes [51]. One subunit proved to be 4F2hc and the other a new member of the gpaAT family which they named xCT. This highly inducible transporter may play a role in the regulation of the immune response by controlling extracellular L-cystine/L-cysteine and intracellular glutathione levels [50].

The rBAT-Associated Transporter $b^{0,+}$AT

The apical influx of L-cystine and cationic amino acids in epithelial cells of the kidney proximal tubule and the small intestine has been known for several years to be mediated by a transporter which includes the 4F2hc-related glycoprotein rBAT [2, 57, 64]. This high affinity, Na^+-independent transport ($b^{0,+}$-type) was known to be defective in patients with the genetic disease cystinuria, a disease in which cystine and other dibasic amino acids fail to be reabsorbed from the tubular fluid (OMIM #220100) [48]. Type I cystinuria, the form of cystinuria which is fully recessive, was often shown to be mediated by mutations in the gene encoding rBAT [7]. As mentioned above, rBAT was originally cloned because it enhanced $b^{0,+}$-type transport when expressed alone in *Xenopus* oocytes where it was shown to associate with an endogenous protein, forming a ~130 kD disulfide-linked heterooligomer. Using this system (exogenous rBAT + endogeous $b^{0,+}$AT), several studies have demonstrated the complex nature of the amino acid exchange mediated by this transporter [6, 8, 11]. The electrogenic uptake of cationic amino acids in exchange with neutral amino acids is stimulated by the membrane potential and the uptake of L-cystine is favored by the high concentration gradient maintained by the intracellular reduction of L-cystine to L-cysteine.

We have identified a new gpaAT family member ($b^{0,+}$AT) in mouse and human EST databases which is highly expressed in small intestine and proximal kidney tubule and specifically associates with rBAT (not 4F2hc) when expressed in *Xenopus* oocytes. Immunofluorescence experiments showed that it is expressed in the apical brush border membrane of the mouse proximal kidney tubule and thus, that it is coexpressed with rBAT in segment 3 where high affinity L-cystine and cationic amino acid transport is thought to take place [39]. In constrast to rBAT, it shows a high level of expression also in the brush border membrane of more proximal segments. Thus, the function of $b^{0,+}$AT in these S1 and S2 segments remains to be investigated.

We have established that both subunits are required for the expression of high affinity L-cystine transport by coexpression experiments in epithelial cell lines. To circumvent the problem of the presence of an endogenous $b^{0,+}$AT transporter in *Xenopus* oocytes, we have constructed a fusion protein in which a linker sequence is inserted between the COOH-terminus of *m*$b^{0,+}$AT and the NH_2-terminus of *h*rBAT. The function of this fusion protein was shown to be independent of the endogenous oocyte $b^{0,+}$AT chain, and thus, kinetic properties of this defined heterooligomer could be measured. As expected, the apparent affinities for L-cystine and L-arginine were high (app. $K_m < 100$ μM) whereas that for L-leucine was lower (app. $K_m \sim 1$ mM). The function of the rBAT-$b^{0,+}$AT transporter in the context of an epithelial cell is indicated in the model shown in Fig. 3.

The cystinuria consortium has analyzed the gene encoding $b^{0,+}$AT (*SLC7A9*) of many nontype I cystinuria patients and found mutations, thereby genetically confirming the role of $b^{0,+}$AT for apical cystine and cationic amino acid transport [13]. Interestingly, in contrast to type I cystinuria, nontype I cystinuria is not fully recessive since heterozygotes demonstrate slight amino aciduria [43, 48]. It will be interesting to investigate why mutations in $b^{0,+}$AT have a more profound functional impact on kidney tubular dibasic amino acid reabsorption than mutations of its rBAT glycoprotein subunit.

Conclusions and Perspectives

The identification and characterization of several glycoprotein-associated amino acid transporters of the LAT

family has shed light on the nature of these proteins, the functions of which are often to increase the range of amino acids transported across membranes. Besides an overall conserved structure and the need for association with a glycoprotein for functional expression, they share an exchanger mode of transport. This point has not been demonstrated formally for all gpaATs, and a stoichiometry of 1:1 has been clearly determined only in the case of xCT. The question of the stoichiometry is important since net overall amino acid import or export is not possible if this value is 1:1. In such a case, net transport of one amino acid is always compensated by the net opposite transport of another amino acid. We favor the hypothesis, that in most cases, there are parallel functioning unidirectional transporters which may sometimes function in a secondary active mode, for instance as Na^+-cotransporters (i.e., system A and ASC). These unidirectional transporters, which control net vectorial transmembrane transport of amino acids thus need to be more tightly regulated than tightly coupled exchangers which function in equilibration and extend the range of amino acids transported. Exchangers, such as the gpaATs of the LAT family, could only control net amino acid flux across a membrane, if their exchange stoichiometries were different from 1:1 and/or could be modulated. It is interesting to note that different members of this exchanger family are able to accomodate all types of amino acids including zwitterionic amino acids cotransported with Na^+, zwitterionic amino acids transported without a requirement for Na^+, cationic and neutral diamino acids, and anionic dicarboxylic amino acids. Most of these transporters accommodate a broad range of amino acids.

The future discovery of more members of the gpaAT family and of the other missing amino acid transporters will allow us to address the question of their cooperation in the control of the different amino acid fluxes which take place locally, between different organs and body compartments, for instance across the blood brain barrier, and across epithelial barriers which face the lumen of the intestine and the kidney tubule.

The authors thank Christian Gasser for the artwork and Christian Bauch for reading the manuscript. The laboratory of F.V. is supported by Swiss National Science Foundation grant 31-49727.96.

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