Heteromeric amino acid transporters: biochemistry, genetics, and physiology

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Chillarón, Josep, Ramón Roca, Alfonso Valencia, Antonio Zorzano, and Manuel Palacín. Heteromeric amino acid transporters: biochemistry, genetics, and physiology. Am J Physiol Renal Physiol 281: F995–F1018, 2001.—The heteromeric amino acid transporters (HATs) are composed of two polypeptides: a heavy subunit (HSHAT) and a light subunit (LSHAT) linked by a disulfide bridge. HSHATs are N-glycosylated type II membrane glycoproteins, whereas LSHATs are nonglycosylated polytopic membrane proteins. The HSHATs have been known since 1992, and the LSHATs have been described in the last three years. HATs represent several of the classic mammalian amino acid transport systems (e.g., L isoforms, yL L isoforms, asc, xc, and b0,+). Members of the HAT family are the molecular bases of inherited primary amino-acidurias cystinuria and lysinuric protein intolerance. In addition to the role in amino acid transport, one HSHAT (the heavy subunit of the cell-surface antigen 4F2 (also named CD98)) is involved in other cell functions that might be related to integrin activation. This review covers the biochemistry, human genetics, and cell physiology of HATs, including the multifunctional character of CD98.

light and heavy subunits; cystinuria; lysinuric protein intolerance; CD98 (4F2) complex and integrins; rBAT

SIX FAMILIES OF AMINO ACID transporters for the cell plasma membrane have been described in mammals (reviewed in Refs. 85, 121, 123), one of which has a heteromeric structure. These heteromeric amino acid transporters (HATs) are composed of a heavy subunit (rBAT or 4F2hc) and the corresponding light subunit, linked by a disulfide bridge (Table 1 and Fig. 1). These transporters were identified after the cloning of rBAT (also named NBAT and D2) from kidney by their functional expression in oocytes (9, 171, 186). Amino acid transport in oocytes was also induced by the expression of the rBAT-homologous protein, the heavy chain of the surface antigen 4F2 (4F2hc; 4F2 is also referred to as CD98) (8, 187). These two heavy subunits of HAT (HSHATs) are type II membrane glycoproteins with a single transmembrane (TM) domain, an intracellular NH2 terminus, and an extracellular domain that shows significant homology with bacterial α-glucosidases. rBAT is mainly expressed in the epithelial cells of the kidney proximal tubule and of the small intestine, where it is located in the brush border. In contrast, 4F2hc is ubiquitous, with a basolateral location in epithelial cells. The first studies of rBAT and 4F2hc have been reviewed extensively (121).

Studies of covalent inactivation by mercural agents demonstrated that 4F2hc needs an accompanying subunit(s) to express transport activity (47; reviewed in Ref. 122). The first light subunits of HAT (LSHATs) were identified in 1998 (LAT-1, yL LAT-1, and yL LAT-2) (79, 101, 128, 133, 175). Since then, four more mammalian LSHAT members have been cloned xcT, LAT-2, asc-1 and b0,+AT (BAT1 in Ref. 27) (7, 18, 27, 52, 47a, 112, 127, 131, 137, 138, 141, 149, 150, 154). The LSHAT
members have recently been reviewed (40, 118, 178, 179). Their general characteristics are the following. First, LSHATs are not N-glycosylated and are highly hydrophobic, with 12 putative TMs (Fig. 1). The lack of N-glycosylation of LAT-1 after translation in vitro has been demonstrated (79). The highly hydrophobic character of LSHATs (molecular mass; 50 kDa) results in an anomalous mobility in SDS-PAGE, compatible with an apparent molecular mass of 35–40 kDa. Second, LSHATs are linked to the corresponding HSHAT by a disulfide bridge. For this reason, HATs are also named glycoprotein-associated amino acid transporters (gpaATs) (178). The intervening cysteine residues are located in the putative extracellular loop II of LSHATs and a few residues toward the COOH terminus from the single putative TM of HSHATs (Fig. 1). Evidence for the formation of these heterodimers (125 kDa) in heterologous expression systems has been obtained for 4F2hc (85 kDa) with LAT-1, 4F2hc (CD98hc) | y⁺LAT-1 | SLC3A2 | y⁺L | 14q11.2 | LPI | y⁺LAT-2 | SLC7A7 | y⁺L | 16q22.1 | LAT-1 | SLC7A5 | L | 16q24.3 | LAT-2 | SLC7A8 | L | 14q11.2 | asc-1 | SLC7A10 | asc | 19q12-13 | LSHATs | SLC3A1 | b⁰⁺AT | 2p16.3-21 | Type I cystinuria | LSHATs | SLC7A9 | 19q12-13 | Non-type I cystinuria

Light subunits of heteromeric amino acid transporters (LSHATs) with the corresponding heavy subunit of heteromeric amino acid transporters (HSHATs) form heteromeric functional amino acid transporters at the plasma membrane. Description of the tissue distribution of LSHATs in mammalian species has been reviewed elsewhere (40, 178, 179). Eleven HSHATs have been identified in vertebrates, 6 for rBAT (human (L11696), rat (M80804), mouse (NM_009205), rabbit (M90086), dog (AF187966), and a partial sequence for the American opossum (X95475)); and 5 for 4F2hc [human (J02939), rat (X89225), mouse (X14309), Chinese hamster (U93712), and zebrafish (AF295374)]. Twenty-two LSHATs with known amino acid transport functions have been reported in vertebrates, 5 for LAT-1 [human (AF077866), rat (AB015432), mouse (AB17189), Bos taurus (AF174615), and Xenopus laevis (Y12716)]; 4 for LAT-2 [human (AF171669), rat (AB024400), mouse (Y19022), rabbit (AF170106), and American opossum (Fernández E, Zorzano A, and Palacín M, unpublished observations)]; 2 for asc-1 [human (NM_019849) and mouse (D87432)]; 3 for y⁺LAT-1 [human (AF092032), rat (AF200684), and mouse (AJ012754)]; 1 for y⁺LAT-2 [human (D87432)]; 2 for xCT [human (AB028891) and mouse (AB023455)]; and 4 for b⁰⁺AT [human (AF141289), rat (AB029559), mouse (NM_021291), and rabbit (AF155119)]. There is finally another LSHAT from Schistosoma mansoni (SPRM1 (L25068)), whose associated amino acid transport function is characteristic of this platelhelmin (161). Only one GenBank accession no. is given for each protein (in parentheses).
LAT-2, or y\textsuperscript{+}LAT-1 (101, 110, 128, 129, 141, 175) and for rBAT (−94 kDa) with b\textsuperscript{0,+}AT (Fernández E, Chillarón J, and Palacín M, unpublished observations), and by coimmunoprecipitation of LAT-1 and 4F2hc (100) and b\textsuperscript{0,+}AT and rBAT (Fernández E, Chillarón J, and Palacín M, unpublished observations) from naturally occurring tissues. Third, LSHAT members need coexpression with the corresponding HSHAT to reach the plasma membrane in heterologous expression systems [LAT-1, LAT-2, asc-1, y\textsuperscript{−}LAT-1, and xCT with 4F2hc (7, 101, 109, 110, 129, 131), and b\textsuperscript{0,+}AT with rBAT (47a)]. Fourth, LSHATs confer specific amino acid transport activity to the heteromeric complex (Table 1). Coexpression of 4F2hc with LAT-1 induces a variant of system L (sodium-dependent transport of neutral amino acids with a large side chain) (79, 101), with LAT-2 induces another variant of system L for neutral amino acids of any size (131, 141, 154), with asc-1 induces system asc (sodium-independent transport for neutral amino acids of small side chain) (52, 112), with xCT induces system x\textsuperscript{−} (sodium-independent transport for cystine and anionic amino acids) (7, 149, 150), and with y\textsuperscript{−}LAT-1 or y\textsuperscript{−}LAT-2 induces system y\textsuperscript{−}L (sodium-independent transport of dibasic amino acids and sodium-dependent transport for neutral amino acids) (78, 128, 175). In contrast to the above-mentioned LSHAT members, b\textsuperscript{0,+}AT induces with rBAT, but not with 4F2hc, system b\textsuperscript{0,+} (sodium-independent transport for dibasic amino acids and neutral amino acids, including cystine) (27, 47a, 104, 127). Fifth, all the amino acid transport activities associated with the LSHATs behave like amino acid exchangers: systems induced by LAT-1, LAT-2, y\textsuperscript{−}LAT-1, y\textsuperscript{−}LAT-2, and xCT, and system b\textsuperscript{0,+} (reviewed in Ref. 12) show a tight coupling exchange of substrates (7, 20, 101, 104, 128, 131, 149, 154), whereas asc-1 might have some unidirectional flux of substrates in addition to the exchange transport (52).

**THE MECHANISM OF EXCHANGE OF HATs**

The amino acid exchange activity of HATs was evidenced before the identification of LSHATs (reviewed in Ref. 124). The seminal observations were described simultaneously and independently by two groups (21, 34), who reported outward positive currents associated with the heteroexchange of neutral (efflux) and dibasic amino acids (influx) via system b\textsuperscript{0,+} in whole or cut-open oocytes expressing rBAT. Further studies demonstrated that system b\textsuperscript{0,+}/rBAT acts as a tertiary active transporter, mediating the electrogenic exchange of dibasic amino acids (influx) for neutral amino acids (efflux) with a stoichiometry of 1:1 (30). This exchange has also been demonstrated in the apical plasma membrane of the proximal tubular cell model of opossum kidney (OK) cells in rBAT-antisense experiments (105) and in chicken brush-border jejunum (174).

System y\textsuperscript{−}L induced by 4F2hc in oocytes also behaves as an electroneutral and asymmetric amino acid exchanger (30): it mediates the efflux of dibasic amino acids and the influx of neutral amino acids plus sodium. The transport of sodium via 4F2hc/y\textsuperscript{−}LAT-1-induced system y\textsuperscript{−}L has recently been demonstrated in oocytes (78). Similarly, the amino acid transport activity x\textsuperscript{−} exchanges the ionic form of cystine for glutamate with a 1:1 stoichiometry (6). This mechanism of exchange has also been demonstrated in oocytes coexpressing 4F2hc and xCT (7, 149). As mentioned above, the exchange of substrates has been demonstrated for all the LSHATs cloned when coexpressed with the corresponding HSHAT.

A functional model for rBAT-induced system b\textsuperscript{0,+} exchange activity was proposed by Coady et al. (33). These authors observed in rabbit rBAT-expressing, cut-open oocytes that aminoisobutyric acid (AIB) induced amino acid currents across system b\textsuperscript{0,+} without being transported itself, thus suggesting variable stoichiometry of exchange. To explain these results, a “double-gated” pore model with a binding site accessible at each side of the membrane was proposed.

Very recently, Torras-Llort et al. (174) studied system b\textsuperscript{0,+} in chicken brush-border jejunum. In these vesicles, accessibility to both sides of the plasma membrane allowed kinetic and simulation analysis of the system b\textsuperscript{0,+} amino acid exchanger. The results were compatible with a sequential mechanism, which implies the formation of a ternary complex (i.e., the transporter bound to a substrate at each side of the membrane). In contrast, the results ruled out a “ping-pong” mechanism (i.e., binding of a substrate on one side of the membrane and then translocation and release of the substrate on the other). The study did not distinguish between ordered and random binding of substrates to the transporter in the sequential mechanism, but the estimated dissociation constants for extracellular (extravesicular or external) or intracellular (intravesicular or internal) substrates suggest that the binding affinity for the extracellular amino acid is higher than for the intracellular substrate. An ordered mechanism, in which the free transporter binds first to the external amino acid and then to the internal one, may account for these results. However, because the binding affinity for the internal amino acid is high (in the micromolar range), the results could also be explained by a random mechanism with a preferential route (i.e., preference for the binding of the external amino acid first). Such preferential behavior might be due to the negative membrane potential, which would favor the binding of cationic amino acids to the transporter from the external side rather than from the internal one. The “double-gated” pore model was not supported in the chicken brush-border jejunum studies because interaction of AIB with system b\textsuperscript{0,+} was not substantiated (174).

The results in chicken brush-border jejunum are compatible with a double-exchange pathway with alternating access (Fig. 2). A similar model was proposed by Dierks et al. (42) for the mitochondrial aspartate/glutamate antiporter, which includes two functional “subunits” or pathways with binding sites alternating at each membrane domain in which the translocation step is under membrane potential control. The functional oligomeric structure of rBAT/b\textsuperscript{0,+} AT system b\textsuperscript{0,+} is unknown, as it is for the other HATs. Interestingly,
Western blot analysis revealed a high-molecular-mass complex (∼250 kDa), in addition to the ∼125-kDa heterodimer, for rBAT (120, 181) and for b0,+AT (Fernández E, Chillarón J, and Palacín M, unpublished observations) in kidney brush-border preparations under nonreducing conditions. This suggests that system b0,+ might be a heterotetramer comprising two heterodimers of rBAT and b0,+AT linked by disulfide bridges. In this scenario, each heterodimer would represent a single pathway of transport with alternating accessibility, and the two heterodimers together would represent the double-exchange pathway. Structural and functional (i.e., with dominant negative mutants) studies to define the functional structural unit of the heteromeric amino acid transporters are presently in progress.

**INHERITED AMINOACIDURIAS**

The transport characteristics of two of the LSHAT-associated transport systems are relevant to inherited aminoacidurias cystinuria and lysinuric protein intolerance (LPI; see Transepithelial transport of amino acids). First, system b0,+ (induced by rBAT and b0,+AT) acts as a tertiary active mechanism of renal reabsorption and intestinal absorption of dibasic amino acids and cystine; it mediates the electronegative exchange of dibasic amino acids (influx) for neutral amino acids (efflux). Second, system y+L (induced by 4F2hc and y+LAT-1) mediates the electroneutral exchange of dibasic amino acids (lysine, arginine, and ornithine; efflux) for neutral amino acids plus sodium (30, 40, 78). It is assumed that this transport system allows the efflux of dibasic amino acids against the membrane potential in many cell types, particularly in the basolateral domain of epithelial cells. The role of rBAT, b0,+AT, and y+LAT-1 in cystinuria and LPI has recently been reviewed (119).

**Cystinuria**

Cystinuria (MIM 220100) is an autosomal-recessive disorder, with an average prevalence of 1 in 7,000 births (153). The disease is caused by the defective transport of cystine and dibasic amino acids across the apical membranes of proximal renal tubular and jejunal epithelial cells. Because of the low solubility of cystine, it precipitates to form kidney calculi that produce obstruction, infection, and, ultimately, renal insufficiency. Cystinuria represents 1–2% of overall renal lithiasis and 6–8% of renal lithiasis in pediatric patients.

Presently, we classify two types of cystinuria types: type I (MIM 220100) and non-type I (MIM 600918) (124). Type I heterozygotes are silent, whereas in non-type I heterozygotes there is a variable degree of urinary hyperexcretion of cystine and dibasic amino acids. Patients with a mixed type, inheriting type I and non-type I alleles from either parent, have also been described (57). Type I cystinuria represents >60% of the cases of the disease.

The amino acid transport activity associated with rBAT (system b0,+ and the expression of rBAT in the brush border of the renal epithelial cells of the proximal tubule and of the small intestine pointed to the rBAT gene (SLC3A1) as a candidate for cystinuria. Mutational and linkage studies demonstrated that mutations in SLC3A1 cause type I cystinuria (23, 24, 54). Over 60 distinct rBAT mutations have been described, including nonsense, missense, splice site, and frameshift mutations, as well as long deletions; mutation M467T is the main type I cystinuria allele found worldwide in 38 nonrelated chromosomes (reviewed in Ref. 124). Cystinuria resembling type I, due to mutations in canine SLC3A1, has been reported in Newfoundland dogs (61).

The gene causing non-type I cystinuria was assigned by linkage analysis to the 19q12–13.1 region (11, 166, 184). In 1999, the non-type I cystinuria gene was identified as SLC7A9 (47a). SLC7A9 was a positional candidate gene for non-type I cystinuria because it has the proper chromosomal location, rBAT-associated amino acid transport activity (system b0,+), and tissue expression (mainly in kidney and small intestine, but also in pancreas and liver). The protein product encoded by SLC7A9 was termed b0,+AT (for b0,+ amino acid transporter).

SLC7A9 is the main, if not the only, non-type I cystinuria gene. In fact, after an exhaustive screening of the open reading frame of SLC7A9 by the International Cystinuria Consortium (49a), 35 distinct mutations were found, accounting for 79% of the carrier chromosomes in 61 non-type I patients, mutation G105R being the main non-type I cystinuria allele (25%). The unexplained alleles might be due to mutations outside the open reading frame of SLC3A1, al-

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**Fig. 2. Model of sequential exchange with a double-transport pathway with alternating access for the heteromeric amino acid transporters.** In this model, the transporters are composed of 2 transport pathways. **Left to right:** 1) binding of a substrate to the outside face (●); higher affinity than the inside face) increases affinity for substrates in the inside face (○); 2) binding of a substrate inside results in the formation of a ternary complex; 3) transporter changes accessibility to each of the two transport pathways; 4) dissociation of the substrate outside reduces the affinity of the substrate inside; 5) dissociation of the substrate inside. This is the simplest model that explains the kinetics results obtained for system b0,+ in chicken brush-border jejunum by Torras-Llort et al. (174).
though other gene(s) might be involved in non-type I
cystinuria.

All the data discussed so far strongly indicate that
rBAT and b0,+,AT are subunits of the amino acid trans-
porter b0,+. However, this view is challenged by the
finding that rBAT has a gradient of expression along
the kidney proximal tubule: segment S3 > S2 > S1 (27,
53, 80, 127, 130, 136), whereas b0,+,AT has the opposite
gradient of expression: S1 > S2 > S3 (27, 104, 127, 136)
[S1 being the small initial part of the proximal convo-
luted tubule (PCT), S2 the rest of PCT plus the cortical
proximal straight tubule (PST), and S3 the terminal
part of the PST located in the outer stripe of the outer
medulla]. Then, an additional LSHAT for rBAT or
HSHAT for b0,+,AT might be available. Identification of
these proteins might help us to understand the mole-
cular bases of cystinuria and why mutations in SLC3A1
are completely recessive, whereas mutations in
SLC7A9 are incompletely recessive (for a detailed dis-
cussion, see Refs. 47a and 49a).

LPI

LPI (MIM 222700) is a rare autosomal-recessive dis-
ease caused by the defective transport of dibasic amino
acids at the basolateral membranes of epithelial cells
in the renal tubules and small intestine (159). LPI is
more prevalent in Finland, but clusters of LPI families
are also known in southern Italy and Japan. The dis-
ease is characterized by reduced intestinal absorption
of dibasic amino acids, increased renal excretion, and
low plasma concentrations of dibasic amino acids,
orotic aciduria, and dysfunction of the urea cycle, lead-
ing to hyperammonemia. Major clinical symptoms in-
clude vomiting, diarrhea, failure to thrive, hepato-
splenomegaly, episodes of hyperammonemic coma, and
osteoporosis. Life-threatening alveolar proteinosis in
the lungs and severe renal involvement were also re-
ported (145, 159). The pathogenic mechanism of sev-
eral clinical complications of LPI, such as alveolar
proteinosis and urea cycle dysfunction, are still un-
clear.

The LPI locus was assigned to chromosome 14q11.2
by linkage analysis (92, 93). The LPI gene was later
identified by candidate positional cloning (14, 176) af-
after identification of y+LAT-1 (SLC7A7 gene) (175).
y+LAT-1 is expressed in target tissues of LPI such as
kidney, lung, and small intestine, among others, and
induces system y+L transport activity when coex-
pressed with 4F2hc (128, 175). The defective system
y+L transport in LPI is restricted to intestine, kidney,
and probably liver and lung. In fact, system y+L is not
altered in LPI erythrocytes or fibroblasts (15, 38), most
probably because of the expression of the y+L trans-
porter isoform y+LAT-2 in these cells.

Twenty-five distinct LPI-associated mutations
spread along the entire SLC7A7 gene have been iden-
tified in 96 LPI patients; only 3 alleles remain to be
explained (reviewed in Ref. 119). All Finnish LPI pa-
patients share the same founder mutation: a splice site
mutation (IVS6–2 AT) that creates a frameshift after
Val298 within putative extracellular loop 4 and a pre-
mature stop-codon 9 amino acid residues thereafter
(109, 176). A genotype-to-phenotype correlation cannot
be established in LPI because of extensive clinical
variability associated with the same genotype (re-
viewed in Ref. 119). Then, other factors in addition to
mutations of SLC7A7 might have a role in the patho-
genesis and clinical manifestations of LPI.

HSHATs

Six sequences are available for mammalian rBAT
(human, rat, mouse, rabbit, dog, and a partial sequence
for the American opossum), sharing 69–89% amino
acid identity and five sequences for vertebrate 4F2hc
(human, rat, mouse, Chinese hamster, and zebrafish),
sharing 41–89% amino acid identity (see GenBank
accession nos. in the legend for Table 1). The rBAT
protein (685 amino acid residues for the human coun-
terpart) is longer than the 4F2hc protein (529 for the
human counterpart), and they share ~25% amino acid
sequence identity (Fig. 3). N-glycosylation was shown
for both proteins (reviewed in Ref. 121).

Membrane topology algorithms predicted that both
proteins would be type II membrane glycoproteins,
with an NH2 terminus inside the cell, a single TM, and
a bulky COOH terminus located outside the cell (re-
viewed in Ref. 121). The cysteine residue participating
in the disulfide bridge with the LSHATs is four to five
amino acids away from the TM, toward the COOH
terminus (Fig. 3). In contrast to this view, Mosckovitz
and co-workers (107), on the basis of accessibility stud-
ies with various antibodies, proposed that rBAT con-
tains at least four TMs, with NH2 and COOH termini
located intracellularly. Recently, however, Fenczik
and co-workers (49) showed with HA-tag constructs
that the NH2 terminus of 4F2hc is intracellular whereas
the COOH terminus is extracellular (49), as expected for
a type II membrane glycoprotein. A further argument in
favor of this structure is the homology of the HSHAT
bulky COOH-terminal domain with α-amylases (9,
134, 171, 172, 186). Indeed, this HSHAT domain shows
some homology only with insect maltase (α-glucosi-
dase) and maltase-like precursors (35–40% amino acid
identity) and with bacterial α-glucosidases (~30%
amino acid identity).

This α-amylase family consists of a large group of
starch hydrolases and related enzymes, comprising
~20 different enzyme specificities, and is presently
known as glycosyl hydrolase family 13 (75). The mem-
bers have a similar architecture, with a catalytic (β/
α)-barrel or TIM-barrel (domain A), interrupted by
a small calcium-binding subdomain (domain B) pror-
truding between the third β-strand (Aβ3) and the third
α-helix (Aα3), and a COOH-terminal domain (domain
C) with an antiparallel β-barrel structure. Major dif-
fferences in amino acid sequence among the α-amylase
family members occur within domain B. Janecek et al.
(75) clustered the α-amylase members in five groups
with >50% sequence identity for domain B and sug-
gested that it varies with enzyme specificity. The group
defined by *Bacillus cereus* oligo1,6-glucosidase (O1,6G) also includes the rBAT proteins. Domain B of O1,6G has a complex topology not shared by other structurally solved α-amylases, which, in turn, have a common α-β architecture known as a two-layer sandwich (CATH; protein structure classification: http://www.biochem.ucl.ac.uk/bsm/cath_new/).

The three-dimensional structure of O1,6G has been refined at 2.0-Å resolution (185). The secondary structural elements of this enzyme are indicated in Fig. 3, together with an alignment with human rBAT and 4F2hc, one putative HSHAT from *Drosophila melanogaster* and two from *Caenorhabditis elegans*. Because of the different levels of sequence similarity for each domain (A, B, and C) between HSHATs and O1,6G, a global alignment has first been performed for the corresponding glycosyl hydrolase family, and, finally, by alignment of predicted secondary structures with Jpred (37). The principal secondary structural elements (PDB file 1UOK) of O1,6G are indicated at the top of each sequence (α-helix; β, extended strand) in the 3 protein domains [TIM barrel (A); protruding domain (B); antiparallel β-barrel (C)], and the sequence is underlined. The secondary structures in the rest of the sequences were also predicted (solid underline) or not (dotted underline) with Jpred (37). Amino acid residues conserved in O1,6G are shown shaded in gray. Residues affected by cystinuria-specific missense mutations in human rBAT are shown in white over black. The cysteine residue conserved in the HSHAT family is boxed. The putative TM domain in HSHAT proteins is underlined in bold. The asterisks indicate amino acid residues in O1,6G suspected of being involved in the catalysis of the enzyme. The motif QPDLN at the COOH-terminal end of domain B of O1,6G is indicated.

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**Fig. 3.** Multialignment of HSHAT proteins and oligo-1,6-glucosidase (O1,6G). Human rBAT and 4F2hc, two hypothetical proteins from *Caenorhabditis elegans* (TyEMBL accession nos. O45298 and Q8XVU3), one hypothetical protein from *Drosophila melanogaster* (Q9VHX3), and O1,6G from *Bacillus cereus* (GenBank accession no. X53507) are shown. The alignment was obtained initially with tcoffee (113), and problematic parts (between Aβ4 and Aβ8) were aligned by threading with FUGUE (155), and finally by alignment of predicted secondary structures with Jpred (37). The principal secondary structural elements (PDB file 1UOK) of O1,6G are indicated at the top of each sequence (α, α-helix; β, extended strand) in the 3 protein domains [TIM barrel (A); protruding domain (B); antiparallel β-barrel (C)], and the sequence is underlined. The secondary structures in the rest of the sequences were also predicted (solid underline) or not (dotted underline) with Jpred (37). Amino acid residues conserved in O1,6G are shown shaded in gray. Residues affected by cystinuria-specific missense mutations in human rBAT are shown in white over black. The cysteine residue conserved in the HSHAT family is boxed. The putative TM domain in HSHAT proteins is underlined in bold. The asterisks indicate amino acid residues in O1,6G suspected of being involved in the catalysis of the enzyme. The motif QPDLN at the COOH-terminal end of domain B of O1,6G is indicated.
bining secondary structure predictions and threading techniques (see legend for Fig. 3 for details). Sequence homology between HSHATs and O1,6G starts with two contiguous tryptophan residues a few amino acid residues away from the cysteine residue involved in the formation of the disulfide bridge with LSHATs. Domain A of O1,6G is highly conserved in HSHATs. Indeed, the secondary structure elements of the \((\beta/\alpha)_8\) barrel, i.e., \(\alpha_1, \alpha_2, \alpha_3, \beta_4, \alpha_5, \beta_5, \alpha_6, \alpha_7, \) and \(\beta_7,\) of the \((\beta/\alpha)_8\) barrel in the protein sequence of most of the vertebrate and invertebrate HSHATs (Fig. 3). This strongly suggests that the structure of the bulky COOH-terminal domain of HSHATs corresponds to a TIM-barrel.

Three catalytic residues (D199, E255, and D329 in O1,6G) and two residues for substrate binding (H103 and H328) within the TIM-barrel constitute the active site of family 13 glycosyl hydrolases (reviewed in Ref. 185). All these residues are in the COOH-terminal face of the TIM-barrel and, together with the protruding

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**Fig. 4.** Membrane topology model of human \(\beta^{0,1}\)AT. The 22 cystinuria-specific mutations affecting single amino acid residues are indicated. The boxed mutations are those associated with a severe phenotype (see text and Table 2 for details). Mutations indicated in bold italics are those associated with a mild phenotype. The rest of the mutations (smaller font) have an unknown or ambiguous phenotype. Conserved amino acid residues in all the LSHAT subfamily members (31 transporters, excluding the mammalian orphan transporters) are indicated inside filled circles or squares. Amino acid residues with small side chain (i.e., Gly, Ser, or Ala) in all of these transporters are indicated in gray circles or squares. The extracellular (EL) and intracellular (IL) loops are numbered. SH, the cysteine residue involved in the disulfide bridge with rBAT; N- and C-, NH2 and COOH terminus, respectively. Amino acid residues within squares are those with small side chain (Sm) in the transmembrane SmxxxSm motifs completely or highly conserved in LSHATs. Thus, with the 31 sequences available within the LSHAT subfamily (excluding the mammalian orphan members), the first motif in TM I and the motifs in TM VI and VIII are present in all of the sequences, the second motif in TM I is only absent in LAT-1 transporters, and the motif in TM VII is only absent in SPRM1. Of the 31 LSHAT subfamily members used to construct this figure, some correspond to \(D.\) melanogaster and \(C.\) elegans hypothetical proteins. The sequences of some of these proteins may be erroneous. Thus T32479 has an extremely long IL4, in T28818 the TM VIII is missing, T21445 and CG12317 are truncated sequences lacking the TM XII and the COOH terminus, and in CG9413 the NH2 terminus and the first part of TM I are missing.
domain B, constitute the active site cleft. Of these TIM-barrel residues of O1,6G, only D199 in the αβ4 region, D329 between αβ7 and αα7, and H105 before domain B can be identified unambiguously in the mammalian rBAT proteins, but none of them in the vertebrate 4F2hc proteins (Fig. 3). Similarly, none of the three invertebrate putative HSHAT proteins share all the active site residues of O1,6G (Fig. 3). In agreement with this, Wells and co-workers (187) did not observe α-glucosidase activity for 4F2hc after expression in Xenopus laevis oocytes.

Janeczk and co-workers (75) reported that domain B, including the COOH-terminal motif QPDLN (residues 167–171 in O1,6G), is conserved in the rBAT proteins but not in the 4F2hc proteins. Indeed, there is complete absence of domain B for 4F2hc and the putative HSHAT O45298 from C. elegans (Fig. 3). In contrast, rBAT and Q9XVu3 from C. elegans and Q9VHX9 from D. melanogaster contain a domain B with the structural features of this domain in O1,6G, including a complete or partial conservation of the COOH-terminal motif. This suggests that O45298 is the C. elegans ortholog of 4F2hc and that Q9XVU3 and Q9VHX9 are the C. elegans and D. melanogaster orthologs of rBAT, respectively. A search through the entire human genome and the genome of D. melanogaster and C. elegans revealed no other putative HSHAT proteins.

The COOH-terminal domain (domain C) of α-amylases corresponds to a β-barrel structure of eight anti-parallel β-strands folded in double Greek key motifs, which is distorted in the sixth strand C66 (185). Sequence alignment, threading, and secondary structure prediction fit the entire O1,6G domain C into the COOH-terminal region of the vertebrate and invertebrate HSHATs, with the exception of C66, which is not clearly predicted in these proteins (Fig. 3). The function of domain C, located far from the active site of O1,6G, is unknown. Finally, the last 30 amino acid residues of the rBAT proteins do not align with the α-amylases.

Structure-Function Relationship Studies

Three studies have dealt with COOH-terminal deletions of HSHATs (19, 39, 102). The first two studies were performed before the identification of LSHATs and therefore rely on the expression of HSHATs in oocytes that, together with endogenous LSHATs, elicited transport of amino acids. Miyamoto et al. (102) showed that a COOH-terminal deletion (Δ511–685) on human rBAT, which eliminates the last α-helix of the TIM-barrel, domain C, and the COOH-terminal tail (Fig. 3), induces a decreased amino acid transport activity in oocytes that resembles that of 4F2hc-induced system y' L-glutamine. The expression of proteins with longer deletions in the COOH terminus of rBAT renders no transport function in oocytes (see Ref. 121 for a longer discussion). This suggests that the COOH-terminal domain of rBAT is relevant for the interaction with endogenous LSHATs (i.e., either b0, y LAT-type or y' LAT-type).

Deora and co-workers (39) studied a series of COOH-terminal deletions of rat rBAT. Surprisingly, expression of these truncated proteins in oocytes yielded an unusual bimodal pattern of the induction of amino acid transport activity. Thus minimal COOH-terminal truncations (Δ658–683, which eliminates the COOH-terminal tail, and Δ615–683, which eliminates the last four β-strands of the domain B and the COOH-terminal tail; Fig. 3) abolished transport activity. The next mutants in the series (Δ588–683, elimination from the last 6 β-strands of domain B; Fig. 3) induced amino acid transport almost like that of the complete rBAT and with the characteristics of rBAT/system b0, y. Further deletions (Δ566–683, elimination from all of domain C, and Δ508–683, elimination from the last α-helix of the TIM-barrel; Fig. 3) abolished amino acid transport induction. There is no obvious reason for the discrepancy of the amino acid transport induction by Δ508–683 rat rBAT (39) and Δ511–685 human rBAT (102). Deora and co-workers (39) studied further the transport-active deletion Δ588–683. The cysteine residue at position 111 in rBAT forms part of the disulfide bridge with the corresponding LSHAT. A mutation to the serine of this residue (C111S) renders a protein that induces 70% of the amino acid transport induced by wild-type rBAT. A notable difference is that the C111S mutant in Δ588–683 rBAT completely abolished its transport activity. This suggests the following. First, the formation of the disulfide bridge with the corresponding LSHAT is not necessary for the functional association with rBAT. This has also been demonstrated for 4F2hc alone and 4F2hc/y LAT-1- or LAT-1-induced transport (47, 129, 175). Thus other interactions beside the disulfide bridge keep the functional transport complex intact. Second, lacking C111 (rat rBAT numbering), the Δ588–683/C111S mutant cannot form a stable complex with the endogenous LSHAT. Thus, in the absence of the disulfide bridge the integrity of domain B and the COOH-terminal tail is necessary for a functional transport complex. Third, the COOH terminus holds rBAT in an active conformation, perhaps by providing the sites for interaction with other rBAT regions or with the light subunit.

The above-mentioned experiments with truncated versions of rBAT could also be interpreted as the result of interactions, depending on the different COOH-terminal deletions, with different oocyte LSHATs. Indeed, Peter and co-workers (126) analyzed the mutations of the three conserved cysteine residues located in the COOH-terminal tail of rBAT (C664, C671, and C683 in rat rBAT; Fig. 4): replacement of C664 by alanine eliminates the functional interaction of rBAT with the putative endogenous y LAT-type subunit and keeps (or causes) the functional interaction with a putative endogenous y LAT-type subunit. Broër and co-workers (19) studied COOH-terminal deletions of 4F2hc coexpressed in oocytes with LAT-1, LAT-2, or y LAT-2. Surprisingly, association of these LSHATs requires different domains. Thus trafficking to the plasma membrane and induction of LAT-1/system L transport activity require only the NH2-terminal tail, the TM
domain of 4F2hc, and 30 extracellular amino acid residues, including the disulfide bridge-forming cysteine residue and the first β-strand of domain B (Fig. 3) (the longest deletion studied in this work). In contrast, functional recognition of LAT-2 and γ-LAT-2 needs the complete extracellular domain of 4F2hc. This suggests that the 4F2hc protein has different interaction sites for its associated light chains. In this study, all truncated versions of 4F2hc delayed the trafficking of LAT-1 to the plasma membrane. Moreover, this trafficking was more severely affected by truncations involving part of the extracellular glucosidase-like domain of 4F2hc than by those that eliminate it almost completely. Some short COOH-terminal truncations resulted in large aggregates that might be responsible for the severe trafficking defect. Finally, the more severe defects in LAT-1 recognition occurred when the last 70 amino acids were removed (i.e., the shortest deletion studied, which eliminates the last 6 β-strands of domain B and the COOH-terminal tail; Fig. 3). This is reminiscent of the study by Deora et al. (39) of truncated versions of rBAT: the activity lost when only small parts of the COOH-terminal domain of the HS-HAT are removed can be regained by larger COOH-terminal deletions. This suggests that the COOH-terminal tail of HSHATs plays a role in the proper folding of rBAT and/or in its interaction with the corresponding LSHAT.

In addition to the amino acid transport function of HSHATs, 4F2hc has been related to integrin function (see CELL PHYSIOLOGY OF THE CD88 COMPLEX). Fenczik and co-workers (49) have examined which domains of 4F2hc play a role in amino acid transport and in regulation of integrins function. By constructing chimeras with 4F2hc and the type II TM protein CD69, the authors showed that the NH₂-terminal and the TM domain of 4F2hc are required for its effects on integrin function, whereas the extracellular glucosidase-like domain is required for the stimulation of LAT-1 amino acid transport. This study together with that mentioned above on truncated versions of 4F2hc (19) point to multiple interactions, both at the NH₂-terminal tail and the TM domain, and at the extracellular glucosidase-like domain, between 4F2hc and LAT-1. Thus the NH₂-terminal tail and the TM domain are sufficient for the functional interaction of 4F2hc with LAT-1, but replacement of the extracellular domain by another prevents this functional interaction.

All but one of the rBAT missense mutations described in type I cystinuria (Fig. 3) are located in the extracellular glucosidase-like domain, which is consistent with its proposed role in amino acid transport. The most obvious role of HSHATs in amino acid transport is to help the trafficking of LSHATs to the plasma membrane. In agreement with this, for several cystinuria-specific rBAT mutations a trafficking defect to the plasma membrane has been substantiated (M467T, M467K) or suggested (T216M, S217R) (31, 143). On the other hand, some cystinuria-specific rBAT mutations affecting transport properties of system b₀⁺,⁺ are presently under study, suggesting a participation of rBAT in the transport mechanism of the holotransporter. Reconstitution studies of HATs will be needed to demonstrate whether isolated LSHATs display amino acid transport activity and to identify the role of HSHATs in the mechanism of transport.

Another unsolved question is why the extracellular domain of HSHATs resembles that of glucosidases but without apparent catalytic activity. One could envisage that the noncatalytic glucosidase-like domain of HSHATs might hold extracellular glucidic structures to locate HATs properly in the plasma membrane. This question should be solved by purification and testing of glucidic binding to the extracellular domain of HSHATs. Homology modeling of HSHATs with α-glucosidases is in progress, but it could be hampered by the low identity of parts of domain A with crystallized α-amylases and the lack of domain B in 4F2hc proteins. Purification and crystallization may be needed to establish the structure of the bulky extracellular region of HSHATs.

LSHATs

There are 23 reported LSHATs with ascribed amino acid transport function: 22 vertebrate sequences that correspond to 7 LSHAT paralogs and the Schistosoma mansoni PRM1 protein (see the beginning of this study and legend for Table 1 for details). Amino acid sequence identity among these LSHATs ranges between 39 and 70% for different paralogs and between 85 and 98% for different mammalian orthologs. In addition, two other orphan LSHAT cDNAs have been cloned in mice (LSHAT-8 and -9) and humans (LSHAT-8) (Bassi MT, Gasol E, Zorzano A, Palacín M, and Borsani G, unpublished observations). Identity of the mammalian orphan LSHATs with the LSHATs with known transport functions drops to 23–29%.

LSHATs belong to the large superfamily of APC transporters (>175 transporters; for amino acids, polyamines, and organocations). Jack and co-workers (74) clustered this superfamily into 10 families. One of these is the LAT family (TC 2.A.3.8), which received its name from the first LSHAT identified (LAT-1; Refs. 79 and 101) and clusters the above-mentioned vertebrate LSHAT members and SPRM1, the yeast high-affinity methionine permease MUP1 (U40316) and the hypothetical yeast protein MUP3 (protein GenBank accession no. P38734) (72), and several hypothetical proteins from C. elegans and D. melanogaster. Jack et al. (74) also identified a signature sequence specific to the LAT family, G[WFY][DNFS][LIV][NH][FYT][LIVAGS][TALIV][EGPS][E[LIVM][X][NDE][PX][RK][NT][LIVM][PK], where X represents any residue. This signature is located in the third putative intracellular loop of the topology model shown in Fig. 4 (see below for discussion of this model).

There is a key structural feature of LSHAT members, the conserved cysteine residue in the putative extracellular loop 2 that participates in the disulfide bridge with the corresponding HSHAT (Fig. 4), which was first functionally identified by Pfeiffer and co-workers (129). This cysteine residue is present in 34
LSHATs: 25 vertebrate sequences and SPRM1 (see above), D. melanogaster midinisks (AF139834), and 4 D. melanogaster (GenBank accession nos. CG1617, CG12317, CG6070, and CG9413) and 3 C. elegans (protein GenBank accession nos. T21445, T23479, and T28818) hypothetical proteins. In contrast, this residue is not conserved in the other LAT family members: yeast MUP1 and MUP3 and in 5 C. elegans (T15226, T16854, T24837, T32821, T31554) hypothetical proteins. We propose to cluster the former group of LAT family members (i.e., those with the conserved cysteine residue) in the LSHAT subfamily. Sequence analysis (173) revealed a specific signature sequence for this subfamily (34 transporters) located between transmembrane (TM) domain I and IL1 (see Fig. 4): [IVFLG(SAT)GIF[VILSTA]PX$_{26}$GS)AST][LYVIF]CSAV [YFSN][AS][E][LI]GSA$(X_5)$SG[GAYW][X][YF], where X represents any residue.

Phylogenetic analysis (173) of the LSHAT subfamily revealed a nodal relationship of CG12317 and CG9413 from D. melanogaster with LAT-1 and b$_{0^{+}}$-AT transporters, respectively, suggesting that these hypothetical proteins correspond to the orthologs of these transporters. In contrast, the rest of the hypothetical proteins from C. elegans and D. melanogaster cluster in two nodes (T32479 and T21445 from C. elegans and CG1607 from D. melanogaster; T28818 from C. elegans and midinisks and CG6070 from D. melanogaster) together with transporters for neutral amino acids of the LSHAT subfamily (i.e., LAT-1, LAT-2, and asc-1). This sequence analysis is therefore not enough to ascribe the amino acid transport function to these C. elegans and D. melanogaster LSHAT members.

Membrane topology predictions revealed that the transporters of the APC family display 10, 12, or 14 TMs (74). Most of the APC families (8 families and the bacterial transporters of the CAT family) display 12 TMs. Figure 4 shows the 12-TM model for human b$_{0^{+}}$-AT. This model is based on TM-HMM algorithms (164) with the multialignment of the 34 transporters of the LSHAT subfamily (i.e., LAT-1, LAT-2, and asc-1). The location of the 22 cystinuria-specific mutations affecting single amino acid residues of b$_{0^{+}}$-AT is shown in Fig. 3. Seventeen mutations involve amino acid residues within the putative TM domains of the protein and the rest within the putative intracellular loops. Thus none of the mutations is located within the putative extracellular loops of b$_{0^{+}}$-AT. This is also the case for the seven missense LPI mutations mentioned above. Similarly, all the transport activity-relevant residues identified in PotE are located in the loops and TM facing the cytoplasmic side of the transporter (82, 83). It has also been shown that the main functional amino acids of the lactose/H$^{+}$ symporter (76, 132) and the metal tetracycline/H$^{+}$ antiporter (192) are located on the cytoplasmic side of the protein. Kashiwagi and co-workers (82) interpreted this asymmetry as the structural basis to provide a quick response of the transporter to any change in cellular substrate concentration.

Six missense b$_{0^{+}}$-AT mutations (A70V, V170M, A182T, A354T, G105R, and R333W) have been tested for function after coexpression with rBAT in HeLa cells (49a). Some of these mutations (V170M, A354T, G105R, and R333W) cause a complete or almost complete loss of function (<10% residual transport activity), whereas the others (A70V and A182T) have only a partial effect (>50% residual activity) (Table 2). It remains to be determined whether these b$_{0^{+}}$-AT mutations affect trafficking to the plasma membrane or whether they inactivate the transporter.

Urinary excretion of cystine and the three dibasic amino acids (arginine, lysine, and ornithine) in heterozygotes bearing the major missense SLC7A9 mutations (G105R, V170M, A182T, R333W) have been shown for function after coexpression with rBAT in HeLa cells (49a). Some of these mutations (V170M, A354T, G105R, and R333W) cause a complete or almost complete loss of function (<10% residual transport activity), whereas the others (A70V and A182T) have only a partial effect (>50% residual activity) (Table 2). It remains to be determined whether these b$_{0^{+}}$-AT mutations affect trafficking to the plasma membrane or whether they inactivate the transporter.

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Mutations have been reported to be a framework for the TM helix-helix association (142), and in this sense, helixes 3 and 6 of AQP1 contain a GlyxxxGly motif (where Gly can be replaced by Ala). Similarly, SmxxxSm motifs (where Sm stands for residues with a small side chain; Gly, Ala, or Ser) are present in the LSHAT subfamily in TM I, V, VI, VII, and VII (Fig. 4). Mutation G259R in b0,−AT and mutation G54V in y−LAT-1 involve SmxxxSm helix-helix association motifs in TM VII and I, respectively. G259R is associated with a severe urinary phenotype in heterozygotes, and G54V is associated with a dramatic loss of transport function (49a, 109). This suggests that residues with small side chains, which are conserved in TMs of LSHAT transporters, may participate in their TM helix-helix associations.

### CELL PHYSIOLOGY OF THE CD98 COMPLEX

In this section, we will use the name CD98 to refer to the 4F2 complex and CD98hc for the 4F2hc, to maintain the nomenclature as used in the papers cited. The light chains are termed LSHATs, as before.

As stated above, LSHATs are believed to mediate amino acid transport itself due to their polytopic structure, whereas the type II protein CD98hc seems to act as a guidance molecule for the light chains on their way to the plasma membrane (110). However, localization and maturation of CD98hc in the absence of light chains are not well studied. At least in oocytes and L cells, CD98hc is expressed at the plasma membrane without a light chain (101, 172).

Overexpression of CD98hc leads to its expression, apparently as a mono-

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**Table 2. Genotype-phenotype correlation in SLC7A7 and SLC7A9 missense mutations**

<table>
<thead>
<tr>
<th>Missense Mutations</th>
<th>Location in Protein (TM Domain)</th>
<th>Residues in LSHAT Members</th>
<th>Urinary Phenotype in Heterozygotes</th>
<th>Transport Defect, Residual Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SLC7A7</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G54V</td>
<td>I</td>
<td>G (All)</td>
<td>?</td>
<td>Severe (0%)</td>
</tr>
<tr>
<td><strong>SLC7A9</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P52L</td>
<td>I</td>
<td>P (All)</td>
<td>Severe</td>
<td>?</td>
</tr>
<tr>
<td>G63R</td>
<td>II</td>
<td>G (30), N (1)</td>
<td>Mild</td>
<td>?</td>
</tr>
<tr>
<td>A70V</td>
<td>II</td>
<td>A (14), V (6), I (5), L (4), S (1), T (1)</td>
<td>Mild</td>
<td>?</td>
</tr>
<tr>
<td>T123M</td>
<td>III</td>
<td>T (16), S (9), C (3), A (2), G (1)</td>
<td>Mild</td>
<td>?</td>
</tr>
<tr>
<td>A126T</td>
<td>III</td>
<td>A (23), Y (5), T (3)</td>
<td>Mild</td>
<td>?</td>
</tr>
<tr>
<td>V170M</td>
<td>IV</td>
<td>V (30), M (1)</td>
<td>Severe</td>
<td>?</td>
</tr>
<tr>
<td>A182T</td>
<td>V</td>
<td>A (16), Y (5), G (3), F (3), I (2), V (2)</td>
<td>Mild</td>
<td>?</td>
</tr>
<tr>
<td>G195R</td>
<td>V</td>
<td>G (All)</td>
<td>Severe</td>
<td>?</td>
</tr>
<tr>
<td>G259R</td>
<td>VII</td>
<td>S (25), G (5), A (1)</td>
<td>Severe</td>
<td>?</td>
</tr>
<tr>
<td>A354T</td>
<td>IX</td>
<td>A (17), S (13), G (1)</td>
<td>Severe</td>
<td>?</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Missense Mutations</th>
<th>Location in Protein (Intracellular Loop)</th>
<th>Residues in LSHAT Members</th>
<th>Urinary Phenotype in Heterozygotes</th>
<th>Transport Defect, Residual Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SLC7A7</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L334R</td>
<td>IV</td>
<td>L (29), I (1), M (1)</td>
<td>?</td>
<td>Severe (0%)</td>
</tr>
<tr>
<td><strong>SLC7A9</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G105R</td>
<td>I</td>
<td>G (All)</td>
<td>?</td>
<td>Severe (0%)</td>
</tr>
<tr>
<td>I241T</td>
<td>III</td>
<td>V (21), I (8), L (1), M (1)</td>
<td>Mild</td>
<td>?</td>
</tr>
<tr>
<td>T123M</td>
<td>IV</td>
<td>R (28), Q (1), N (1), E (1)</td>
<td>Severe</td>
<td>?</td>
</tr>
</tbody>
</table>

Only missense mutations with available phenotype are described. The nos. in parentheses indicate the number of LSHAT members having the particular amino acid residue at the corresponding position among 31 sequences (i.e., the LSHAT subfamily members, with the exception of the mammalian orphan transporters). Heterozygotes excreting cystine and the sum of cystine and the 3 dibasic aminoacids above 5 times the average of these two parameters in controls are classified as having a severe urinary phenotype. In contrast those below these limits are classified as having a mild urinary phenotype. Residual transport activity is expressed in % of the transport activity of wild type y−LAT-1 (measured in oocytes) or b0,−AT (measured in HeLa cells). Modified from Ref. 69.
mer, at the plasma membrane (47, 49). However, no study has measured the amount of plasma membrane free CD98hc in the normal in vivo situation (for instance, in activated T cells, proximal tubule cells, intestinal epithelial cells, etc.), which is important to an understanding of some of the data that will be discussed below.

CD98 was identified in the 1980s as an early activation antigen of T and B cells (60, 99, 134). Since those studies, many different functions besides amino acid transport have been ascribed to the complex, from cellular activation and division (41, 167, 191) to cell adhesion, fusion, and differentiation (114, 117, 170, 182). A recent careful review from Devés and Boyd (40) offered the first systematic overview of the huge amount of data dealing with these apparently disconnected functions. In this section, we will focus on the recent work performed in the fields of integrin activation, viral and cell fusion and differentiation, T cell activation, and oncogenesis.

**Integrin Activation**

Integrins are heterodimers of specific combinations of α- and β-subunits, both of them type I membrane proteins with large extracellular domains and short COOH-terminal cytoplasmic tails. The extracellular domains mediate extracellular matrix and cell-cell adhesion. These interactions produce changes in the cytoplasm affecting the cytoskeleton, signal transduction pathways, and gene expression profiles. These processes are known as “outside-in” signaling (32, 152). In turn, intracellular signaling can change affinity and/or avidity of integrins for its substrates, a process called “inside-out” signaling, affinity modulation, or integrin activation (64). Integrin activation is thought to be mediated by intracellular effectors that might directly interact with integrin cytoplasmic tails (28, 36). Fenczik et al. (48) developed a genetic cloning strategy to isolate these potential effectors. In their system (Fig. 5), a Chinese hamster ovary cell line stably expresses a constitutively activated chimeric integrin complex. Overexpression of a β-integrin cytoplasmic tail located at the plasma membrane leads to dominant suppression of integrin activation, most likely by titration of intracellular effector molecules. CD98hc was isolated as one of these possible effectors because its overexpression led to the complementation of the dominant suppression (CDS) caused by the β-integrin cytoplasmic tail (48). The cytoplasmic tail of CD98hc was necessary for CDS, as revealed by a deletion mutant. As whole antibodies against CD98hc, but not Fab fragments, were able to stimulate integrin-dependent cell adhesion, it seems that CD98hc crosslinking could regulate avidity of integrins.

The same laboratory went further in the characterization of CD98hc-mediated CDS (194). They found that solubilized CD98 binds in vitro to β1A- and β3-integrin tails (but not to β1D or β2). This binding correlated with the lack of CD98hc-mediated CDS caused by β1D- or β3-integrin cytoplasmic tails. β1A-

**Viral and Cell Fusion and Differentiation**

Membrane fusion leading to multinucleated cell formation is a physiological process specially relevant for osteoclastogenesis and myogenesis. Moreover, many enveloped viruses, such as paramyxoviruses, induce syncitium cell formation; others, like the human immunodeficiency virus, require fusion mechanisms to enter their cellular hosts.

In the early 1990s, Ito and colleagues (73) isolated monoclonal antibodies (MAbs) that enhanced cell fusion of the Newcastle disease virus. These MAbs immunoprecipitated either CD98hc or the α 3-integrin subunit (114), named fusion regulatory protein-1 and -2, respectively. Since then, the aforementioned authors have extended their studies to paramyxovirus-induced syncitium cell formation and human immunodeficiency virus entry into monocyte/macrophage cells (115, 117), isolating new anti-CD98hc MAbs that are able to either induce or inhibit those processes. The fact that different anti-CD98hc MAbs have opposing effects on fusion events suggests that the MAbs can...
induce or fix conformations of the extracellular domain of CD98hc, competent or not to transduce cell fusion signals into the cell. Moreover, in regard to the work of Ginsberg and co-workers (48, 49, 194), it is worth mentioning that fusion induced by anti CD98hc mAbs was blocked by fibronectin and anti-β₁-integrin antibodies, indicating functional and/or physical interactions between the integrin system and CD98hc.

The role of CD98hc was further investigated by the generation of stable cell lines expressing a chimera in which the cytoplasmic domain of human CD98hc was replaced by the cytoplasmic domain of the hemagglutinin-neuraminidase from the human parainfluenza virus type 2 and the mutant C330S (which does not impair either disulfide linking to the LSHATs or amino acid transport (129)). Both mutants suppressed the cell fusion-enhancing activity of anti-CD98hc MAbs (117). The mechanisms of these dominant-negative effects are not known. If LSHATs are required for cell fusion, the mutant CD98hc may titrate them in a way not competent for fusion. The conformation of the CD98hc extracellular domain may also change to that of a fusion-incompetent molecule. The binding to the MAbs, however, was not affected. Titration of β-integrin cytoplasmic tails (194) by the C330S mutant could also explain the dominant-negative effect, but this mechanism cannot be invoked for the chimera.

**Fig. 5.** Complementation of dominant suppression by CD98hc. A: expression cloning strategy used a Chinese hamster ovary cell line stably expressing a chimaeric integrin (αβ) constitutively activated (i.e., in a conformation that binds ligand L with high affinity). The activation is thought to occur through the binding to cytoplasmic integrin tails of a putative “integrin activating complex” (IAC). B: overexpression of a TAC-β tail chimera at the plasma membrane (1) leads to a dose-dependent suppression of integrin activation, probably by titration of the IAC. C: overexpression of the CD98hc (2) leads to the complementation of the dominant suppression caused by the TAC-β chimera. The authors showed that CD98hc is able to bind β-integrin tails (49, 194), which might be a possible mechanism for this complementation. Stoichiometry of Tac-β chimeras and Tac-β-CD98hc has not been determined experimentally. Adapted from Ref. 48.
More recently, Ito’s group (62) has concentrated its efforts on the osteoclast differentiation pathway. Anti-CD98hc MAbs induced homotypic cell aggregation and multinucleated giant cell formation of monocytes without any other fusogen (62). These polykaryocytes displayed several (but not all) of the exclusive features of the osteoclasts. Two recent reports have begun to delineate monocyte-to-osteoclast signaling pathways elicited by the anti-CD98hc MAbs. The first (103) highlights the importance of induction of c-src expression and activation by the MAbs. This protein kinase is widely believed to play a role in cell differentiation (17). Targeted disruption of the c-src gene causes a form of osteopetrosis whereby osteoclasts are present but inactive (165), indicating that c-src is involved in osteoclastogenesis. Transcription of c-src is dependent on Sp1, which is also upregulated by anti-CD98hc MAbs. The use of a panel of inhibitors suggested the involvement of a tyrosine kinase-Ras-Map kinase-Sp1 pathway in the anti-CD98hc MAb induction of c-src in monocytes. As expected, an anti-CD98hc MAb that inhibited polykaryocyte formation (in the presence of an anti-CD98hc active MAb) also suppressed Sp1 and c-src expression.

The second report links the two known routes of osteoclastogenesis, the CD98-mediated pathway and that mediated by the osteoclast differentiation factor (ODF; a member of the cytokine family) (106) by showing that the latter is suppressed by an inhibitory anti-CD98hc MAb and that the former is inhibited by osteoclast inhibitory factor (a secreted member of the tumor necrosis factor receptor family), the classic inhibitor of the ODF-mediated pathway. The expression of the ODF receptor increases on incubation with the active anti-CD98hc MAbs.

The authors did not investigate the role of the integrin system in osteoclast differentiation. One might speculate that similar results to those seen in viral fusion would have been observed, suggesting the intimate relationship between CD98 and integrins. In this sense, Suga and colleagues (168) reported recently that crosslinking of CD98 by MAbs mediated cell aggregation and adhesion of lymphocytes, most likely by increasing the avidity of the α1β2 integrin for intercellular adhesion molecule. The activation was dependent on phosphatidylinositol (PI)3-kinase and on the persistent activation of the Ras-related small GTPase Rap1. Moreover, in vitro fertilization of murine eggs (which express CD98 on the surface) is inhibited by anti-CD98hc antibodies. Fertilization is also inhibited by the recombinant soluble disintegrin domain of A disintegrin and metalloprotease-3 protein (10, 195), which seems to interact with β1-integrins on the egg surface. This interaction is also inhibited by anti-CD98 antibodies (170).

It becomes evident from the above results that the anti-CD98hc MAbs could somehow mimic natural ligands for this protein, involved in cell fusion and adhesion processes. CD98hc is a possible receptor for galectin-3 (44), a 26-kDa β-galactoside binding protein of the galectin family (65). This protein is secreted by monocytes/macrophages (151) and epithelial cells (96) and may have a role in cell-cycle control, prevention of T cell apoptosis, activation of several cell types, including lymphocytes and monocytes/macrophages, and as a mediator of cell-cell and cell-extracellular matrix adhesion (67, 86, 97). Very recently, galectin-3 has been shown to be a chemotaxtrant for monocytes and macrophages (144) and to induce uptake of extracellular calcium in T cells (43). More studies are needed to define the roles of galectin-3-CD98hc interactions and to identify other possible ligands of the CD98 complex.

T Cell Activation

Activation of T lymphocytes depends on two signals. One is mediated by the CD3 complex after interaction between the T cell receptor and the myosin heavy chain-peptide complex (90). The second signal is independent of antigen. This less-characterized signal can be mediated by different T cell membrane proteins and/or cell adhesion molecules and ligands, including antibodies against integrins (177, 189), together with anti CD3 antibodies. CD98 is involved in this costimulatory signal, because some anti-CD98 antibodies can costimulate T lymphocytes, whereas others can inhibit them (41, 111). Little is known, however, about the mechanisms of these effects. Recently, Warren et al. (183) screened antibodies for their ability to costimulate T cells together with anti-CD3 antibodies and found a new CD98-specific antibody. This antibody alone induced EDTA-sensitive aggregation of T cells, a typical feature of cellular adhesion. More importantly, anti-integrin antibodies that inhibit costimulation mediated by integrins, but not by nonintegrins, were able to inhibit anti-CD98 antibody-mediated costimulation, again indicating a functional interaction between CD98 and integrins.

Oncogenic Potential of CD98

Besides its expression in epithelial cells, activated B and T cells, and monocytes/macrophages, CD98hc is highly expressed in proliferative normal tissues and also in almost all tumor cells (125, 191). Overexpression of human and rat CD98hc has transforming activity in NIH3T3 cells (156). More importantly, this requires tight association with the LSHATs, because the rat CD98hc C103S mutant, which partially impairs association with light chain and amino acid transport, showed much lower reduced anchorage-independent growth than wild-type and C325S. The latter showed greater transforming activity than the wild-type control. Moreover, tumorigenicity of C103S transfecant Balb3T3 clones in nude mice was negligible compared with wild-type and C325S mutant. Amino acid transport activity and the proliferation rate of all transfectants remained the same, suggesting they are both dispensable for the tumorigenic effect. The enhanced effect of the C325S mutant might be caused by a conformational change caused by the mutation on the extracellular domain of CD98hc. The authors also reported (58) a positive correlation between progressive
COOH-terminal deletions of the CD98hc extracellular domain and tumorigenic potential. Anti-CD98hc MAb inhibits anchorage-independent growth of the mutants and wild-type (only until the epitopes recognized by the MAb were lost). At this point, it is pertinent to remember the dominant-negative behavior of the human C33OS mutant (homologous to the rat C32S mutant) on the anti-CD98hc-mediated enhancing effect on paramyxovirus syncitium cell formation (117). Whether this is related to its enhanced tumorigenic effect remains to be studied.

The question arises as to whether a similar mechanism of CD98 action underlies all of these different functions. A key CD98-integrin interaction may well be essential for cell fusion/differentiation, oncogenesis, and cell activation. Cytoplasmic and TM of CD98hc seem to play a role in inside-out integrin signaling, and the extracellular domain is important for amino acid transport (48, 49, 194), although this may be light-subunit specific (19). However, it is not clear whether association with the light chain is necessary for integrin activation mediated by CD98hc. The disulfide bond between heavy and light chains of CD98, but not amino acid transport, is important for the oncogenic potential of the protein, perhaps via some steric restrictions imposed by this link. An analogous uncoupling between amino acid transport activity and disulfide bond formation between heavy and light subunits has been reported by Wagner et al. (180): the human CD98hc-LAT-1 complex induced the expression of a nonselective cation channel in oocytes; the human CD98hc C109S mutant only slightly affected amino acid transport but totally inactivated the expression of the associated channel. Further experiments may elucidate the possible role of the integrin system in CD98-mediated osteoclastogenesis and tumorigenesis.

CELL PHYSIOLOGY OF THE LSHAT PROTEINS

LAT-1: Role in Normal and Tumorigenic Growth and Proliferation

LAT-1 protein was cloned as a truncated form (E16/T41), rapidly induced and degraded on stimulation of primary lymphocytes, a pattern of expression indicative of an early-activation antigen (55). It was later shown to be highly expressed in many tumor cell lines, in proliferating tissues, and in primary human tumors but at barely detectable levels in adult tissues except brain, ovary, and placenta. It was proposed as a possible tumor marker (188), and recently its message has been found to be differentially expressed in the hepatoblastoma cell line HepG2 after exposure to the teratogenic agent 2,3,7,8-tetrachlorodibenzo-p-dioxin (146), with a concomitant increase in system L transport. The identification of LAT-1 as an amino acid transporter (79, 101) has allowed the study of the regulation of LAT-1-mediated system L activity in a variety of cell systems and its relationship with cell proliferation and tumorigenesis.

LAT-1 mRNA and system L respond to the amino acid availability in primary rat hepatoma cells, suggesting adaptive regulation (157). A decrease in arginine, but not glutamine, levels leads to the upregulation of LAT-1 message and system L transport, but not of 4F2hc message. Neither other amino acids nor the changes in expression of other amino acid transporters (specifically other 4F2hc partners) were studied (25). Arginine is not a substrate of LAT-1, perhaps indicating a complex interplay between amino acid concentrations at either side of the membrane and the expression of the different amino acid transporters. Interestingly, arginine deprivation-induced upregulation was found in tumor cells and in γ-glutamyl-transpeptidase (GGT)-positive transformed cells, where constitutively high levels of LAT-1 mRNA and system L activity were found. These findings were extended in a recent paper in which the levels of LAT-1 and system L were compared in nontransformed mouse hepatocytes vs. NIH3T3 fibroblasts (26). On a cellular basis, LAT-1 and system L activity were lower in mouse hepatocytes, where system L activity was increased solely by the transfection of LAT-1. This indicates either that 4F2hc was not limiting in that system or that LAT-1 could displace other light chain binding to 4F2hc. The first possibility seems more likely because cotransfection of 4F2hc and LAT-1 did not increase the transport levels. LAT-1-transfected hepatocytes had a growth advantage in low-arginine medium compared to nontransfected controls. This growth advantage was not due to changes in the cell cycle and could be ascribed to different proliferation rates, saturation densities, adhesion, or cell survival, although no further experiments were performed to address these issues. The fibroblasts displayed higher levels of LAT-1 and system L, which were increased only by the cotransfection of both 4F2hc and LAT-1. The authors speculated that LAT-1 upregulation in low-arginine media and the constitutive high levels of system L in tumor cells may be adaptations (and a positively selected mutation in the tumors) to limited nutrient media, such as those in the tumor microenvironment, where amino acid availability is low (68). This is analogous to the role of GGT, which is upregulated early during hepatic carcinogenesis. In this microenvironment, levels of cystine are low and glutathione acts as a source of cysteine via GGT.

Masuko's group (58, 156) reported the oncogenic capacity of overexpressed wild-type and mutant forms of 4F2hc in NIH3T3 cells. This ability required a disulfide link with a light chain (Refs. 58 and 156 and see Oncogenic Potential of CD98), the identity of which was not investigated. A good candidate for this light chain could be LAT-1, given the high levels of system L in this system and the possibility that increases in system L give a growth advantage to the cells. However, no increase in amino acid transport was obtained on 4F2hc overexpression. Very recently, Brüeer and coworkers (19) have shown that truncated 4F2hc forms retaining the cysteine important for heterodimer formation are still fully able to bind LAT-1 (but not other isoforms, like LAT-2 and y’LAT-2) and induce system L transport in oocytes.
The possible link between 4F2hc tumorigenic potential and the growth advantage through LAT-1 overexpression needs further study. The different 4F2 complexes expressed at the plasma membrane should be quantified and correlated with amino acid transport activities. Heteromeric and monomeric (if it exists) 4F2hc protein levels should be compared on the surface and in intracellular membranes of normal and tumor cell lines and tissues. Similar considerations apply to adaptive regulation: many other proteins are also regulated by amino acid deprivation, including the CAT1 cationic amino acid transporter (2), aspartate synthase (56), ornithine decarboxylase (29), system A (16), c-jun and c-myc (66), etc. This is consistent, for instance, with the general upregulation of amino acid transport activities and protein synthesis in hepatic tumors (13) and with the positive effects of amino acid transport on tumor invasiveness and proliferation (160). The availability of transcriptomics and proteomics technology may help in delineating general responses to individual amino acid availability and in mapping changes in transporter expression in proliferating cells and tumors and during development. Such studies are needed to obtain a general picture of amino acid transport regulation, which will help in the rational design of experiments to uncover the signaling pathways for these effects (e.g., the intriguing upregulation of LAT-1 expression by low arginine levels).

Recently, a general response to amino acid deprivation has been found in yeast (98). In mammalian cells there appears to be a similar signaling pathway, with the PI3-kinase-related mammalian target of rapamycin (mTOR) as its key point (84). mTOR directly or indirectly regulates two translational regulators, eukaryotic initiation factor 4E binding protein (eIF-4E BP1) and p70 S6 kinase (see Ref. 140 for review). In general, amino acid deprivation deactivates p70 S6 kinase and dephosphorylates eIF-4E BP1 (both events need mTOR activity), leading to a decrease in the rate of translation (59). In most studies, the authors found that the amino acids having a major individual impact in mimicking the effect of general amino acid deprivation were branched amino acids, specially leucine (50, 190). To date, the only branched amino acid transporters are ATB0^+, a sodium- and chloride-dependent amino acid transporter, and some LSHAT proteins, such as LAT-1 and 2 and y^+LAT-1 and -2 (19, 79, 131, 162, 175). This prompted the idea that some 4F2 complexes might act as sensors of amino acid availability, although this speculation is not supported by evidence. Thus the pathway from amino acid deprivation to mTOR remains a mystery. Regulation of protein synthesis is also important at the whole body level. Insulin increases general translation in peripheral tissues after a meal (87). Amino acids, especially leucine and arginine, are required for this effect, which is again mediated by an mTOR-dependent pathway. Actually, amino acids alone can produce the same effect as insulin. This could have physiological significance especially after a high-protein meal, when amino acid levels in blood are increased and can substantially contribute to the increase in protein synthesis, besides the insulin effect (169). Moreover, the positive effect of branched-chain amino acids (specially leucine) on protein turnover in muscle is well known (22).

Finally, it is worth mentioning that an increase in protein synthesis is essential for entry to mitosis (94). It will be interesting to investigate whether this could relate to the effect of LAT-1 expression and activity on growth and proliferation.

**xCT: Protection Against Oxidative Stress**

In most mammalian cell lines (4, 5), \( \text{x}_\text{c}^- \) amino acid transport activity exchanges the anionic form of cysteine for glutamate with a 1:1 stoichiometry. This system accounts for the entry of cysteine and the exit of glutamate due to the very low intracellular levels of cysteine, which is rapidly reduced to cysteine and incorporated into glutathione and proteins, and the high intracellular levels of glutamate. Transport of cysteine is a rate-limiting step in glutathione synthesis (6). The activity of \( \text{x}_\text{c}^- \) is induced by agents that reduce intracellular glutathione, like oxygen and electrophilic agents, and by cystine depletion. In mouse macrophages, \( \text{x}_\text{c}^- \) activity is low, but it is activated by lipopolysaccharides (LPS), which also increases intracellular levels of glutathione (147).

Sato and colleagues (149) took advantage of this latter property for the expression cloning of the xCT protein that mediates \( \text{x}_\text{c}^- \) amino acid transport. Cloning provided the molecular tool for studying the regulation of \( \text{x}_\text{c}^- \) activity and its role in glutathione synthesis. xCT mRNA is highly expressed in the brain but is absent from other adult tissues. Activation of mouse peritoneal macrophages by LPS (and other agents) raises xCT mRNA levels with a concomitant increase in \( \text{x}_\text{c}^- \) activity. These increases are regulated by oxygen levels: hypoxia diminished xCT expression and activity, and LPS-induced transport increased until oxygen levels reached 20% and then began to decrease when they reached 50% (148). Response to oxygen was specific to xCT because LPS-induced arginine transport, mediated by MCAT-2B/system y^+ activity, was not sensitive to oxygen. Hypoxia also caused a decrease in glutathione content but not of glutamylcysteine synthetase activity, which points to cysteine transport via xCT as the rate-limiting step for the generation of glutathione. The authors conclude that xCT upregulation provides the antioxidant defense for macrophages, especially in regions of inflammation.

Nitric oxide (NO)-mediated upregulation of xCT has also been reported in other cell models (95). However, in LPS-activated macrophages, NO production is not induced, indicating that it is not involved in LPS activation of \( \text{x}_\text{c}^- \) activity. Analysis of the xCT promoter revealed putative nuclear factor-\( \kappa \)B and electrophilic response element (EpRE) binding sites, but their participation in LPS activation of xCT was excluded. On the other hand, diethylmaleate (an electrophil agent) induction of xCT transport is mediated, in part, by EpRE (71).
xCT in the brain is highly expressed in astrocytes, which protect neurons from damage (91), and coculture experiments have shown that neuronal glutathione is maintained by astrocytes (45). Moreover, embryonic neuronal/glial cultures depend on xₐ activity for viability (116). A recent report shows that L-lactate at high concentrations can act as a competitive inhibitor of xₐ transport in cultured rat astrocytes (88), concomitant with a decrease in glutathione content. The possible physiological importance of this observation stems from the known accumulation of lactate (up to 30 mM) in the brain during ischemia, head trauma, and hyperglycemia. A disturbed antioxidant capacity in neurons via decreased glutathione amounts in astrocytes could contribute to neuron injury.

**Transepithelial Transport of Amino Acids**

The reader is referred to the excellent review by Verrey and co-workers (179) on the role of HSHAT and LSHAT protein families in transepithelial transport. Therein, the role of the exchange mechanism and the asymmetry of transport by these proteins in an understanding of transepithelial transport is highlighted.

Most of our knowledge about transepithelial transport of amino acids comes from early experiments in vivo (using microperfused tubules, kidney cortex slices, membrane vesicles, etc.) (158). The results were interpreted in the light of experiments with heterologous expression of amino acid transporters mainly in the X. laevis oocyte system, immunolocalization and in situ hybridization studies in tissues, and genetic data from the study of cystinuria and lysinuria (see INHERITED AMINOACIDURIAS) (118, 121). However, there is still a gap between former physiological studies and the molecular biological approaches. For instance, despite the discovery of two cystinuria genes, reabsorption of cystine is still unsolved in molecular terms: high-affinity transport of cystine in the S3 segment of the nephron is mediated by rBAT, but the identity of its partner LSHAT is controversial because b₀⁺⁺AT is scarce in this segment (see Cystinuria). On the other hand, the protein responsible for the low-affinity transport of cystine at the S1 segment is not known. A good candidate is b₀⁺⁺AT, because it is expressed at the appropriate site; however, it mediates high-affinity transport when heterologously expressed with rBAT (which, in turn, is barely detectable in the S1 segment!) (see Ref. 47a for review). Cystine transport is ensured because of its rapid reduction to cysteine in the cytosol. To complete reabsorption, cysteine is transported, in part, to the blood through a basolateral transport system(s), which is unknown. Moreover, although cationic amino acids can be reabsorbed by apical b₀⁺⁺-like system in S3, a common apical influx of cystine and cationic amino acids is not demonstrated in S1 or S2 (see Ref. 124 for review), opening the possibility of a different apical cationic transporter in these segments.

Surprisingly, no polarized cell model has been used in a systematic way to study transepithelial transport in molecular terms. The well-known Madin Darby canine kidney cell model appears as one possibility: Verrey’s group has successfully stably expressed system b₀⁺⁺AT in these cells (127). A complementary approach is to study a polarized cell model in which these transporters are already present. Our group reported the expression of rBAT in the proximal tubule-like OK cells. The b₀⁺⁺-like transport activity was demonstrated in these cells, and it was shown to be dependent on the intrinsic asymmetry of the transporter. Therefore, a facilitative neutral amino acid transporter (T) must be present at the basolateral membrane. A tertiary active transport mechanism accounts for the reabsorption of dibasic amino acids (AA⁺⁺) and cystine (CysL) accounts for the high accumulation of neutral amino acids (AA⁺⁺) in the cell, which provide the driving force for the entry of cystine and dibasic amino acids through system b₀⁺⁺ (rBAT-b₀⁺⁺AT). Dibasic amino acid and cystine influx are favored by the negative membrane potential and the rapid reduction of cystine to cysteine (CSH), respectively. Net efflux of dibasic amino acids is accounted by exchange with neutral amino acids plus sodium via system y¹⁺L (4F2hc-y¹⁵LAT-1) at the basolateral membrane. The pool of intracellular neutral amino acids (including cysteine) can be exchanged with the extracellular neutral amino acid pool via the basolateral system L (4F2hc-LAT-2). As long as this exchange is 1:1, the neutral amino acid individual pools, but not the total pool, will change depending on the concentrations of the different amino acids at either side of the basolateral membrane and on the intrinsic asymmetry of the transporter. Therefore, a facilitative neutral amino acid transporter (T) must be present at the basolateral membrane to explain net transport of these amino acids. The opossum proteins that are at least partially cloned are shown inside white ovals. ATPase, Na⁺⁻K⁺ ATPase. Adapted from Ref. 105.
on rBAT expression using an antisense strategy (105). This demonstrates the usefulness of the model in the study of amino acid transport using conventional molecular biology techniques. Murér’s group (81) has also used these cells successfully for transient transfection of phosphate transporters to study its polarized transport. So far, we have found y’L and L activities on the basolateral side of OK cells that matched y’LAT-1 and LAT-2-induced activities in oocytes, respectively, and we have cloned the opossum cDNAs for those proteins by homology (Fernández E and Palacián M, unpublished observations). We are presently performing antisense experiments to elucidate the role of these transporters in transepithelial transport of amino acids in OK cells. Although the function of y’LAT-1 is obvious due to its identification as the LPI gene (see LPI), the function of LAT-2 is still unknown.

In Fig. 6, a model for OK transepithelial amino acid transport is shown. The cloned opossum transporters are indicated. As pointed out in Verrey’s review (179), at least a sodium-dependent, neutral amino acid transporter on the apical side and a net efflux transporter on the basolateral side have to be invoked to fully explain neutral and basic (and cysteine) transepithelial transport. There are some candidates for the first (162) but no clues for the efflux transporter. The model should apply to the kidney and most likely also to the intestine. The information available for other epithelial tissues, such as liver, placenta, and the blood-brain barrier, is scarce, although this situation is beginning to change (3, 89).

**Unexpected Substrates for LSHAT Proteins**

The substrate specificity of LSHAT proteins has been extended in intriguing ways. Transport of T3 and T4 hormones by oocytes cojected with 4F2hc and LAT-1 is saturable, with a Michaelis-Menten coefficient in the high-affinity range (<10 μM), and is inhibited by prototypic system L substrates, tryptophan, and the amino acid analog 2-amino-2-nbtorphan-carboxylic acid (BCH) (139). T3 and T4 are transported by other pathways [organic anion transporters Oatp (1) and Ntcp (51), and the recently cloned system T (85)], so the physiological relevance of the LAT-1 pathway is unclear. It should be remembered (see LAT-1: Role in Normal and Tumorigenic Growth and Proliferation) that LAT-1 and 4F2hc are highly expressed in proliferating cells and the key role of thyroid hormones in development is well known.

L-DOPA, a precursor of dopamine, seems to be transported by 4F2hc-LAT-1 and also by rBAT in different cellular models (77, 163). Especially relevant could be the transport of L-DOPA via LAT-1 at the blood-brain barrier, where LAT-1 is highly expressed (12). In a cell model of the blood-brain barrier (MBE4 cells), L-DOPA transport was strongly inhibited by Phe, Leu, and BCH, all LAT-1 substrates. In the kidney, dopamine is thought to be an autocrine-paracrine signal stimulating cAMP accumulation in tubule cells expressing L-amino acid decarboxylase (which converts L-DOPA to dopamine). BCH inhibited apical sodium-independent transport of L-DOPA in LLC-PK1 cells, indicating the presence of LAT-1or -2 at the apical border of these L-amino acid decarboxylase-positive cells. This finding is at odds with the known basolateral localization of 4F2hc, the partner of LAT-1 and -2, in tubule cells (135).

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