Function and structure of heterodimeric amino acid transporters

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Wagner, Carsten A., Florian Lang, and Stefan Bröer. Function and structure of heterodimeric amino acid transporters. Am J Physiol Cell Physiol 281: C1077–C1093, 2001.—Heterodimeric amino acid transporters are comprised of two subunits, a polytopic membrane protein (light chain) and an associated type II membrane protein (heavy chain). The heavy chain rbAT (related to b0,− amino acid transporter) associates with the light chain b0,+AT (b0,+ amino acid transporter) to form the amino acid transport system b0,+ whereas the homologous heavy chain 4F2hc interacts with several light chains to form system L (with LAT1 and LAT2), system y+,L (with y+LAT1 and y+LAT2), system x− (with xAT), or system asc (with asc1). The association of light chains with the two heavy chains is not unambiguous. rbAT may interact with LAT2 and y+LAT1 and vice versa; 4F2hc may interact with b0,+AT when overexpressed. 4F2hc is necessary for trafficking of the light chain to the plasma membrane, whereas the light chains are thought to determine the transport characteristics of the respective heterodimer. In contrast to 4F2hc, mutations in rbAT suggest that rbAT itself takes part in the transport besides serving for the trafficking of the light chain to the cell surface. Heavy and light subunits are linked together by a disulfide bridge. The disulfide bridge, however, is not necessary for the trafficking of rbAT or 4F2 heterodimers to the membrane or for the functioning of the transporter. However, there is experimental evidence that the disulfide bridge in the 4F2hc/LAT1 heterodimer plays a role in the regulation of a cation channel. These results highlight complex interactions between the different subunits of heterodimeric amino acid transporters and suggest that despite high grades of homology, the interactions between rbAT and 4F2hc and their respective partners may be different.

heavy and light chains; disulfide bridge; cystinuria; lysinuric protein intolerance

AMINO ACID TRANSPORTERS ACCOMPLISH in parallel with peptide transporters the uptake of amino acids from food in the small intestine, the release into blood, and subsequent uptake of amino acids from the blood into tissues such as liver or skeletal muscle or the reabsorption of amino acids from the urine along the kidney nephron. In the central nervous system, amino acid transporters regulate the transport of amino acids across the blood-brain barrier or are involved in the reuptake of neurotransmitter amino acids such as glycine, aspartate, or glutamate from the synaptic cleft and are important for the metabolic coupling of astrocytes and neurons. In the placenta, amino acid transporters supply the fetal blood with nutrients from the maternal side and help detoxify the fetal blood. Other amino acid transporters are involved in basic cellular functions such as cell volume regulation, the synthesis
of glutathione (GSH), the provision of amino acids for protein synthesis, and energy metabolism.

According to their specialized functions, amino acid transporters fall into different families that are distinguished by the functional properties (specificity of amino acids transported, transport mechanism, coupling to ions) and their molecular similarity or dissimilarity. Many of these amino acid transporters have been first described as transport systems in tissues or cell cultures and were only over the past few years identified on the molecular level. Very recently, a new large family of heterodimeric amino acid transporters has emerged that is unique in its functional and molecular properties. Unlike any other amino acid transporter known so far, these transporters consist of two subunits, a light chain and a heavy chain. Functionally, all heterodimeric amino acid transporters prefer antiport mechanisms leading to exchange of amino acids.

A few recent reviews described the cloning and some functional characteristics of the heterodimeric amino acid transporters (97, 149, 150). However, in the meantime, new subunits have been identified, and progress has been made identifying functional properties and understanding the physiological role of these transporters. This review will, therefore, give a short overview of the heterodimeric amino acid transporters known thus far, their functional properties, their postulated physiological role, their pathophysiological significance, and molecular interaction of the subunits. Because most transporters have been also cloned from human tissue, the discussion of functional properties of these clones will refer to data obtained from the human clones where possible.

Several nomenclatures have been proposed for the new subunits (117, 149). This review, however, will use the nomenclature most widely accepted, which is based on the nomenclature of the different amino acid transport activities as introduced and described by Christensen et al. (29, 30) and also used in a recent review by Palacin et al. (98). Besides historical reference, this nomenclature bears the advantage that the name of the transporter subunit indicates the transport activity expressed by the protein. In general, Na$^+$-independent transport activities are described with a capital letter, and Na$^+$-independent transport activities are described with a lowercase letter (note the exception of system L, which is Na$^+$ independent).

**STRUCTURAL FEATURES**

**The Heavy Chains rbAT and 4F2hc**

The first subunit of the heterodimeric amino acid transporter family to be identified was rbAT (related to $\text{b}^{0,+}$ amino acid transporter; see System $\text{y}^{+}$) (5), which was isolated by expression cloning independently by three groups (7, 138, 157). On the basis of its homology, the second heavy subunit, the 4F2 heavy chain (4F2hc), was identified (5, 158) and characterized as either part of system $\text{y}^{-}$L or system L (5, 15, 19, 20). However, 4F2hc had been previously identified and cloned as a surface antigen on activated lymphocytes and was later renamed CD98 (62, 76, 114, 139).

Two transcripts of rbAT mRNA have been identified: a short (~2.3 kb) and a long (~4 kb) transcript differing in 3’-untranslated region (79, 159). The coding sequence of both variants is identical.

The human heavy chain rbAT (SLC3A1) and 4F2hc (SLC3A2) consist of 685 (~85 kDa, glycosylated; 78 kDa, unglycosylated) and 529 (~94 kDa, glycosylated; 72 kDa unglycosylated) (5–7, 54, 87, 98, 155, 157, 158) amino acids, respectively, and have an identity of ~30% (~50% similarity) (5). Analysis of the amino acid sequences suggests that both heavy chains have only one transmembrane helix, that the NH$_2$ terminus is intracellularly localized (5, 7), and, hence, the COOH terminus is extracellularly located. Both proteins have, therefore, been classified as type II membrane proteins. The topology has not been rigorously probed. Studies with epitope-specific antibodies suggest that rbAT may have four transmembrane helices (86). Ample glycosylation of rbAT and 4F2hc clearly indicates that a large part of the COOH terminus is located extracellularly. More studies to resolve this problem are needed. A part of rbAT and 4F2 is structurally related to glycosidases, but glycosidase activity has not yet been demonstrated (157).

On the basis of the structural model of only one transmembrane helix that seemed to be unable to form a transmembrane transporter pore and the lack of amino acid transport activity upon expression of 4F2, and, in particular, rbAT in most cell lines, it was soon postulated that additional subunits were needed for a functional amino acid transporter (18, 45, 98, 99).

**The Light Chains**

Seven lights chains (LAT1, LAT2, $\text{y}^{-}$LAT1, $\text{y}^{-}$LAT2, ascAT1, xCT, and $\text{b}^{0,+}$AT) have been identified (see also Figs. 1 and 2 and Table 1). On the basis of homology and hydropathy plots, the light chains have 12 transmembrane domains with the NH$_2$ and COOH termini located intracellularly (3, 4, 25, 47, 52, 68, 80, 103, 104, 108, 116, 117, 121, 126, 130, 142). Although the topology has not been addressed experimentally, it is beyond doubt that in contrast to the heavy chains, the light chains display the typical helix bundle structure of membrane transport proteins. The light chains belong to the large superfamilly of amino acid/polyamine/choline transporters that occur in nematodes, yeast, bacteria, and mammals (for review, see Ref. 149 and http://www.biology.ucsd.edu/~msaier/transport/).

The most closely related family of transporters related to the light subunits is the family of CAT transporters encoding cationic amino acid transporters of the system $\text{y}^{-}$ family (149). Despite the sequence similarity, no interaction between CAT family transporters and 4F2hc or rbAT has been observed (Brüer, unpublished data). In contrast to the light chain family members, CAT-type transporters are thought to span the membrane 14 times. The first 12 helices are similar in both families, suggesting that the CAT transporters have
Fig. 1. Alignment for human light chains of heteromeric amino acid transporters (Genpept Database, Australian National Genomic Information Service). For the amino acid sequence and alignment of the heavy chain b0,\textsubscript{AT} (related to b0,\textsubscript{amino acid transporter}) and 4F2hc, see Palacin et al. (98). LAT1 (accession no. AAD20464), LAT2 (accession no. AAF20381), y\textsubscript{LAT1} (accession no. AAC83706), y\textsubscript{LAT2} (accession no. BBA13376), b0,\textsubscript{AT} (accession no. AAD55898), asc1 (accession no. BAB03213), and xCT (accession no. BAA82628). The following codes are used in the consensus line below the alignment: uppercase letters, conserved residues; lowercase letters, residues conserved in all but 1 sequence; 3, serine or threonine; 4, lysine or arginine; 5, aromatic amino acid; 6, branched chain amino acid; \#cysteine conserved in all light chains involved in the disulfide bridge with the respective heavy chain.
two additional helices on the COOH terminus. Whether these allow 4F2hc-independent trafficking is not known. All light subunits share at least 40% identity (see Fig. 2) with the largest differences in the NH₂ and COOH termini (Fig. 1). Little information on functionally or structurally important residues is available at this point. Mutation of G54 to valine and L334 to arginine in the y¹LAT1 light chain generates a non-functional transporter, thereby causing the phenotype of lysinuric protein intolerance (see Lysinuric Protein Intolerance). The protein is still translocated to the plasma membrane. Both residues are highly conserved in all light chain sequences; G54 resides in helix 1, whereas L334 is located in the loop between helices 8 and 9 (47, 89). Several mutations in the b₀⁺AT subunit causing non-type I cystinuria lead also to highly diminished or completely abolished transport activity in rbAT-b₀⁺AT-transfected cells (48, 50).

Interactions Between the Subunits

The most prominent structure involved in the interaction between heavy and light chains is a disulfide bridge between C109 in 4F2hc and C114 in rbAT, respectively, and a conserved cysteine between the transmembrane helices TM3 and TM4 in the different light subunits (80, 90, 105, 155). However, the functional role of the disulfide bridge remains elusive. It appears to be needed neither for the trafficking of the transporter to the membrane nor for the function of the amino acid holotransporter, as shown for the 4F2hc/LAT1 and rbAT heteromers expressed in Xenopus oocytes (34, 90, 105, 154). Pfeiffer et al. (105) found a

Fig. 2. Family tree of the light subunits of the heteromeric amino acid transporters. Range of homology is denoted by bar (10% homology).

Table 1. Amino acid transport systems expressed by members of the heteromeric amino acid transporter family

<table>
<thead>
<tr>
<th>Transport System</th>
<th>Light Subunit</th>
<th>Heavy Subunit</th>
<th>Tissue/Localization</th>
<th>Function</th>
<th>Pathophysiology</th>
<th>Reference No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>L LAT1</td>
<td>4F2hc (SLC3A2)</td>
<td>Kidney, spleen, thymus, liver, small intestine, placenta, testis, brain, heart, lung, blood-brain barrier, leukocytes/basolateral</td>
<td>Na⁺-independent exchange of large neutral aa, transport of thyroid hormones; BCH inhibitable</td>
<td>9, 66, 68, 90, 112, 121</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L LAT2</td>
<td>4F2hc (SLC3A2)</td>
<td>Kidney, placenta, ovary, small intestine, brain, liver, spleen, prostate, testis, skeletal muscle, heart, lung/basolateral</td>
<td>Na⁺-independent exchange of smaller neutral aa; BCH inhibitable</td>
<td>4, 108, 116, 121</td>
<td></td>
<td></td>
</tr>
<tr>
<td>y¹LAT1</td>
<td>4F2hc (SLC3A2)</td>
<td>Kidney, small intestine, leukocytes, lung, erythrocytes, placenta/basolateral</td>
<td>Na⁺ + large neutral aa/dibasic aa exchange; Lysinuric protein intolerance (y¹LAT1)</td>
<td>11, 104, 142</td>
<td></td>
<td></td>
</tr>
<tr>
<td>y¹LAT2</td>
<td>4F2hc (SLC3A2)</td>
<td>Brain (glia, neurons), small intestine, testis, parotis, heart, kidney, lung, liver/basolateral</td>
<td>Na⁺ + neutral aa/dibasic aa exchange; glutamine/arginine exchange</td>
<td>16, 142</td>
<td></td>
<td></td>
</tr>
<tr>
<td>xᶜ xCT</td>
<td>4F2hc (SLC3A2)</td>
<td>Macrophages, liver, kidney, brain, retinal pigment cells/basolateral</td>
<td>Glutamate/cystine exchange</td>
<td>3, 12, 126</td>
<td></td>
<td></td>
</tr>
<tr>
<td>asc ascAT1</td>
<td>4F2hc (SLC3A2)</td>
<td>Brain, lung, placenta, small intestine, kidney/basolateral</td>
<td>Na⁺-independent exchange of small neutral aa, also D-serine, D-glycine</td>
<td>52, 91</td>
<td></td>
<td></td>
</tr>
<tr>
<td>b₀⁺⁺ b₀⁺⁺AT</td>
<td>rbAT (SLC3A1)</td>
<td>Kidney, small intestine, brain/apical</td>
<td>Na⁺-independent exchange of neutral/dibasic aa; cystine, arginine, lysine, and ornithine reabsorption; Cystinuria type I (rbAT) and non-type I (b₀⁺⁺AT)</td>
<td>25, 47, 103, 117</td>
<td></td>
<td></td>
</tr>
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</table>

All transport systems are composed of 2 subunits, a light chain and a heavy chain. The name in parentheses gives the Human Genome Organization nomenclature for the respective gene. BCH, 2-aminobicyclo(2,2,1)heptane-2-carboxylic acid; aa, amino acid.
reduction in expressed transport activity after abolishing the disulfide bridge between 4F2 and Xenopus LAT1; however, other groups could not find such a decrease in transport activity or surface expression (90, 154). Surprisingly, it appears that the disulfide bridge allows crosslinking to light chains, which may not be the physiological partners of the corresponding heavy chain. For example, 4F2hc, when overexpressed, interacts with \(^{b_0}AT\) in mammalian cells (115, 117) and with the oocyte endogenous \(^{b_0}AT\)-like light chain (15). The physiological significance of these observations has yet to be established because 4F2hc has a predominantly basolateral localization, whereas \(^{b_0}AT\) is found on the apical membrane (see System \(^{b_0}\)). The function of the disulfide bridge became more obvious when progressive COOH-terminal truncations of the rat \(^{b_0}AT\) were studied (34). A truncation (\( \Delta 615-683 \)) initially led to the complete abolishment of transport activity, which was resumed with further truncations (\( \Delta 588-683 \)) and eventually ceased again with larger truncations (\( \Delta 508-683 \)). In contrast to the wild type, the truncated protein that showed transport activity (\( \Delta 588-683 \)) was very sensitive to the mutation of the disulfide bridge forming cysteine (34). The effect can be rationalized, assuming that the COOH terminus is involved in noncovalent binding of the light chain. Once the binding domain has been destroyed, the covalent disulfide bridge, although less discriminatory, becomes crucial for the interaction. In contrast, Miyamoto et al. (84) performed similar experiments, producing larger truncations of human \(^{b_0}AT\) with different results. The smallest truncation (\( \Delta 511-685 \)) was the only truncation inducing transport activity. However, the transport activity resembled system \(y^1L\) rather than \(^{b_0}AT\), showing \(Na^+\)-independent arginine and \(Na^+\)-dependent leucine transport. Also, Peter et al. (101) reported on a short COOH-terminal rat \(^{b_0}AT\) truncation producing \(y^1L\) activity. Again, these data suggest that once the COOH terminus is removed, recognition of the \(^{b_0}AT\)-like light chain is abolished, and other light chains are bound instead via the less specific disulfide bridge (Fig. 3). Overexpression fosters this effect, even in the presence of wild-type heavy and light chains. In line with these observations, a \(Na^+\)-dependent transport activity has been detected in Xenopus oocytes expressing \(^{b_0}AT\), the properties of which are at variance with the characteristics of all light chains cloned to date. The physiological correlate of this transport activity is not known (Ref. 34 and Wagner, unpublished data). Moreover, \(^{b_0}AT\) is able to translocate light chains LAT1, LAT2, and \(y^1LAT1\) to the plasma membrane when coexpressed with these light chains in oocytes, although these proteins are thought to reside on different membranes in vivo. Mutation of the \(^{b_0}AT\) C114 residue abolishes the partial \(Na^+\) dependence and unspecific interaction with LAT1 and \(y^1LAT1\) without altering the properties of the \(^{b_0}AT\) transporter. The conservation of the cysteines involved in the formation of the disulfide bridge in all heavy and light chains, however, suggests that the observed promiscuity could have physiological relevance. A likely place for the occurrence of these unusual heterodimers is in nonepithelial cells expressing different combinations of heavy and light chains (see FUNCTIONAL ASPECTS).

The significance of the heavy chain COOH terminus in light chain recognition is supported by functional analysis of truncated versions of 4F2hc. Removal of the COOH-terminal 70 amino acids caused a complete loss of interaction with light chain \(y^1LAT2\) and \(LAT2\). By contrast, light chain LAT1 was still translocated to the plasma membrane by a 4F2hc remnant of 133 amino acids, almost as efficient as by the wild-type protein (14), suggesting that recognition of the several 4F2hc-associated light chains requires different domains. Further experiments indicated that the extracellular portion, but not the transmembrane or cytoplasmic parts of 4F2hc, is necessary for interaction with LAT1 (49). The cytoplasmic and transmembrane regions, however, seem to be essential for interaction with integrins (Ref. 49, see REGULATION OF HETERODIMERIC AMINO

Fig. 3. Proposed hypothetical model for the interaction of \(^{b_0}AT\) heavy chain with its light chain \(^{b_0}AT\). The light chain \(^{b_0}AT\) spans the membrane 12 times, whereas the heavy chain \(^{b_0}AT\) only once. Both subunits are covalently linked by a disulfide bridge. Additional interactions between the 2 subunits occur between the COOH terminus of \(^{b_0}AT\) and yet unidentified sites in the \(^{b_0}AT\) subunit. These interactions contribute to the functional characteristics of system \(^{b_0}\).
Acid Transporters and Possible Role in Cell Growth, Differentiation, and Cancer.

Recent observations suggest that the disulfide bridge is important for the regulation of a 4F2-associated cation channel (154). The pharmacology of this cation conductance was unique and resembled only partly the other described cation conductance identified at the basolateral site of the distal tubule and A6 cells (21, 151). In contrast with other transporter-associated conductances (e.g., 17, 46), the 4F2/LAT1-induced cation conductance is not gated by transporter substrates, suggesting either two different permeation pathways or the induction of an endogenous Xenopus cation channel.

Together, it appears that noncovalent bonds dominate the interaction between light and heavy chains. The function of the disulfide bridge remains elusive. It may stabilize the complex in the plasma membrane or could be involved in regulation of associated proteins.

Most mutations in rbAT affect the trafficking of the associated light chain to the plasma membrane, whereas mutations in the b0,AT light chain result in nonfunctional transporters (47). Similarly, 4F2hc translocates as many as six different light chains to the plasma membrane, each displaying different substrate specificity. Hence, it seems to be obvious that the specificity and the characteristics of the respective amino acid transport systems are mainly determined by their light chains. However, recent investigations of rbAT mutants suggest that the rbAT heavy chain may also be involved in or modulate the transport characteristics of the holotransporter. Two mutations of the rbAT subunit alter the exchange mode and the apparent affinity for neutral amino acids of the system b0,AT transporter. One of these mutations changes a COOH-terminal cysteine residue, C673, and alters the apparent affinity for neutral amino acids. In agreement with these results, it was found that mutation of cysteines in the COOH-terminal part of rbAT rendered amino acid transport less sensitive to inhibition by N-ethylmaleimide, which suggests that these cysteines may form part of the translocation pathway (102).

Further evidence for the involvement of rbAT in the transport process is the observation that the kinetic constants and substrate specificity of the b0,AT-mediated amino acid transport differ depending on the coexpressed heavy chain (115, 117). When coexpressed with 4F2 in mammalian cells, b0,AT-mediated glutamine transport, whereas when coexpressed with rbAT, glutamine was not recognized as a substrate. Michaelis-Menten constant (Km) values for all substrates were significantly lower in the presence of 4F2 compared with coexpression with rbAT (115).

Although more difficult to rationalize, but in line with the notion of rbAT being a functional domain of the b0,AT transporter, it was found that mutations in b0,AT causing non-type I cystinuria were all located in the transmembrane or cytosolic domains. This could suggest that the extracellular loops of the b0,AT light chains are less important for transport function than some rbAT mutations. Moreover, all light chains are nonglycosylated, although the corresponding motifs occur in extracellular loops (67).

Functional Aspects

System L

System L conveys the Na+-independent transport of large branched and aromatic neutral amino acids in almost all types of cells. System L is differentiated against related transporters by its ability to transport the two model substances, 2-aminobicyclo-(2,2,1)heptane-2-carboxylic acid (BCH) and 3-aminobicyclo(3,2,1)octane-3-carboxylic acid. On the basis of the affinity for its substrate, two subtypes have been described; L1, with a high affinity in the micromolar range, and L2, with a lower affinity in the millimolar range (156). System L transport activity has been described in a variety of cells and organs.

The first evidence that system L is associated with expression of 4F2 came from Xenopus laevis oocyte expression experiments in which Bröer et al. (19, 20) observed that poly(A) RNA-induced system L activity was abolished by 4F2hc antisense cRNA or antisense oligonucleotides. In 1998, two groups independently identified the first light subunit interacting with 4F2 to form system L (68, 80). Kanai et al. (68) used an expression cloning approach, whereas Spindler et al. (134) identified system L while screening for aldosterone-induced genes in the Xenopus A6 cell line. The isolated amino acid permease-related gene (ASUR4) did not induce any transport activity upon expression in Xenopus oocytes. When coexpressed with 4F2, however, system L activity was induced (80). The cloned transporter was eventually named LAT1. Subsequently, a second cDNA was identified, and, on the basis of its homology, named LAT2 (4, 108, 116, 121, 130). Other groups identified LAT1 as the light chain forming part of the CD98 lymphocyte surface antigen (78, 90, 145).

LAT1 is widely expressed in nonepithelial cells such as brain, spleen, thymus, testis, skin, liver, placenta, skeletal muscle, and stomach (68, 90, 112). In the brain, LAT1 expression is found predominantly in the endothelial cells forming the blood-brain barrier (9, 66). The human LAT1 forms a protein of 507 amino acids, having a molecular mass of 55 kDa (68, 80, 112). 4F2/LAT1 induces Na+-independent transport of large neutral amino acids with K_m values in the micromolar range (68, 80, 112). Amino acid transport is inhibited by BCH, as expected for system L (68, 80). The K_m values differ depending on the site of the membrane studied. Phenytoylalnine appears to be a good release substrate but leucine a very poor one, whereas for uptake, leucine is a very good substrate (150). All studies indicate that LAT1 is an obligatory exchanger, at least when expressed in oocytes.

4F2 brings LAT1 to the plasma membrane. In the absence of 4F2, LAT1 is found in intracellular compartments, whereas 4F2 can reach the plasma membrane independently (80, 90). The pattern of surface expression of 4F2 was altered in the presence of LAT1, confining it to cell-cell adhesion sites (90).
The second isoform for system L, LAT2, a protein of 535 amino acids, is highly expressed in polarized epithelia, suggesting an important role in transepithelial amino acid transport (4, 108, 116, 121, 130). The mRNA was identified in kidney, small intestine, testis, prostate, ovary, brain, skeletal muscle, and placenta. Immunolocalization showed basolateral staining in the mouse kidney proximal tubule and small intestine and colocalization with 4F2hc (121). The heavy subunit 4F2 is necessary for trafficking of LAT2 to the oocyte membrane (108, 130). Expression of LAT2 and 4F2 induces amino acid transport with system L characteristics, namely, $Na^+$-independent transport of neutral amino acids sensitive to classic system L inhibitors (108, 121, 130). However, amino acid specificity and apparent affinity are different for LAT2 compared with LAT1. LAT2 also transports small neutral amino acids such as $L$-alanine, $L$-glycine, $L$-cysteine, and $L$-serine and also glutamine, all of which are poor substrates for LAT1. The apparent affinities for all substrates are severalfold higher compared with those of LAT1 (108, 116, 121, 130). Similar to LAT1, several features of the LAT2 transporter are not fully understood and are at variance with observations in mammalian cells. Some substrates, particularly leucine, show lower affinity for LAT2 than for LAT1, suggesting that LAT2 might be the correlate of the system L2 defined in the liver (108). Expression of LAT2 and 4F2 induces amino acid transport with system L characteristics, namely, $Na^+$-independent transport of neutral amino acids sensitive to classic system L inhibitors (108, 121, 130). However, amino acid specificity and apparent affinity are different for LAT2 compared with LAT1. LAT2 also transports small neutral amino acids such as $L$-alanine, $L$-glycine, $L$-cysteine, and $L$-serine and also glutamine, all of which are poor substrates for LAT1. The apparent affinities for all substrates are severalfold higher compared with those of LAT1 (108, 116, 121, 130). Similar to LAT1, several features of the LAT2 transporter are not fully understood and are at variance with observations in mammalian cells. Some substrates, particularly leucine, show lower affinity for LAT2 than for LAT1, suggesting that LAT2 might be the correlate of the system L2 defined in the liver (108). However, studies in mammalian cells indicate $K_m$ values in the millimolar range, whereas those measured for LAT2 are still well below 1 mM for most substrates (121, 130). Moreover, it is controversial whether or not LAT2 mediates uniport of amino acids (108, 116, 121, 130). Considering the basolateral expression in polarized epithelia as the proximal tubule and small intestine and the ability of LAT2 to transport small neutral amino acids, a role in the transepithelial transport of these amino acids has been suggested (108, 121, 130). In particular, the transport of cysteine could provide the basolateral efflux pathway of this amino acid, which is intracellularly formed from cystine taken up by the apical system $\mathbf{b}^0$ that is defective in cystinuria (108).

In the placenta, system L activity was identified at the apical and basolateral sites of the syncytiotrophoblast, with characteristics of LAT1 at the apical site and LAT2-mediated transport at the basolateral site. However, there has been no protein localization carried out to confirm the apical localization of LAT1. The transport of tryptophan by system L is an important feature of the syncytiotrophoblast. This amino acid is needed as a substrate for the enzyme indoleamine 2,3-dioxygenase, which is responsible for suppressing the maternal immune response to the allogenic murine fetus (72, 73). L-Methyltryptophan, a blocker of indoleamine 2,3-dioxygenase activity, is a transport inhibitor of system L-mediated tryptophan uptake; blocking of system L by other inhibitors also abolished indoleamine 2,3-dioxygenase activity, suggesting that system L-mediated tryptophan uptake is rate limiting for the activity of the enzyme and plays an important role in maintaining pregnancy (72, 73).

System $\mathbf{y}^+\mathbf{L}$

System $\mathbf{y}^+\mathbf{L}$ was first functionally described in erythrocytes (37, 39). Further investigation and reevaluation of earlier data revealed its presence in placenta (42, 53, 94), platelets (82), skin fibroblasts (33, 131), hepatocytes (118), small intestine (36), and kidney (118, 119). In polarized epithelia, system $\mathbf{y}^+\mathbf{L}$ is basolateral and allows for the efflux of dibasic amino acids in exchange for preferably large neutral amino acids and $Na^+$. The transport of dibasic amino acids occurs with high affinity with apparent $K_m$ values in the range of 5–10 µM (38, 39). In the absence of $Na^+$, exchange of intracellular dibasic with extracellular dibasic amino acids occurs (39). Neutral amino acids are only poorly transported in the absence of $Na^+$, and the transporter is less able to differentiate different neutral amino acids. $Li^+$ potentiates neutral amino acid binding but there is also a loss in selectivity (38). This transport pattern distinguishes system $\mathbf{y}^+\mathbf{L}$ from the dibasic amino acid transport system $\mathbf{y}^+$ (38, 39). On a molecular basis, two isoforms of system $\mathbf{y}^+\mathbf{L}$ have recently been identified, $\mathbf{y}^+\text{LAT1}$ and $\mathbf{y}^+\text{LAT2}$ (104, 142). Both subunits interact with the 4F2hc to form the systems $\mathbf{y}^+\text{L1}$ and $\mathbf{y}^+\text{L2}$ (16, 45, 67, 104, 142).

Human $\mathbf{y}^+\text{LAT1}$ is a protein consisting of 511 amino acids with a predicted molecular mass of ~56 kDa (104, 142). Its mRNA was found mainly in kidney and small intestine, and, to a lesser extent, in peripheral blood lymphocytes, liver, pancreas, epididymis, testis, ovary, placenta, lung, and thyroid. Functionally, $\mathbf{y}^+\text{LAT1}$ obeys an obligatory exchange mechanism, transporting dibasic amino acids in the absence of $Na^+$ and neutral amino acids in the presence of $Na^+$ (67, 104, 142). $Na^+$ serves to increase the affinity for neutral amino acids without altering the maximal velocity of the transporter (67, 142). In the absence of $Na^+$, protons may substitute for $Na^+$ and drive transport, also increasing the affinity for neutral amino acids (67). $Na^+$ and $H^+$ are both cotransported in a 1:1 stoichiometry with neutral amino acids. However, $H^+$ transport occurs only under nonphysiological conditions, i.e., in the absence of extracellular $Na^+$ (67). It seems that $\mathbf{y}^+\text{LAT1}$ preferentially mediates the efflux of arginine, which may be important in the kidney, where arginine is produced from citrulline and released into the blood to supply the rest of the body with this amino acid (16, 104). Mutations in $\mathbf{y}^+\text{LAT1}$ cause the autosomal recessive disease lysinuric protein intolerance (see Lysinuric Protein Intolerance) (10, 143).

The second isoform, $\mathbf{y}^+\text{LAT2}$, constitutes a protein of 515 amino acids with ~56 kDa molecular mass (142). Expression (mRNA) was found in brain, testis, and parotis and was found weaker in small intestine, kidney, and heart. A very weak signal was obtained from lung and liver (16). In the brain, $\mathbf{y}^+\text{LAT2}$ was expressed in both neurons and astrocytes. Expression was much stronger in the embryonic brain and declined in adult brain (16). Recently, expression in skin fibroblasts has been reported (33). $\mathbf{y}^+\text{LAT2}$ displays the same mechanism as $\mathbf{y}^+\text{LAT1}$ but differs in sub-
strate specificity (16). Besides cationic amino acids, it prefers large neutral amino acids with bulky side chains. The transport of y⁺LAT2 displays strong asymmetry for its substrates: arginine is a good substrate for uptake and efflux, whereas leucine and glutamine are easily taken up but are not released. For those substrates, a 1:1 stoichiometry was measured. This points to a function of y⁺LAT2 as an electroneutral arginine/glutamine exchanger (16). y⁺LAT2 bears some resemblance to system x⁻ (see System x⁻) in that it is able to transport glutamate at a low rate. Considering its pattern of expression, y⁺LAT2 may play a role in neurons taking up glutamine as a precursor for glutamate synthesis. It could also play a role at the blood-brain barrier by mediating arginine uptake to meet brain requirements in exchange for glutamine to maintain nitrogen balance. However, expression in endothelial cells has yet to be proven (16).

Neither of the two cloned isoforms of system y⁺L display the high affinity for dibasic amino acids as reported initially from erythrocytes and other tissues (16, 38, 105, 142). Furthermore, erythrocytes from patients with a genetic defect in the SLC7A7 gene encoding for y⁺LAT1 (lysinuric protein intolerance) have normal system y⁺L activity and do not express the second y⁺L isoform y⁺LAT2 (11). This suggests that a third, not yet identified, isoform of system y⁺L may exist.

System asc

The first description of system asc came from erythrocytes, where it was identified as a Na⁺-independent transport system for short chain neutral amino acids (2, 43, 146). The identification of the 4F2 light chain (4F2lc) family eventually resulted in the isolation of a new light chain with properties close to those expected for system asc and was hence named asc1 (52). The asc1 cDNA encodes a protein of 523 amino acids with a molecular mass of ~53 kDa and is most closely related to LAT2 (see Fig. 1). The human gene was mapped to chromosome 19q12-q13.1 (91). RNA was found in human kidney, brain, placenta, heart, skeletal muscle, lung, liver, and pancreas (91). Studies on the mouse isoform indicate expression in brain, kidney, placenta, small intestine, and lung (52). However, expression appears to be strongest in brain and kidney.

System asc conveys the Na⁺-independent high-affinity transport of small neutral amino acids such as L-serine, L-alanine, L-cysteine, L-glycine, and L-threonine. The transport is strongly inhibited by different alanine analogs such as 2-aminoisobutyric acid, which is also a substrate. However, α-(methylamino)isobutyric acid has no effect on transport (52). Functionally important, system asc also accepts, with a high affinity, the D-isomers D-serine and D-alanine. D-Serine is also a substrate. However, since it is required for the activation of the glutamate N-methyl-d-aspartate receptor. Furthermore, L-serine and L-glycine may play an important role as trophic factors for cerebellar Purkinje cells (55). Importantly, the affinity of asc1 for D-serine lies within the physiological concentration of D-serine in the cerebrospinal fluid (52). In contrast to all other members of the 4F2lc family, significant uniport activity was detected in asc1/4F2hc-expressing Xenopus oocytes (52).

System x⁻

GSH is a key element for intracellular buffering of free radicals. Its synthesis depends critically on the availability of cystine and cysteine, which needs to be taken up by the cell. System x⁻ is the major amino acid transport system providing the cell with cystine in exchange for glutamate in a Na⁺-independent fashion (29). Chemical or physical stress has been shown to induce the activity of this system in a variety of cells such as macrophages, neuronal or glial cells, fibroblasts, kidney cells, pancreas cells, and hepatocytes (65, 125). Because the uptake of cystine and its subsequent reduction to cysteine are the rate-limiting step for glutathione synthesis, system x⁻ activity directly regulates intracellular GSH concentrations (126). Cystine is only transported in its anionic form; therefore, cystine/glutamate transport by system x⁻ is electro-neutral (126).

Expression cloning identified xCT and 4F2hc as the two subunits forming system x⁻ (126). xCT has 501 amino acids, a molecular mass of ~55 kDa, and is predicted to have 12 transmembrane domains (3, 12, 126). xCT was transcriptionally upregulated after the chemical reduction of cellular GSH by treatment with lipopolysaccharide or by nitric oxide in murine macrophages and retinal pigment cells (12, 124). Induction in macrophages was also dependent on ambient oxygen concentrations (124). As expected, expression of 4F2 and xCT induced Na⁺-independent exchange of glutamate and cysteine; other amino acids were poor substrates for the obligatory exchanger (3, 126).

System b⁰⁺

System b⁰⁺ was first described by Van Winkle et al. (147, 148) in mouse blastocysts as a Na⁺-independent transport system for neutral and dibasic amino acids. It has attained much interest over the last few years because a defect in human kidney system b⁰⁺ causes the inherited hyperaminoaciduria cystinuria (see Cystinuria). Human b⁰⁺ transport activity is characterized by the Na⁺-dependent transport of neutral and dibasic amino acids, including cystine, which is not a substrate of the mouse b⁰⁺ system in blastocysts (148). Vesicle studies from proximal tubules and microperfusion experiments demonstrated that a high-affinity system for cystine was shared with dibasic amino acids, and a low-affinity transport system was not shared (51, 81, 129, 152, 153). In the jejunum, only one common high-affinity system was described (96). These studies agree with flux studies demonstrating the expression of a b⁰⁺-like transport activity in the small intestine (77, 88). The high-affinity transport system in the kidney involved heteroexchange of cysteine for lysine (81) and was localized to the straight
portion of the proximal tubule (S3 segment) (127). Cystine transport competed with several neutral amino acids (152, 153). It was also concluded that cysteine was not a substrate of these transport systems and was taken up by an alternative system and/or converted to cystine in the lumen (152, 153).

On a molecular basis, rbAT (also named NBAT or D2) was first identified by expression cloning as a possible subunit of this system (6, 7, 74, 138, 157). Expression of rbAT in oocytes induced Na\(^+\)-independent transport of neutral and dibasic amino acids and cystine. The transport system that is induced by rbAT expression in oocytes has a high affinity for dibasic amino acids and cystine in the 20–80 \(\mu\)M range and a lower affinity for neutral amino acids in the 0.2 mM high millimolar range (6, 7, 22, unpublished data). The fact that the transport of neutral amino acids caused outward currents (net outward movement of positive charges) and the transport of dibasic amino acid caused inward currents (net inward movement of positive charges) led to the discovery that system \(b_0^{+}\) was an obligatory exchanger (22, 27, 31). The transport of cystine and the suppression of cystine transport in the OK cell line after a partial knock out of rbAT (85) led to the subsequent identification of rbAT as a gene involved in cystinuria (see below).

rbAT interacts with the recently cloned light chain \(b_0^{+}\)AT to form the complete transporter for system \(b_0^{+}\) (25, 48, 103). The human \(b_0^{+}\)AT protein has only 487 amino acids, with a predicted molecular mass of 54 kDa (25, 47, 103, 117). \(b_0^{+}\)AT mRNA is expressed in kidney and small intestine, and, to a smaller extent, in heart, liver, placenta, and lung (25, 47, 103, 117). Immunostaining in the kidney, however, revealed different localization along the nephron. rbAT protein was found in the apical membrane of the renal proximal tubule, increasing from the S1 to the S3 segment and in the microvilli of the small intestine (25, 69, 87, 103, 106). \(b_0^{+}\)AT protein, however, is also expressed in the apical membrane of the proximal tubule, but expression levels decrease from the S1 to the S3 segment (25, 103, 115). Given the promiscuity of the light chain/heavy chain interaction as outlined above, it is tempting to speculate that rbAT and \(b_0^{+}\)AT subunits may have additional partners in the proximal tubule. The OK and LLC-PK\(_1\) kidney cell lines and the colon carcinoma Caco-2 cell line also express rbAT (85, 141, 157, 159). It therefore seems beyond doubt that the above-described high-affinity cystine transport observed in kidney and intestine is mediated by the rbAT/\(b_0^{+}\)AT heterodimer. Surprisingly, the mouse \(b_0^{+}\)AT subunit induces cystine transport when coexpressed with rbAT, suggesting the presence of yet unknown light chains in mouse blastocysts. Besides expression in kidney and intestine, rbAT mRNA or protein has been localized in pancreas (6), heart (159), adrenal gland (92), brain stem, and spinal cord (92, 106, 159). However, in part of the brain cells, rbAT was localized to intracellular membranes (92, 106). Some of the neurons also contained constitutive nitric oxide synthase, and it was therefore discussed whether rbAT may be involved in providing arginine for nitric oxide synthesis (107, 136). The relevance of these observations has to be discussed in view of much more limited distribution of the \(b_0^{+}\) subunit, supporting the notion that rbAT may interact with other light chains in these cell types. Vice versa, \(b_0^{+}\)AT may interact with 4F2hc in liver cells.

The functional characterization of the rbAT/\(b_0^{+}\)AT heterodimer proved to be difficult because the usual expression system, \(X. laevis\) oocytes, exhibit a very strong endogenous \(b_0^{+}\)-like transport activity upon expression of rbAT alone, which is almost indistinguishable from its mammalian counterpart. Pfeiffer et al. (103), therefore, constructed a fusion protein linking the NH\(_2\) terminus of rbAT with the COOH terminus of \(b_0^{+}\)AT. This protein induced \(b_0^{+}\)-like transport activity in \(X. laevis\) oocytes and demonstrated Na\(^+\)-independent, high-affinity transport of dibasic amino acids and cystine and low-affinity transport of neutral amino acids. Feliciadalo et al. (47) and Chairengdua et al. (25) alternatively demonstrated functional expression of the two subunits in COS cells and showed that each protein reached the plasma membrane only in the presence of both subunits and exhibited the same features of \(b_0^{+}\)-like transport activity as expected.

Transport by the rbAT/\(b_0^{+}\)AT heterodimer obeys an obligatory exchange mechanism for neutral and dibasic amino acids. Three factors contribute to the asymmetry of the transporter to accomplish the reabsorption of dibasic amino acids and cystine. The affinity for dibasic amino acids and cystine is severalfold higher than for neutral amino acids (7, 22, 103, unpublished data), the negative membrane potential favors the inward transport of dibasic amino acids in exchange for neutral amino acids, and there is a strong chemical gradient for cystine. Cystine is rapidly reduced to cysteine intracellularly, which may leave the cell basolaterally through the LAT2 transporter (Fig. 4) (27, 108, 135).

REGULATION OF HETERODIMERIC AMINO ACID TRANSPORTERS AND POSSIBLE ROLE IN CELL GROWTH, DIFFERENTIATION, AND CANCER

Little is known about regulation of the heterodimeric amino acid transporters. Besides the fact that the first member of this family, LAT1, was identified as an aldosterone-regulated gene (134) in the \(X. laevis\) cell line A6, the significance of this still unclear, studies on the regulation of these transporters have only started. Even though it is well documented that some of the described transport activities are highly regulated (29), the most interesting question, how this regulation occurs, still needs to be addressed. A few recent studies shed some light on this question. Because heterodimeric transporters are constituted of two different proteins, synthesis of both subunits might be regulated. Indeed, some studies indicate that 4F2 is the main regulator of activity, whereas other studies indicate that regulation occurs on the level of the light chain. Kudo et al. (71) studied regulation of systems L,
y\(^{-}\)L, and y\(^{+}\) in human placental trophoblasts, which are known to induce these systems after taking to culture. They found an increase only in 4F2 and CAT1 mRNA but not in LAT1, LAT2, y\(^{-}\)LAT1, and y\(^{-}\)LAT2. Unfortunately, nothing is known about protein levels and a possible translocation/trafficking of existing protein (71). This finding would suggest that regulation involves mainly 4F2. A second study in the kidney, however, comes to a different conclusion. In the kidney, activation of the basolateral, rate-limiting system y\(^{-}\)L, but not system L, was observed in dexamethasone-treated rats. Schwartfeger et al. (128) found upregulation of 4F2 protein expression and a similar increase in y\(^{-}\)LAT1 mRNA, suggesting that the enhanced expression of both subunits is necessary for upregulation of y\(^{-}\)L transport activity. Furthermore, regulation of LAT1 was observed in hepatocytes in which 2,3,7,8-tetrachlorodibenzo-p-dioxin increased LAT1 transcription and system L amino acid transport (123). However, no information about 4F2 regulation was provided. The regulation of system x\(_{-}\) seems to follow a more complicated pattern. Whereas induction of system x\(_{-}\) by bacterial lipopolysaccharides involves transcriptional upregulation of both subunits xCT and 4F2hc, the regulation by ambient oxygen concentrations or nitric oxide affects only xCT expression levels (12, 124).

On the other hand, expression of LAT1 is regulated by amino acid availability. Surprisingly, arginine, not a substrate of LAT1, has the most profound effect on transcription. The regulation is lost in transformed cell lines (24). Another piece of evidence for an involvement of 4F2/LAT1 in the control of amino acid fluxes is that overexpression of 4F2 causes transformation of BALB/3T3 cells (132) and that activation of lymphocytes is accompanied by an upregulation of 4F2 (20, 140). Induction of 4F2 in lymphocytes occurs very early upon prolifeative stimuli such as Ca\(^{2+}\) or protein kinase C activation (140). The light chain LAT1 is highly expressed in a number of tumor cell lines (68, 90, 114) and appears to be concomitantly regulated with 4F2. Different antibodies directed against 4F2 block lymphocyte and tumor cell proliferation (38). Furthermore, there seems to be a correlation between the expression of 4F2 and the proliferative activity of cells, as described for skin keratinocytes, melanoma cells, Langherans cells, and intestinal epithelia (38). Besides a possible role in cell proliferation, a role in cell differentiation has been proposed. In addition, overexpression of 4F2/LAT1 seems to induce malignant transformation in NIH/3T3 fibroblasts (61, 132). The common point for these observations may be the fact that amino acid availability may control mRNA synthesis and proliferation (64) and that the flow of amino acids through 4F2/LAT1 is a requisite for the described events (37). At variance with this proposed function, all studies have indicated thus far that LAT1 obeys an obligatory antiport mechanism that is not compatible with a net uptake of essential amino acids, which has been demonstrated, e.g., at the blood-brain barrier (100) and which is necessary for cell growth. However, transport rates determined in cultured mammalian cells are usually severalfold higher than metabolic rates. A small percentage of unipart activity might, therefore, suffice to sustain cell growth. The low temperature used in oocyte experiments might obscure such a low activity of the studied transport systems. In the case of the oblig-
atomy antiporter ASCT2, it was recently shown that alanine displayed significant uniport activity (13).

4F2 has also been reported to associate with integrins and regulate their activation through the cytoplasmic and transmembrane regions (26, 48, 49). Integrins are involved in a variety of cellular functions such as cell growth, migration, and tumor metastasis. This interaction may involve the induction of membrane protein clustering, allowing for interaction among proteins, and, therefore, inducing intracellular signaling (37). The above-mentioned ability of 4F2hc/LAT1 to cause malignant transformation when overexpressed, which is difficult to reconcile with an increase in amino acid transport only, might, therefore, be conditional on the ability of 4F2hc to interact with other proteins, such as integrins. Moreover, it could be demonstrated that 4F2hc is the fusion regulatory protein-1 that plays an important role in the fusion of cells and may be required for virus-induced cell fusion (95, 144).

However, it may be the case that the virus misuses a cell surface protein for attachment. Antibody binding to 4F2 or CD98 triggers the activation of protein kinases, the mechanism of which is not understood (137). It is tempting to speculate that the heterodimeric construction of this family of transporters, rather than being necessary for amino acid transport itself, ensures an integration of events in cell proliferation and differentiation with a change in amino acid transport activity.

Role of Heterodimeric Amino Acid Transporters in Monogenic Diseases

In contrast to the diffuse role of 4F2/LAT1 in cell proliferation, a loss of function carried out by members of the heterodimeric amino acid transporters results in well-described failures of reabsorption of amino acids in kidney and intestine, known as cystinuria and lysinuric protein intolerance, respectively. However, the phenotypes of these two diseases, which have been genetically linked to the rbAT/b0,+AT complex and the γ-LAT1 subunit, respectively, shed light on the function of these transporters and their interaction with other transporters. Brief descriptions of both diseases are given below, and the role of the involved subunits is discussed. For a more detailed review of these diseases, refer to a recent review (97).

Cystinuria

Cystinuria is an inherited hyperaminoaciduria (OMIM 220100 and OMIM 600918) of cystine and the dibasic amino acids arginine, lysine, and ornithine. Because the solubility of cystine is very low, particularly in acid fluids, cystine precipitates and forms recurrent cystine kidney stones. These stones can occur beginning very early in childhood and continue, leading to progressive renal failure due to recurrent urinary infections and slow destruction of renal parenchyma. Cystinuria accounts for 1–2% of nephrolithiasis in adult patients and for ~6–8% in pediatric patients. The estimated prevalence is 1 in 7,000 newborns, with a higher prevalence in Libyan Jews of ~1 in 2,500 and a lower prevalence in Sweden, with ~1 in 100,000 newborns (97).

The transport defect lies in the brush-border membrane of the renal proximal tubule and small intestine and leads to reduced renal and intestinal reabsorption of cystine, arginine, ornithine, and lysine. Because these amino acids can be formed by metabolism or taken up by other transporters, the defect does not cause malnutrition. Early functional data suggested the existence of three distinct subtypes of cystinuria according to the excretion patterns and intestinal absorption of the amino acids in heterozygotes, i.e., the unaffected siblings of a cystinuria patient carrying only one affected allele (120). Heterozygous siblings from non-type I cystinuria patients show elevated urinary excretion levels and reduced intestinal absorption of cystine and dibasic amino acids, whereas heterozygous siblings from type I cystinuria patients show no defects. On a molecular level, however, only two forms of cystinuria can be distinguished thus far: cystinuria type I, caused by mutations in the heavy subunit rbAT (SLC3A1) (1, 23, 41, 44, 56, 83, 109, 111, 122), and cystinuria non-type I, with mutations in the light subunit b0,+AT (SLC7A9) (47). More than 60% of all cystinuria cases worldwide are type I (97). The fact that unaffected carriers of type I cystinuria show a normal amino acid excretion pattern defines this type as autosomal recessively inherited. In contrast, heterozygotes for SLC7A9 mutations have elevated amino acid excretion, which led to the assumption of an incomplete autosomal recessive inheritance.

Up to now, >50 mutations and 10 polymorphisms in the SLC3A1 gene have been identified, giving rise to large deletions, premature stop codons, or missense mutations (1, 8, 23, 35, 41, 44, 56–60, 63, 83, 110, 111, 113, 122). The two most common mutations are M467T or M467K and T216M; the M467T/K mutation is predominantly found in Spanish and Italian patients (24, 36), whereas the T216M mutation is mainly identified in Greek patients (1). These results suggest a population-specific distribution, as previously described for cystic fibrosis transmembrane conductance regulator mutations in cystic fibrosis (1). Of these missense mutations, only a few have been functionally characterized. M467T and M467K show a reduced expression in Xenopus oocytes that is fully restored either with increasing injected RNA concentrations or prolonged periods of expression. The mutant is not fully glycosylated and, therefore, causes a defective trafficking of the otherwise fully functional subunit to the membrane (28). Subsequently, a number of studies examined seven other missense mutations on the basis of only their expression rate and concluded these to be also trafficking mutants (83, 111, 122). However, two mutations, R365W and C673S, were recently identified that also altered the functional characteristics of the b0,+ -like transport system, suggesting that rbAT is not only important for the trafficking of the transporter to the membrane. For some mutations, no functional defect could be identified, which may be due to the oocyte
expression changes in a protein leading to translocation defects can be partially overcome by reducing the incubation temperature.

In the light subunit SLC7A9 (b0,+AT), 35 mutations were found (47, 50); the most common, V170M, completely abolished amino acid transport when cotransfected with rbAT into COS cells (47). Pont et al. (50) suggested a correlation between the phenotype and the mutation, showing that some mutations with residual transport activity correlated with lower amino acid excretion levels and thus with a less severe disease phenotype. The milder mutations affected amino acids that were not highly conserved, whereas the severe mutations were found in highly conserved regions (50). Recently, the V170M mutation, which is found mainly in Libyan Jews, has been traced back to one possible founder who lived around the time the Jews settled in Libya after their expulsion from the Iberian Peninsula (32).

**Lysinuric Protein Intolerance**

Lysinuric protein intolerance (OMIM 222700) is characterized by the defective transport of the cationic amino acids lysine, arginine, and ornithine at the basolateral side of the epithelia in the proximal tubule and small intestine (119). The consequent renal loss and reduced intestinal absorption of these amino acids result in an impaired urea cycle with low blood urea. Therefore, the defect manifests itself with a variety of symptoms such as hyperammonemia after high protein meals, the failure to thrive, hepatosplenomegaly, osteoporosis, pulmonary problems (alveolar proteinosis), and mental deteriorations. Furthermore, lysinuric protein intolerance patients may also suffer from hematological and immunological problems such as hemophagocytic lymphohistiocytosis (fever, hepatosplenomegaly, hypofibrinogenemia, hypertriglyceridemia, cytopenia) and impaired B cell activity with severe recurrent infections (40, 75). The transport defect has also been described in skin fibroblasts (131). However, a recent report did not find altered amino acid transport in skin fibroblasts from lysinuric protein intolerance patients (33). When untreated, the patients will die. The rare autosomal recessive disease is particularly frequent in Finland (89).

Mutations in the SLC7A7 (solute carrier 7, family 7) gene encoding the y+LAT1 protein were found to be causative for this disease. Eighteen mutations, i.e., deletions, frame shifts, missense mutations, and nonsense mutations have been identified thus far in patients (10, 70, 89, 93, 133, 143). Functional studies with some mutations expressed in *Xenopus* oocytes revealed that only y+LAT1 missense mutations reached the plasma membrane but showed little transport activity. Truncations and frameshifts produced a protein that was unable to reach the plasma membrane (89).

No disease has been identified that is associated with mutations in 4F2. Given its wide distribution and functional importance in amino acid transport across cell membranes and across blood-tissue barriers, it seems likely that nonfunctional mutations result in death at embryonic stages.

**OPEN QUESTIONS**

Work on the role and structure of the heterodimeric amino acid transporters has just begun. Many problems need to be solved, only a few of which are mentioned below.

**Structural Aspects**

Besides the question of whether all members of this new family have been identified, it will be most interesting to understand how the subunits interact and what confers the specificity of this interaction. Having closely related subunits with very distinct transport characteristics will allow better understanding as to which structural features determine the specificity of each transport system.

**Functional Aspects**

In parallel, we need to learn about the physiological role of the transporters in the different tissues and how transport activity is regulated. The effects of 4F2 heterodimers on ion channel activity, cell proliferation, cell fusion, and cell adhesion are still incompletely described, and the associated proteins are not defined. It is likely that not all light chains and perhaps some heavy chains have not been identified yet. In particular, light chains with increased uniport activity are expected to be expressed at blood-tissue barriers and at the basolateral side of the kidney.

**Involvement in Health and Diseases**

Failure of amino acid reabsorption is the most prominent consequence of amino acid transport failure. Heterodimeric transporters could be important in the maintenance of pregnancy, proliferation of tumor cells, transport of hormones (i.e., thyroid hormones), and drugs. Although amino acid transport is the best-defined function of 4F2h/c/e, there is clear evidence that it is involved in cell proliferation, activation, cell fusion, and differentiation. Provision of an integrated view of these functions is required to cope with the complexity of heterodimeric amino acid transporters and to understand the role each transporter and transport system is playing in its cellular context.

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