Amino acid transport of \(y^+L\)-type by heterodimers of 4F2hc/CD98 and members of the glycoprotein-associated amino acid transporter family

Rahel Pfeiffer, Grégoire Rossier\(^1\), Benjamin Spindler, Christian Meier, Lukas Kühn\(^1\) and François Verrey\(^2\)

Institute of Physiology, University of Zürich, Winterthurerstrasse 190, CH-8057 Zürich and \(^1\)Swiss Institute for Experimental Cancer Research, CH-1066 Epalinges, Switzerland

\(^2\)Corresponding author
e-mail: Verrey@physiol.unizh.ch

Amino acid transport across cellular membranes is mediated by multiple transporters with overlapping specificities. We recently have identified the vertebrate proteins which mediate Na\(^+\)-independent exchange of large neutral amino acids corresponding to transport system L. This transporter consists of a novel amino acid permease-related protein (LAT1 or AmAT-L-lc) which for surface expression and function requires formation of disulfide-linked heterodimers with the glycosylated heavy chain of the h4F2/CD98 surface antigen. We show that h4F2hc also associates with other mammalian light chains, e.g. y\(^+\)LAT1 from mouse and human which are \(\sim 48\%\) identical with LAT1 and thus belong to the same family of glycoprotein-associated amino acid transporters. The novel heterodimers form exchangers which mediate the cellular efflux of cationic amino acids and the Na\(^+\)-dependent uptake of large neutral amino acids. These transport characteristics and kinetic and pharmacological fingerprints identify them as y\(^+\)L-type transport systems. The mRNA encoding y\(^+\)LAT1 is detectable in most adult tissues and expressed at high levels in kidney cortex and intestine. This suggests that the y\(^+\)LAT1–4F2hc heterodimer, besides participating in amino acid uptake/secretion in many cell types, is the basolateral amino acid exchanger involved in trans-epithelial reabsorption of cationic amino acids; hence, its defect might be the cause of the human genetic disease lysinuric protein intolerance.

**Keywords:** amino acid exchanger/epithelial transport/lysinuric protein intolerance/\(\textit{Xenopus}\) oocytes

Introduction

The surface glycoproteins rBAT and h4F2hc/CD98 have been shown to induce amino acid transport of the b\(^{0,+}\) and L and/or y\(^+\)L type, respectively, when expressed in \(\textit{Xenopus}\) oocytes or some other heterologous expression systems (Bertran et al., 1992; Wells et al., 1992; Broer et al., 1995, 1997, 1998). For both glycoproteins, it has been proposed that they are not themselves transporters but rather 'activate' endogenous systems (Bertran et al., 1992; Wells et al., 1992; Broer et al., 1998). Both glycoproteins associate covalently with so-called light chains which migrate on SDS–PAGE at \(\sim 40\) kDa (Haynes et al., 1981; Hemler and Strominger, 1982; Lüscher et al., 1985; Wang and Tate, 1995). The human heterodimeric surface glycoprotein h4F2hc and its murine homologue CD98 were first detected in activated lymphocytes (Haynes et al., 1981) and subsequently shown to be expressed widely (Yagita et al., 1986; Parmacek et al., 1989; Dixon et al., 1990), with an exclusively basolateral localization in intestine and kidney epithelia (Quackenbush et al., 1986) (B.Sordat, personal communication). However, serious attempts to obtain sequence information from their light chains following biochemical purification have failed (C.Bron, personal communication), a problem compatible with the presence of a mixture of different polypeptides.

Recently, searching for early aldosterone-regulated gene products, we cloned from \(\textit{Xenopus}\) A6 cells a cDNA (ASUR4) encoding a highly lipophilic, permease-related protein which migrates at \(\sim 40\) kDa on SDS–PAGE (theoretical mol. wt 55.5 kDa) (Spindler et al., 1997). When ASUR4 is co-expressed together with h4F2hc in \(\textit{Xenopus}\) oocytes, they form disulfide-linked heterodimers which localize at the cell surface and mediate the Na\(^+\)-independent transport of large neutral amino acids, as expected for the L-type amino acid transport system (Mastroberardino et al., 1998). Its human homologue E16 has very similar transport properties and, thus, represents the first light chain of h4F2hc identified so far. We here term this transporter hLAT1 (human L-type amino acid transporter 1), instead of using our former designation hAmAT-L-lc, to conform with the nomenclature proposed by Christensen et al. (1994) which was also adopted by Kanai et al. (1998). Interestingly, a related \(\textit{Shistosoma mansoni}\) protein (SPRM1) also bound covalently to h4F2hc, but induced an amino acid transport of different specificity and Na\(^+\) dependence, thus representing the first non-mammalian member of a new family of glycoprotein-associated amino acid transporters (gpa-AT).

In 1992, Devés et al. first described in human erythrocytes a broad scope amino acid exchange system (y\(^+\)L) which, in the absence of Na\(^+\), mediates the exchange of cationic amino acids and, in the presence of Na\(^+\), the exchange of intracellular cationic amino acids with a preference for large extracellular neutral amino acids (Devés et al., 1992, 1993; Angelo and Devés, 1994; Fei et al., 1995). This complex transport pattern clearly differs from the transport of cationic amino acids produced by the y\(^-\)type transporters of the chloramphenicol acetyltransferase (CAT) family, the first of which had been cloned as a receptor for the ecotropic retrovirus (Albritton et al., 1989). Subsequently, the amino acid transport activity detected upon expression of h4F2hc in \(\textit{Xenopus}\) oocytes has been recognized as being of the y\(^+\)L type (Kim et al., 1991; Wang et al., 1991; Chilaron et al., 1996). A y\(^+\)L transport system may be involved in the
Fig. 1. Alignment of the yL^1 LAT amino acid sequences with hLAT1 and SPRM1. Amino acids identical to those of mouse yL^1 LAT1 are in bold. Putative transmembrane domains (TMDs), the position of which represents a compromise based on the predictions obtained for the different sequences, are numbered from 1 to 12. The cysteine residue involved in the disulfide bond with h4F2hc is indicated by the letter C.

In this report, we characterize the mouse and human homologues of a new member of the gpa-AT family which we identified by database screening. When co-expressed with h4F2hc in Xenopus oocytes, this light chain (yL^1 LAT1) forms disulfide-linked heterodimers and produces amino acid transport of the yL^1 L type.

The first identified light chain of h4F2hc/CD98, the hLAT1 (E16), mediates L-type amino acid transport (Na^+/H^+ exchange of large neutral amino acids) upon co-expression with h4F2hc in Xenopus oocytes (Mastroberardino et al., 1998). Using its sequence, we performed database searches (Blast programs, NCBI) and identified cDNA sequences corresponding to at least four additional mammalian polypeptides of the same light chain family. The level of identity between their sequences ranges from 42–72%. In this study, we characterize the structure and function of one of the new family members for which we obtained both mouse and human expressed sequence tag (EST) cDNAs from the IMAGE consortium (Lennon et al., 1996). The amino acid sequence of the mouse and human proteins are strongly similar (90.4% identity; 98.6% similarity) and show ~48% identity with hLAT1 (Figure 1). Upon co-expression with h4F2hc, they produce yL^1-L-type amino acid transport (see below). Thus, we named them m- and hyL^1 LAT1. The suffix ‘1’ has been added, since the closest related light chain in the database, the human HA7016 cDNA (product of the KIAA0245 gene, 72% identity) (Nagase et al., 1996), appears to produce a similar type of transport (R.Pfeiffer, unpublished results) and might be named hyL^1 LAT2. We have identified two mouse cDNA clones for yL^1 LAT1 (a and b), which differ in their 5'-untranslated region and originate from kidney and heart cDNA libraries, respectively. Their encoded proteins differ by a single amino acid (S versus T) at position 4. The functional experiments reported in this study were performed using the kidney cDNA (yL^1 LAT1a). The closest non-mammalian protein is the S.mansoni SPRM1 (~40% identity) (Figure 1). This transporter associates with human 4F2hc and displays an amino acid transport with properties resembling both the mammalian L and yL^1 L system (Mastroberardino et al., 1998). The m- and hyL^1 LAT1 are 510 and 511 amino acids long, respectively, with a calculated mol. wt of ~56 kDa. The primary structure of the different gpa-AT family members suggests the conservation of a 12 transmembrane domain (TMD) topology with intracellular N- and C-termini (TMpred Server, ISREC). A conserved cysteine residue in the second extracellular loop between TMD 3 and 4 is involved in the disulfide bond with h4F2hc (Pfeiffer et al., 1998).

The next most related protein to the light chain family basolateral extrusion step for the (re)absorption of dibasic amino acids across small intestine and kidney proximal tubule epithelia (Angelo and Devès, 1994; Chillaron et al., 1996).

In this report, we characterize the mouse and human homologues of a new member of the gpa-AT family which we identified by database screening. When co-expressed with h4F2hc in Xenopus oocytes, this light chain (yL^1 LAT1) forms disulfide-linked heterodimers and produces amino acid transport of the yL^1 L type.

**Results**

The first identified light chain of h4F2hc/CD98, the hLAT1 (E16), mediates L-type amino acid transport (Na^+/H^+ exchange of large neutral amino acids) upon co-expression with h4F2hc in Xenopus oocytes (Mastroberardino et al., 1998). Using its sequence, we performed database searches (Blast programs, NCBI) and identified cDNA sequences corresponding to at least four additional mammalian polypeptides of the same light chain family. The level of identity between their sequences ranges from 42–72%. In this study, we characterize the structure and function of one of the new family members for which we obtained both mouse and human expressed sequence tag (EST) cDNAs from the IMAGE consortium (Lennon et al., 1996). The amino acid sequence of the mouse and human proteins are strongly similar (90.4% identity; 98.6% similarity) and show ~48% identity with hLAT1 (Figure 1). Upon co-expression with h4F2hc, they produce yL^1-L-type amino acid transport (see below). Thus, we named them m- and hyL^1 LAT1. The suffix ‘1’ has been added, since the closest related light chain in the database, the human HA7016 cDNA (product of the KIAA0245 gene, 72% identity) (Nagase et al., 1996), appears to produce a similar type of transport (R.Pfeiffer, unpublished results) and might be named hyL^1 LAT2. We have identified two mouse cDNA clones for yL^1 LAT1 (a and b), which differ in their 5'-untranslated region and originate from kidney and heart cDNA libraries, respectively. Their encoded proteins differ by a single amino acid (S versus T) at position 4. The functional experiments reported in this study were performed using the kidney cDNA (yL^1 LAT1a). The closest non-mammalian protein is the S.mansoni SPRM1 (~40% identity) (Figure 1). This transporter associates with human 4F2hc and displays an amino acid transport with properties resembling both the mammalian L and yL^1 L system (Mastroberardino et al., 1998). The m- and hyL^1 LAT1 are 510 and 511 amino acids long, respectively, with a calculated mol. wt of ~56 kDa. The primary structure of the different gpa-AT family members suggests the conservation of a 12 transmembrane domain (TMD) topology with intracellular N- and C-termini (TMpred Server, ISREC). A conserved cysteine residue in the second extracellular loop between TMD 3 and 4 is involved in the disulfide bond with h4F2hc (Pfeiffer et al., 1998).
is the yeast MUP1 methionine permease (~25% identity) which has a hydrophobic profile similar to that of the gpa-A Ts (not shown). The CAT-type transporters, originally known as ecotropic virus receptors and which produce y'-type transport of cationic amino acids, represent more remote mammalian relatives of the gpa-A T family. The level of identity of y'-LAT1 with rCAT-1 and hCAT-2A is ~21 and 25%, respectively. Sequence alignments (not shown) show several large gaps, and CAT proteins are substantially longer (624 and 657 amino acids) than y'-LAT1. They also have a higher number of putative TMDs (14 versus 12).

y'-LAT1 forms a covalently linked heterodimer with h4F2hc

We have shown previously that hLAT1 is covalently associated with h4F2hc (Mastroberardino et al., 1998). Using immunoprecipitation of proteins expressed in Xenopus oocytes, we tested whether y'-LAT1 also associates with h4F2hc and thus represents yet another light chain forming a heterodimer with this glycoprotein heavy chain (Figure 2). Immunoprecipitation of h4F2hc expressed alone produced, independently of sample reduction, a broad band migrating at ~80 kDa on SDS–PAGE which is typical for the terminally glycosylated form of h4F2hc (Figure 2, lanes 1 and 6). In contrast, immunoprecipitation of y'-LAT1 revealed a band migrating at ~40 kDa, as expected for a h4F2hc light chain (Haynes et al., 1981; Hemler and Strominger, 1982) (lanes 11 and 13). The fact that this protein of nearly 56 kDa migrates faster than expected on SDS–PAGE is not unusual for a highly lipophilic membrane protein. Either antibody precipitated both chains when h4F2hc and m- or h y'-LAT1 were co-expressed in the oocytes. In the presence of β-mercaptoethanol, the chains were essentially separated on the gel, while in the absence of reduction, the chains remained associated as heterodimers and migrated as a single band at ~130 kDa. In the presence of a light chain and sample reduction (lanes 3 and 12), an additional band of 66 kDa became prominent which most probably corresponds to the core-glycosylated form of h4F2hc. This suggests a retardation of the maturation process of the heavy chain when light chains are co-expressed. The co-immunoprecipitation results demonstrate that h4F2hc forms disulfide-linked heterodimers with m- and h y'-LAT1 upon co-expression in Xenopus oocytes.

Transport properties of y'-LAT1

To test whether y'-LAT1 functions as an amino acid transporter, we measured the uptake of radioactively labelled amino acids in Xenopus oocytes expressing either y'-LAT1 or h4F2hc alone or both together. Initial experiments showed that expression of y'-LAT1 or h4F2hc alone did not increase the amino acid uptake compared with uninjected oocytes when measured 24 h after cRNA injection (Mastroberardino et al., 1998; data not shown). However, when both chains were co-expressed, a 2- to 7-fold increase in amino acid transport was observed. All subsequent experiments were performed 24 h after cRNA injection, and control values from oocytes injected with h4F2hc alone were subtracted.

Figure 3 shows the uptake of different amino acids added at a concentration of 100 μM. The cationic amino acids L-arginine and L-lysine were transported in the presence of Na + as well as in its absence. However, transport of the neutral amino acids L-leucine, L-glutamine and L-methionine was highly Na + dependent. L-Alanine and L-phenylalanine, at a concentration of 100 μM, were transported at a much lower rate, but also in an Na + dependent manner. L-Histidine was transported similarly in the presence and absence of Na + when the experiments were performed at the usual pH of 7.4. However, the ratio of Na + dependent to Na + independent L-histidine uptake was strongly increased at pH 8.0 (data not shown), indicating that both the neutral and the cationic forms are transported, but that only the transport of the neutral form is Na + dependent. L-Glutamic acid and L-tryptophan were not transported above background levels.
alanine (higher affinity for L-leucine, see below) is typical and NEM did not block L-arginine uptake. L-Leucine uptake was and different inhibitors/competitors. L-Arginine (Fig. 4. Inhibition of L-arginine and L-leucine uptake: effect of Na⁺/H⁺11001 for three representative amino acids: L-arginine as a/ H11006 amino-2-norbornane-carboxylic acid (BCH) and methyl-/ H11005 and to a lesser extent in its absence. BCH, MeAIB and NEM had no effect on L-leucine uptake. L-Arginine was inhibited by an excess of system L- and A-specific substrates 2-/ H11001/4F2hc and mouse or human y/LAT1 were performed/ cRNA alone (several days of expression) and which displayed a weak y/L-type amino acid transport.

Tissue distribution of y/LAT1 mRNA

On Northern blot, my/LAT1 produced a single ~2.3 kb band on mouse kidney poly(A)+ RNA (Figure 7A). A signal corresponding to an mRNA of the same size was also detected in rat kidney and small intestine. Northern
hybridization on total RNA [lower sensitivity than with poly(A)+] of different rat tissues showed a signal only in kidney cortex. In particular, no signal was detected in kidney medulla and in colon. Hybridization of a commercial mouse RNA dot-blot with a my+LAT1 probe (Figure 7B) confirmed the high level of expression in intestine (RNA from whole intestine) and kidney, further suggesting that this amino acid transporter has a function in transepithelial (re)absorption. Lower levels of my+LAT1 RNA were detected in most tested tissues. The signals in brain, skeletal muscle, heart and uterus were too close to background levels to decide whether my+LAT1 is expressed in these tissues at low levels, possibly in subpopulations of cells. In summary, y+LAT1 appears to be expressed in most tissues, but at the highest level in kidney cortex and the small intestine.

**Discussion**

The mouse and human y+LAT1 homologues characterized in this study represent a second mammalian ‘light chain’ belonging to the newly described gpα-AT family of permease-related amino acid transporters which covalently associate with a glycoprotein ‘heavy chain’ to function at the cell surface (Mastroberardino et al., 1998; Figure 2). It is likely that different light chains, which comprise this family, associate with the heavy chain via an equivalent domain. Indeed, they are structurally very similar and display, according to predictions of their 12 TMD topology with intracellular N- and C-termini, a single common cysteine residue (Cys152 for my+LAT1) facing the extracellular space. We have shown for the related light chains ASUR4 (Xenopus LAT1) and SPRM1 that this cysteine residue, located between the putative TMDs 3 and 4, forms a disulfide bridge with Cys109 of h4F2hc (Pfeiffer et al., 1998). Light chains of the same family, which can be expressed in the same cell (Broer et al., 1998), might thus compete with each other for binding to the heavy chain. Immunofluorescence experiments performed with the related chain SPRM1 have shown that a function of the heavy chain is to allow surface expression of the light chain. In the absence of heavy chain, the light chain remains in an intracellular compartment, probably the endoplasmic reticulum (Mastroberardino et al., 1998). In contrast, surface expression of the heavy chain appears to be independent of co-expression of a light chain (Teixeira et al., 1987; Teixeira, 1990; Mastroberardino et al., 1998).

The type II glycoprotein heavy chain 4F2hc and the related rBAT display, within their extracellular domain, a region of strong similarity with glycosidases, the function of which has not been elucidated (Wells and Hediger, 1992), and might fulfil other functions besides facilitating expression of amino acid transporters at the correct surface membrane. For instance, a role in integrin activation has been ascribed to CD98 (Fenczik et al., 1997). The structural similarity of 4F2hc/CD98 to the rBAT protein, a glycoprotein which induces b°,H+-type transport when expressed in Xenopus oocytes, which is involved in apical amino acid transport and is known to associate with a 40 kDa protein (Wang and Tate, 1995), suggests that rBAT might also associate with light chain(s) of the gpα-AT family. Mutations in the gene encoding this rBAT light chain are expected to explain those cases of cystinuria which are not due to mutations in the rBAT gene. However, this light chain has not been identified as yet and neither y+LAT nor LAT co-precipitated with rBAT (data not shown).

The pattern of amino acid transport via gpα-AT-type
Fig. 6. Stimulation of L-arginine efflux by extracellular L-arginine and L-leucine. Oocytes were loaded with 1 μM L-[3H]arginine and, after a brief wash, incubated in buffer with or without Na+/H+-11001 (continuous line, −Na+/H+-11001; dotted line, +Na+/H+-11001; ○, oocytes expressing h4F2hc alone; •, oocytes coexpressing h4F2hc and my LAT1) without amino acid (A) or with 1 mM cold L-arginine (B) or L-leucine (C). Three aliquots of the incubation buffer were taken at the indicated time points. No efflux was observed in absence of extracellular amino acid. L-[3H]Arginine efflux mediated by h4F2hc and my LAT1 was stimulated by extracellular L-arginine in the presence and absence of Na+ and to a lesser extent and partially Na+-dependently by L-leucine. Means ± SEMs pooled from two independent experiments are shown.

exchangers displayed by a cell membrane thus depends on the presence of heavy chain(s) and on the nature of the expressed gpa-AT light chains. The tissue distribution of 4F2hc is broad (Parmacek et al., 1989), both in epithelial and non-epithelial cells, while rBAT, which has been shown to be defective in type I cystinuria, is expressed mostly in the apical membrane of kidney proximal tubule and small intestine (Pickel et al., 1993). In kidney proximal tubule, 4F2hc has been shown to be localized at the basolateral membrane (Quackenbush et al., 1986; Chillaron et al., 1996; B.Sordat, personal communication). Based on the mRNA analysis, the tissue distribution of the light chains is different for each one. For instance, h4F2hc shows a particularly strong expression in the kidney and intestine compared with h5LAT2 and LAT1 (Figure 6; B.Spindler and F.Verrey, unpublished results). It will be of interest to test whether the simultaneous expression of different light chains in the same cells is coordinated, and whether structural interactions or a functional network exist.

The analysis of transport properties induced in oocytes by the injection of 4F2hc plus y+LAT cRNA has to a large extent confirmed expectations based on the description of system y+L by Devés and colleagues in erythrocytes (Devés et al., 1992). There is, however, a major difference in the apparent affinity for extracellular L-arginine in the presence of Na+ which for y+LAT1 was two orders of magnitude lower than that reported for the erythrocyte y+L transport [apparent Km this study: 341 μM; Ki estimation in Devés et al. (1998): 3 μM]. This suggests that y+LAT1 is not the erythrocyte y+L transport system.
to which the related chain y\^1LAT2 [HA7016 cDNA, product of the KIAA0245 gene (Nagase et al., 1996)] appears to correspond better (our preliminary results). Qualitatively similar results to those obtained by co-expression of y\^1LAT1 and h4F2hc have been obtained previously by the expression of the heavy chain 4F2hc alone in Xenopus oocytes, though only after prolonged incubation and at lower rates (Bertran et al., 1992; Wells et al., 1992). This suggests that Xenopus oocytes express an endogenous y\^1L-type light chain which can associate with exogenous h4F2hc to produce this transport. The fact that Xenopus and even Schistosoma light chains of the gpa-AT family can associate with human 4F2hc is known for the Xenopus type L light chain ASUR4 (XLAT1) and SPRM1, respectively (Mastroberardino et al., 1998). Furthermore, surface expression of light chains has been shown to depend on association with the glycoprotein heavy chain, a situation which is reminiscent of that of the endogenous Na,K-ATPase α catalytic subunit of Xenopus oocytes which can be stabilized and brought to the cell surface upon expression of exogenous Na,K-ATPase β glycoprotein (Geering et al., 1989).

The analysis of the transport properties of y\^1LAT1 reported here can be summarized as follows: in physiological high Na\(^+\) conditions, apparent affinity for neutral amino acids (L-leucine) is an order of magnitude higher than for cationic amino acids (L-arginine) such that this transport system is expected to mediate preferentially the cellular uptake of neutral amino acids. In contrast, cellular efflux of L-leucine is not mediated by this transport system in our experimental conditions, while L-arginine efflux is stimulated readily by extracellular L-arginine or by L-leucine in the presence of Na\(^+\) (though somewhat less efficiently than by L-arginine). Thus, (electroneutral) exchange of intracellular cationic amino acid against extracellular neutral amino acids plus Na\(^+\) ions appears to be the most likely physiological function of this exchanger. It remains to be investigated to what extent the functional asymmetry is due to the different ionic compositions of the cellular and extracellular fluids and/or to a structural asymmetry of the binding sites in the different transporter conformations. The results of the transport experiments support the notion that, in physiological conditions, the y\^1LAT1 system mediates essentially the efflux of cationic amino acids in exchange for neutral amino acids (plus Na\(^+\)).

This pattern of transport corresponds to that expected for the basolateral exchanger of the intestine and kidney proximal tubule which mediates the serosal efflux step of transepithelial cationic amino acid (re)absorption (Angelo and Devés, 1994; Chillaron et al., 1996). This step is rate limiting and is known to be defective in lysinuric protein intolerance (LPI) (Rajantie et al., 1981; Smith et al., 1987). Hence, y\^1LAT1 is a strong candidate for being the defective transporter in this genetic condition, a hypothesis which is supported by the tissue distribution of its mRNA which we show here to be heavily expressed in mouse/rat small intestine and kidney cortex. The presence of the mRNA of y\^1LAT1 in many non-epithelial tissues is compatible with the fact that in LPI amino acid transport defects have also been detected in non-epithelial cells such as skin fibroblasts (Smith et al., 1987).

### Materials and methods

#### Sequence analysis and cRNA synthesis

The cDNAs for m- and h\^1LAT1 obtained from the IMAGE consortium JEST accession Nos m\^1LAT1a: AA276085 (mouse kidney library), h\^1LAT1: AA393488 (human testis library) were sequenced on both strands (Microsynth, Balgach, Switzerland; ISREC sequencing facility, respectively). Their complete sequences are available under DDBJ/EMBL/GenBank accession Nos AJ012754 and AJ130718. An additional cDNA from a mouse heart library (AA500631) encoding m\^1LAT1b seems to be a splice variant at the level of the 5' untranslated sequence (DDBJ/EMBL/GenBank accession No. AJ130943). There are also two differences between the two mouse clones at the level of the nucleotides within the coding sequence leading to a single amino acid change at position 4: Thr for Ser. Sequence alignments were performed using the align program at http://cartan.gmd.de/ToPLign.html. The open reading frames of y\^1LAT1 and h4F2hc (followed by their 3' ends by 35 nucleotides and a ClaI site, and 33 nucleotides and a XhoI site, respectively, and on their 3' ends by 31 nucleotides and a BamHI site, and 35 nucleotides and a HindIII site, respectively) were amplified by PCR using the Vent\(^+\) (New England Biolabs) polymerase and transferred to the pS5Dseasy vector (Puoti et al., 1997). For cRNA synthesis, plasmids containing the cDNAs of h4F2hc (vector pSpor) and y\^1LAT1's (pS5Dseasy) were linearized using the restriction sites HindIII and BglII, respectively. cRNA was synthesized with T7 and SP6 RNA polymerase (Promega), respectively, according to standard protocols.

#### Xenopus laevis oocytes

Oocytes were treated with collagenase A for 2–3 h at room temperature in Ca\(^2+\)-free buffer containing 20.5 mM NaCl, 2 mM KCl, 1 mM MgCl\(_2\) and 10 mM HEPES pH 7.4 to remove follicular cells and then kept at 16°C in ND96 buffer containing 96 mM NaCl, 2 mM KCl, 1 mM MgCl\(_2\), 1.8 mM CaCl\(_2\), 5 mM HEPES pH 7.4.

#### Amino acid uptake and efflux in Xenopus oocytes

Oocytes were injected with 5 ng of (each) cRNA dissolved in 33 nl of water and kept for 24 h at 16°C in ND96 buffer. Before the experiments, they were washed six times with uptake buffer containing 100 mM NaCl (+[Na]-buffer), choline-Cl ([–Na]-buffer) or LiCl, 2 mM KCl, 1 mM MgCl\(_2\), 1 mM CaCl\(_2\), 10 mM HEPES pH 7.4. Six oocytes per uptake experiment were pre-incubated at 22°C for 2 min. The buffer was then replaced by the respective uptake buffer supplemented with amino acid at the indicated concentration and the corresponding L-[\(^{14}\)C]glutamine. Uptake experiments were performed for 1 min because pilot experiments had shown linear amino acid uptake during this time. The oocytes were washed five times with 3 ml of (–Na)-buffer and distributed to individual vials. After oocyte lysis in 2% SDS, radioactivity was counted by liquid scintillation. Uptakes for dose-response curves were performed at five different amino acid concentrations, sigmoidal curves corresponding to Michaelis–Menten kinetics were fitted to the experimental data and amino acid concentrations for half-maximal activation (apparent \(K_M\)) were derived.

For efflux experiments, oocytes were loaded for 17 min with 1 \(\mu\)M L-[\(^{14}\)C]arginine or L-[\(^{14}\)C]leucine in (–Na)- or (+Na)-buffer, respectively. After a brief wash, eight oocytes were transferred to 300 \(\mu\)l of (–Na)-or (+Na)-buffer without amino acid or 1 mM cold L-arginine or L-leucine. Three aliquots of 2 of the buffer were taken at 0, 5, 10, 20 and 30 min and radioactivity was counted by liquid scintillation.

#### Labelling of oocytes and immunoprecipitation

Injection of cRNAs in oocytes was as for uptake experiments. After injection, oocytes were incubated for 48 h in ND96 buffer supplemented with 1 mM/mL L\(^{15}\)S]methionine. Then, oocytes were washed twice in ND96 buffer and lysed in oocyte lysis buffer (20 \(\mu\)l/oocyte) containing 120 mM NaCl, 50 mM Tris–HCl pH 8.0, 0.5% NP-40 supplemented with protease inhibitors. Lysates were vortexed for 20 s and incubated briefly on ice. The lysate was then centrifuged for 10 min at 12 000 r.p.m. in an Eppendorf centrifuge at 4°C. The supernatant was frozen in liquid nitrogen. Incorporated radioactivity was determined by scintillation counting of trichloroacetic acid (TCA) precipitates from aliquots.

For 4F2hc precipitation, the monoclonal antibody described originally by Haynes et al. (1981) was used. A polyclonal antibody against my\^1LAT1 was raised in rabbits using as antigen a synthetic peptide comprising the amino acids 12–27 of the N-terminal tail coupled to keyhole limpet haemocyanin (Eurogentec, Seraing, Belgium). Antibodies were pre-bound to protein G plus/protein A–agarose (Calbiochem) for
2 h at room temperature in oocyte lysis buffer. The beads were then added to the pre-cleared lysate (equal amounts of counts, incubated twice for 30 min at 4°C with protein G plus/protein A-agarose beads) which was rotated overnight at 4°C. Beads were washed five times in buffer containing 100 mM NaCl, 20 mM Tris–HCl pH 8.0, 1 mM EDTA, 500 mM LiCl, 0.5% NP-40, and five times in buffer containing 100 mM NaCl, 20 mM Tris–HCl pH 8.0, 1 mM EDTA, 0.5% NP-40. They were resuspended in SDS–PAGE sample buffer and heated to 65°C for 15 min.

β-Mercaptoethanol was added where indicated and SDS–PAGE analysis was performed. Gels were stained in Coomassie Blue and fixed. After incubation in Amplify (Amersham), the gels were dried and exposed to film at −80°C with an intensifying screen.

**Northern blot analysis**

Total RNA (10 µg) or poly(A) RNA (7 µg) and RNA standards (Promega) were run on 1% agarose/formaldehyde minigels, transferred to Genescreen membranes (NEN Dupont) and immobilized with UV light according to standard protocols. my−1 LAT1-specific probes labelled with [α-32P]dCTP to a specific activity of 2×10^9 c.p.m./µg DNA were generated by random priming (Oligolabeling kit, Pharmacia). Hybridization and washes of mouse RNA dot-blot (Clontech) and Northern blots were performed according to standard protocols. Blots were exposed, scanned and signals quantified using a PhosphorImager and the Imagequant software (Molecular Dynamics).

**Acknowledgements**

The authors thank Christian Gasser for artwork, Heini Murer and Jürg Biber for use of their Xenopus oocyte facilities, and Jürg Biber and Olaf Hattenhauer for the poly(A)^+ Northern blot. The laboratory of F.V. is supported by Swiss National Science Foundation grant 31-49727/96. The project is also supported by a grant of the Swiss Cancer League to L.K.

**References**


Received September 30, 1998; revised November 5, 1998; accepted November 6, 1998