Association of 4F2hc with light chains LAT1, LAT2 or y$^+$LAT2 requires different domains

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Heterodimeric amino acid transporters are comprised of a type-II membrane protein named the heavy chain (4F2hc or rBAT) that may associate with a number of different polytopic membrane proteins, called light chains. It is thought that the heavy chain is mainly involved in the trafficking of the complex to the plasma membrane, whereas the transport process itself is catalysed by the light chain. The 4F2 heavy chain (4F2hc) associates with at least six different light chains to induce distinct amino acid-transport activites. To test if the light chains are specifically recognized and to identify domains involved in the recognition of light chains, C-terminally truncated mutants of 4F2hc were constructed and co-expressed with the light chains LAT1, LAT2 and y$^+$LAT2. The truncated isoform T1, comprised of only 133 amino acids that form the cytosolic N-terminus and the transmembrane helix, displayed only a slight reduction in its ability to promote LAT1 expression at the membrane surface compared with the 529 amino acid wild-type 4F2hc protein. Co-expression of increasingly larger 4F2hc mutants caused a delayed translocation of LAT1. In contrast to the weak effects of 4F2hc truncations on LAT1 expression, surface expression of LAT2 and y$^+$LAT2 was almost completely lost with all truncated heavy chains. Co-expression of LAT1 together with the other light chains did not result in displacement of LAT2 and y$^+$LAT2. The results suggest that extracellular domains of the heavy chain are responsible mainly for recognition of light chains other than LAT1 and that the extracellular domain ensures proper translocation to the plasma membrane.

Key words: amino acid transport, heterodimeric transporter, protein trafficking.

INTRODUCTION

Heterodimeric amino acid transporters form a large family of antiporters which are involved in the intercellular and inter-organ transfer of amino acids [1,2]. The transporters are constituted of a heavy chain, which as suggested by its hydropathy plot is a type-II membrane protein with only one transmembrane helix. The light chain in contrast is a typical helix-bundle protein. Current evidence suggests that the heavy chain is necessary for trafficking of the complex to the plasma membrane, whereas the light chain most probably catalyses the transport itself [3–5]. To date, two different heavy chains are known, referred to as rBAT and 4F2hc. Also, seven different light chain cDNAs have been characterized (LAT1 and LAT2, encoding two isoforms corresponding to system L, y$^+$LAT1 and y$^+$LAT2, encoding two isoforms of system y$^+$L, xCT, encoding a glutamate/cystine antiporter, asc, encoding system asc, and b$^{0,+}$AT, encoding the light chain of system b$^{0,+}$); however, all but one (b$^{0,+}$AT) are associated with 4F2hc (see [1,2] for reviews). Although heavy and light chains are covalently connected to each other by a disulphide bridge, this structural feature is not necessary for surface expression of the light chain; however, it appears to prevent dissociation of the complex in the plasma membrane [3,5–7]. It is not known how 4F2hc interacts with different light chains when they are expressed in the cell at the same time.

The aim of this study was to identify domains of 4F2hc which are involved in the recognition of different light chains. Our results indicate that the ubiquitous light chain LAT1 does not require the extracellular domain for interaction with 4F2hc, whereas other light chains, e.g. y$^+$LAT2 and LAT2, do require this domain for association with 4F2hc. LAT1 might therefore be considered as the default transporter, whereas other light chains require different elements for recognition.

EXPERIMENTAL

Materials

L-[U-^{14}C]isoleucine (11.4 GBq/mmol), L-[U-^{14}C]arginine (10.3 GBq/mmol) and L-[U-^{14}C]alanine (5.6 GBq/mmol) were purchased from Amersham Buchler (Braunschweig, Germany). The RNA cap structure analogue 7 mG(5′)ppp(5′)G, restriction enzymes, nucleotides and RNA polymerases were from Life Technologies (Mulgrave, VIC, Australia). Collagenase (EC 3.4.24.3; 0.3 units/mg from *Clostridium histolyticum*) and proteinase inhibitor Pefabloc were from Roche (Castle Hill, NSW, Australia); lots were tested for their suitability for oocyte expression. All other chemicals were of analytical grade and purchased from Merck (Kilsyth, VIC, Australia) or ICN Biomedicals (Aurora, OH, U.S.A.).

Expression in *Xenopus laevis* oocytes

For expression studies, the human 4F2hc cDNA in plasmid pSP65T was used [8]. For *in vitro* transcription, plasmid DNA was linearized with *Hin*dIII and transcribed *in vitro* with SP6 RNA polymerase in the presence of a cap analogue. The rat LAT1 and human y$^+$LAT2 light chains were used as described before [6,9]. The mouse LAT2 cDNA was isolated during the course of this work from a mouse kidney cDNA library; its sequence is identical with published mouse LAT2 sequences [10].

Abbreviation used: cRNA, *in vitro*-transcribed RNA.

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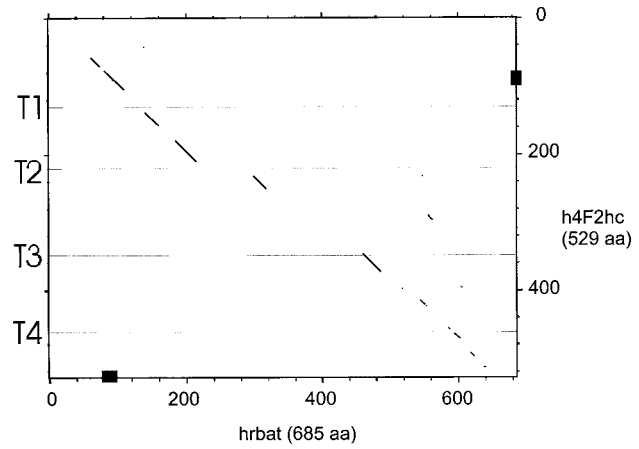

Figure 1 Dot-plot comparison between the human rBAT and the human 4F2hc proteins

The amino acid sequence of human rBAT (*x*-axis) and human 4F2hc (*y*-axis) were compared and regions of similarity are indicated by a dot (see [18]); the numbers refer to amino acid residues. A window of 20 amino acids was used and the stringency set to 13. Comparison of two identical proteins would result in a straight diagonal line across the plot. The location of the transmembrane helix in the human 4F2hc and rBAT proteins is shown by black boxes on both axes. The schematic structures of rBAT and 4F2hc are therefore represented by the *x*- and *y*-axes, respectively. The sizes of the truncated 4F2 heavy chains are given by horizontal lines (T1–T4).

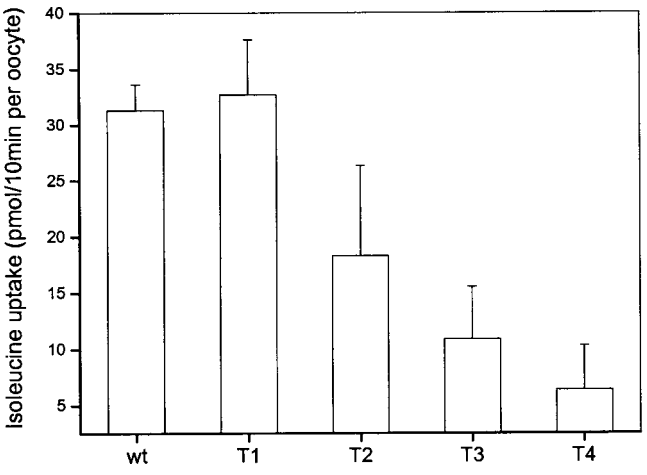

Figure 2 Transport activity of LAT1 associated with truncated and wild-type 4F2hc

Oocytes were injected with 10 ng of cRNA encoding wild-type 4F2hc (wt) or truncated versions (T1–T4) and 10 ng of LAT1 cRNA. After an expression period of 2 days, isoleucine uptake (100 μM substrate) was determined over an incubation period of 10 min. Each bar represents the mean uptake activity of seven oocytes, the activity of non-injected oocytes being subtracted already. The Figure represents a total of eight experiments performed with different incubation times.

It was linearized with *Not*I and transcribed *in vitro* using T7 polymerase. After determination of the amount of *in vitro*-transcribed RNA (cRNA) by measuring absorption at 260 nm, integrity of the transcript was verified by denaturing agarose-gel electrophoresis.

X. laevis females were purchased from the South African *Xenopus* facility (Knysna, Republic of South Africa). Oocytes (stages V and VI) were isolated as described in [11] and allowed to recover overnight. They were microinjected with 10 nl of cRNA in water at a concentration of 1 μg/μl, using a microinjection device (World Precision Instruments, Sarasota, FL, U.S.A.) or remained uninjected in the controls.

Uptake experiments were performed as described recently [11], and ND96 (96 mM NaCl/2 mM KCl/1.8 mM $CaCl_2$/1 mM $MgCl_2$/5 mM Hepes; titrated with NaOH to pH 7.4) was used as incubation buffer. The concentration of ^{14}C-labelled compounds was taken into account for the adjustment of final substrate concentrations.

All data are given as means $\pm$ S.E.M. All measurements were performed with equal numbers of cRNA- and water-injected oocytes. In experiments with labelled compounds, all values represent net uptake rates, calculated as (mean uptake rate of seven cRNA-injected oocytes) $-$ (mean uptake rate of seven non-injected oocytes) = (mean net uptake rate), using Gauss' law of error propagation for the calculation of the final S.D. values.

Site-directed mutagenesis and epitope tagging

Site-directed mutagenesis was performed without subcloning by using the QuikChange site-directed mutagenesis kit (Stratagene, La Jolla, CA, U.S.A.). Briefly, two complementary primers were constructed in which the desired mutation was flanked by 10–15 nucleotides corresponding to the human 4F2hc or rat LAT1 cDNA sequences. The primers were used to amplify the complete cDNA-containing vector in 12 PCR cycles. Subsequently, the template DNA was removed by digestion with *Dpn*I. Plasmid was isolated from transformed bacteria and sequenced to verify the mutation. To avoid second-site mutations, which might have been introduced during the amplification reaction, the transport activity of three independent clones was determined.

The following oligonucleotides were used (only sense primers are shown, antisense primers have the complementary sequence) to introduce stop codons at amino acid positions 125, 213, 345 or 452 of the human 4F2hc sequence [8]: NS133, 5′-CTT CAG GCC TTC tAG GGC CAC GGC G-3′; NS221, 5′-TGG TTC TCC ACT tAG GTT GAC ACT G-3′; NS353, 5′-CTC CGA CTC TAC tAG CTG ATG CTC T-3′; NS460, 5′-TGG GAC CAG AAT tAG CGT TTT CTG G-3′ (the mutated bases are shown in lower case).

To detect the light chain LAT1, the Myc epitope EEKLISEEDL was inserted into the rat LAT1 [12] sequence behind the start codon, by PCR amplification using an oligonucleotide that included the epitope sequence (underlined): mycrLAT1, 5′-C GAG AGC ATG GAA CAA AAA CTC ATC TCA GAA GAG GAT CTG GCG GTC GCG GGC GCA-3′. The antisense primer was the same as used recently for cloning of LAT1 [6].

Western blotting

A total of ten oocytes were lysed in 300 μl of homogenization buffer (10 mM NaCl/1 % Triton X-100/1 mM Pefabloc/20 mM Tris/HCl, pH 7.6) by trituration. The egg yolk was pelleted by centrifugation at 15000 ***g*** in a microcentrifuge for 10 min at 4 °C. The supernatant was removed with a pipette, avoiding contamination from floating lipids. A sample of the supernatant (40 μl) was loaded on to a 10 % polyacrylamide gel and separated at constant voltage (200 V) for 2 h. Proteins were blotted overnight on to nitrocellulose membranes (Schleicher & Schuell, Dassel, Germany). Membranes were treated for 1 h with blocking buffer (5 % non-fat milk in PBS/0.15 % Tween 20, pH 7.4) The epitope-tagged LAT1 protein was subsequently detected using as a primary antibody mouse anti-c-Myc 9E10 (1:2000; Santa Cruz Biotechnology, Santa Cruz, CA, U.S.A.) and as a secondary

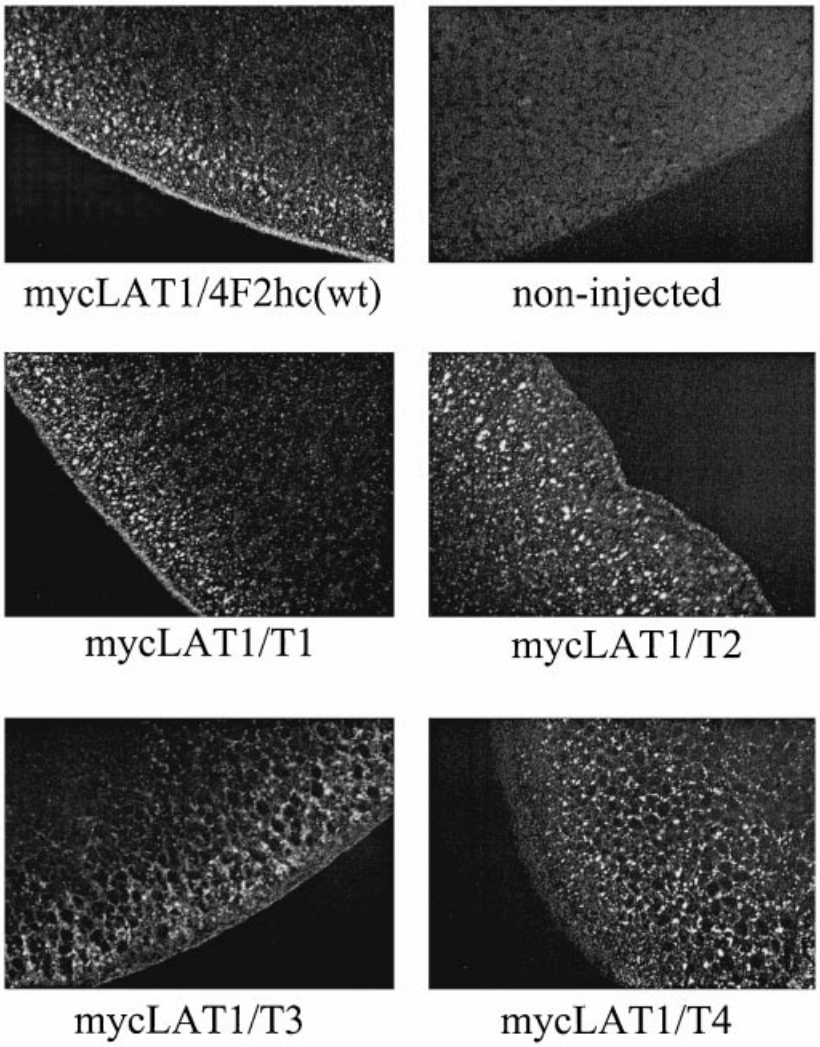

Figure 3 Localization of epitope-tagged LAT1 in oocytes expressing truncated and wild-type 4F2hc

Oocytes were injected with 10 ng of cRNA encoding wild-type 4F2hc (wt) or truncated versions (T1–T4) and 10 ng of epitope-tagged LAT1 cRNA. After an expression period of 2 days, oocytes were manually devitellinized and fixed, and subsequently epitope-tagged LAT1 was detected with antibodies recognizing the Myc epitope. Non-injected oocytes were analysed in the same way to identify any endogenous immunoreactivity. One of two experiments is shown.

antibody sheep anti-mouse Ig coupled to horseradish peroxidase (1:2000; Amersham Life Science, Little Chalfont, Bucks., U.K.). Antibodies were each incubated for 1 h at room temperature in 2.5% non-fat milk in PBS/0.15% Tween 20. Subsequently, membranes were washed four times for 10 min each in PBS. For immunodetection, an enhanced chemiluminescence kit (Amersham Life Science) was used, and the blots were exposed to Kodak X-OMAT film (Sigma, Deisenhofen, Germany).

Immunohistochemistry

Oocytes were devitellinized with tweezers after incubation in hypertonic medium (200 mM potassium aspartate/10 mM Hepes, pH 7.4), and fixed in Dent's solution (80% methanol/20% DMSO) overnight at −20 °C. The fixative was washed out with a graded series of ethanol solutions (90% and 70% in water, then 50% and 30% in PBS). Oocytes were then washed three times in PBS before incubation with the primary antibody (mouse anti-c-Myc 9E10, 1:1000; Santa Cruz Biotechnology) in the presence of 10% goat serum at 4 °C for 12 h. After washing with PBS, incubation with secondary Alexa 546 goat anti-mouse IgG antibody (1:200; Molecular Probes, Eugene, OR, U.S.A.) was performed at room temperature for 1 h. After further washing with PBS, stained oocytes were post-fixed with 3.7% paraformaldehyde for 30 min. The embedding procedure in acryl-resin (Technovit 7100; Heraeus Kulzer, Wehrheim, Germany) was carried out according to manufacturer's instructions. Embedded oocytes were cut into 5 μm sections and analysed with a fluorescence microscope (Nikon Optiphot, Düsseldorf, Germany).

RESULTS

Transport activity of truncated 4F2hc proteins

To investigate the function of the extracellular domain of the heavy chain 4F2hc, stop codons were introduced into its sequence. The human 4F2hc forms a protein of 529 amino acids. It is related to the rBAT protein which, however, recognizes the

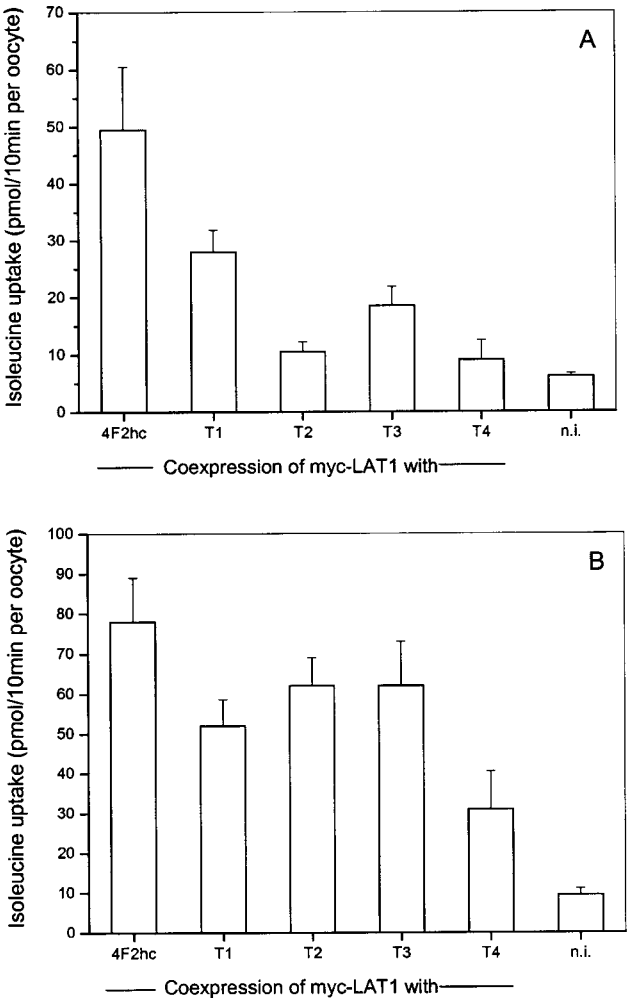

Figure 4 LAT1 transport activity after increased expression periods

Oocytes were injected with 10 ng of cRNA encoding wild-type 4F2hc or truncated versions and 10 ng of epitope-tagged LAT1 cRNA. After expression periods of 2 (**A**) and 4 (**B**) days, isoleucine uptake (100 μM substrate) was determined over an incubation period of 10 min. Each bar represents the mean uptake activity of seven oocytes. The Figure represents a total of four experiments with similar results. n.i., not injected.

$b^{0,+}$AT light chain. To identify domains which are conserved between rBAT and 4F2hc a dot-plot analysis was performed (Figure 1). The largest truncation results in a protein of 133 amino acids which includes the transmembrane helix and about 30 extracellular amino acids (referred to as 4F2hcT1 in the following). The second largest truncation forms a protein of 221 amino acids (4F2hcT2). The position was chosen as it includes a stretch of amino acids which is fairly conserved between 4F2hc and rBAT (about 30 % identical amino acid residues). The third truncation forms a protein of 353 amino acids (4F2hcT3), which includes a small second stretch of amino acids that is conserved between rBAT and 4F2hc and a large stretch of amino acids that are completely unrelated between 4F2hc and rBAT (less than 20 % identical amino acids). The fourth truncation (460 amino acids, 4F2hcT4) again includes a stretch of amino acids of high similarity (51 % identity between rBAT and 4F2hc).

Initially, the association of wild-type and truncated 4F2hc proteins with the light-chain isoform LAT1 was investigated by determining isoleucine uptake (100 μM) in oocytes that had been co-injected with 10 ng each of heavy and light chain cRNA. Rather surprisingly we found that the shortest protein (4F2hcT1, 133 amino acids) was as active as the wild-type 4F2hc in promoting LAT1 surface expression, when measured on the second day of expression (Figure 2). In other oocyte batches, T1 was slightly less active (see Figures 6 and 7, below), but never fell below 50 % of the activity of wild-type 4F2hc. Increasingly larger truncations (T2, T3 and T4) displayed incremental decreases in transport activity.

Localization of epitope-tagged 4F2hc/LAT1 complexes in *Xenopus laevis* oocytes

The low transport activity observed in some of the truncated 4F2hc proteins might result from a less efficient translation, rapid degradation or a trafficking defect. To discriminate between these possibilities a Myc epitope was fused to the N-terminus of LAT1. Co-injecting 6 ng of the epitope-tagged Myc-LAT1 with 6 ng of 4F2hc induced an isoleucine-uptake activity of 43 ± 7 pmol/10 min per oocyte compared with 57 ± 12 pmol/10 min per oocyte as determined for the wild-type LAT1. Similar to the wild-type LAT1, increasing the injected amount to 12 ng for each cRNA did not cause a significant further increase in transport activity (results not shown).

Immunohistochemical analysis of oocytes expressing epitope-tagged LAT1 together with 4F2hc mutants revealed that a significant amount of the 4F2hcT1–LAT1 complex resided in the plasma membrane on the second day of expression, whereas 4F2hcT2, T3 and T4 showed severe trafficking defects, guiding the epitope-tagged LAT1 protein only into submembraneous compartments (Figure 3).

The trafficking defect was corroborated by an analysis of the transport activity after increased expression periods. When determined on the fourth day of expression, truncated forms T1–T3 promoted significant transport activities when co-injected with epitope-tagged LAT1, which amounted to about 80 % of that of the wild type. In contrast, the T4–Myc-LAT1 complex, although missing only 69 amino acids, still had low transport activity on the fourth day (Figure 4B) when compared with transport activities on the second day (Figure 4A).

To study the interaction between truncated heavy chain and epitope-tagged LAT1, crude extracts of oocytes were analysed by Western blotting under oxidizing conditions to allow the formation of disulphide bridges between heavy and light chains (Figure 5). Co-injection of full-length 4F2hc with LAT1 caused immunoreactivity to appear at molecular masses of 120 kDa and 45 kDa, representing the heterodimer and the tagged light chain, respectively. The T1 and T2 heterodimers appeared at 55 kDa and 64 kDa, respectively. Very weak bands could be detected at 85 kDa and 105 kDa, representing the heterodimers of T3 and T4. However, most protein associated with T3 and T4 formed large aggregates above 200 kDa. When a reducing gel with the same samples was blotted, the aggregates dissappeared and the immunoreactivity was found at 45 kDa, representing the monomer. This suggests that the large aggregates are indeed comprised of heterodimers, rather than being light-chain multimers, forming even larger complexes.

Interaction of truncated 4F2hc mutants with different light chains

The 4F2hc protein, in addition to LAT1, guides a number of other light chains to the plasma membrane. We therefore investigated whether the truncations had a similar effect on the promotion of light chains other than LAT1. At variance with the behaviour of LAT1 we found that activities of the light chains y^+LAT2 and LAT2 were exquisitely sensitive to any truncation

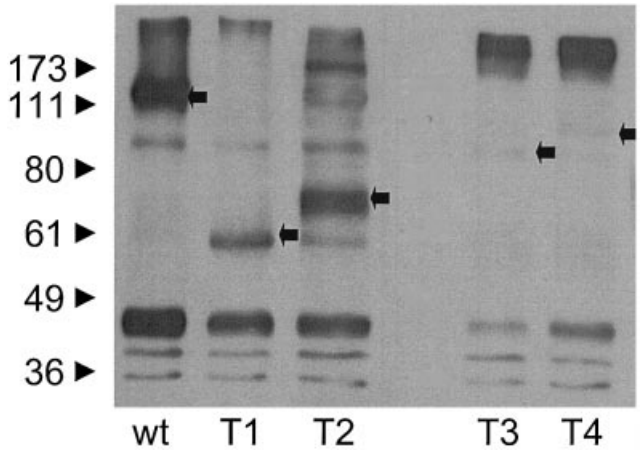

Figure 5 Western blot of oocytes expressing wild-type and truncated 4F2hc together with epitope-tagged LAT1

Oocytes were injected with 10 ng of cRNA encoding wild-type 4F2hc (wt) or truncated versions and 10 ng of epitope-tagged LAT1 cRNA. After an expression period of 2 days, transport activity was determined in seven oocytes of each batch. The remaining ten oocytes were lysed. Aliquots of the extract were separated by SDS/PAGE under oxidizing conditions and subsequently blotted on to nitrocellulose membranes. Epitope-tagged LAT1 was detected with an anti-Myc monoclonal antibody. The arrows indicate the positions of the heterodimers formed by wild-type 4F2hc and its truncated versions. The sizes of marker proteins are shown on the left.

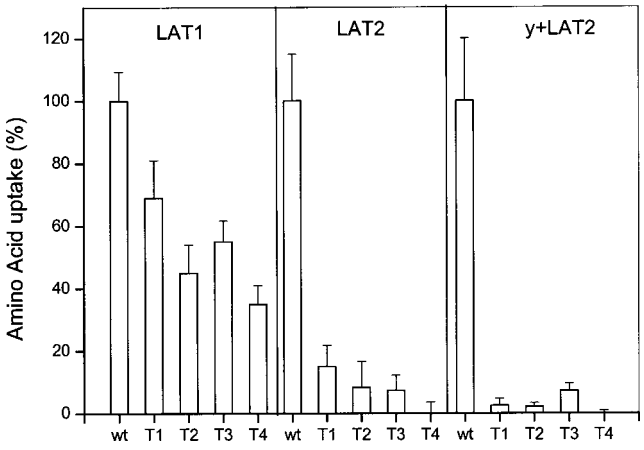

Figure 6 Interaction of wild-type and truncated 4F2hc with different light chains

Oocytes were injected with 10 ng of cRNA encoding wild-type 4F2hc (wt) or truncated versions and 10 ng of either LAT1, LAT2 or y^+LAT2 cRNA. After an expression period of 2 days, transport activity of LAT1 was determined using 100 μM [^{14}C]isoleucine as a substrate, LAT2 activity was determined using 100 μM [^{14}C]alanine, and y^+LAT2 activity was determined using 50 μM [^{14}C]arginine. Sodium-free ND96 buffer was used in all assays. Each bar represents the mean uptake activity of seven oocytes, the activity of non-injected oocytes having been subtracted already. The uptake activity is given as the relative transport activity of that elicited by co-expression with wild-type 4F2hc. The Figure represents a total of three experiments.

of the 4F2hc protein, when activity was determined on the second day (Figure 6). Even on the third day of expression no further increase in the activity of LAT2 or y^+LAT2 could be detected (results not shown).

Mammalian cells express more than one light chain at a time. To investigate possible competition between different light chains, LAT1, LAT2 and y^+LAT2 were co-expressed in the same oocyte

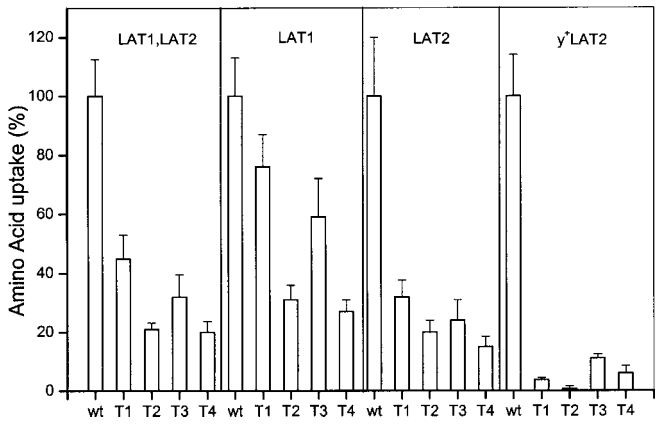

Figure 7 Interaction of wild-type and truncated 4F2hc with different simultaneously injected light chains

Oocytes were injected with 10 ng of cRNA encoding wild-type 4F2hc (wt) or truncated versions and 5 ng each of LAT1, LAT2 and y^+LAT2 cRNA. After an expression period of 2 days transport activity of LAT1 plus LAT2 was determined using 100 μM [^{14}C]isoleucine as a substrate, LAT1 activity was determined by using 100 μM [^{14}C]isoleucine in the presence of 10 mM alanine, LAT2 activity was determined by using 100 μM [^{14}C]alanine, and y^+LAT2 activity was determined using 50 μM [^{14}C]arginine. All transport activities were determined in the absence of sodium ions. Each bar represents the mean uptake activity of seven oocytes, the activity of non-injected oocytes having been subtracted already. The uptake activity is given as the relative transport activity of that elicited by co-expression with wild-type 4F2hc. The Figure represents a total of three experiments.

together with truncated 4F2hc proteins. To maintain the injected volume, the injected amount of light-chain cRNA had to be reduced to 5 ng. However, 5 ng of cRNA is still a saturating amount for expression (results not shown). To determine the transport activity of each light chain selectively, we took advantage of the differences in substrate specificity and ion dependence. The activity of y^+LAT2 was measured as Na^+-independent uptake of arginine (100 μM substrate), an amino acid that is not transported by LAT1 or LAT2. The activity of LAT2 was determined selectively by measuring alanine uptake (100 μM) in Na^+-free transport buffer. Alanine is not recognized by LAT1 and is not transported via y^+LAT2 in the absence of Na^+ [9]. The activity of LAT1 was calculated from the difference of Na^+-independent isoleucine uptake in the presence and absence of 10 mM alanine. The excess of alanine suppresses LAT2 activity and the absence of sodium ions excludes participation of y^+LAT2. When evaluated under these conditions, expression of LAT1 was almost unaffected in the presence of 4F2hcT1, whereas activity of LAT2 and y^+LAT2 was severely impaired, irrespective of the co-expressed truncated 4F2hc unit (Figure 7). The differences in light-chain recognition were therefore retained even in the presence of multiple light chains. The uptake activity observed for each light chain was similar to that in oocytes expressing the light chains separately. For example, the arginine-uptake activity of y^+LAT2 amounted to 37 ± 7 pmol/10 min when expressed alone compared with 32 ± 5 pmol/10 min in the presence of the two other light chains. LAT1 activity was found to be 15 ± 2 pmol/10 min and 19 ± 3 pmol/10 min in the presence and absence of other light chains, respectively. The corresponding figures for LAT2 were 7 ± 1.5 pmol/10 min and 8 ± 1.5 pmol/10 min, respectively.

The 4F2hc-related protein rBAT, when expressed in oocytes, interacts with an endogenous light chain that mediates the Na^+-independent transport of neutral and basic amino acids. To investigate whether the truncated isoforms might activate an

endogenous light chain, all truncations were expressed in the absence of cloned light chains. None of the deletions induced any transport activity above that of wild-type 4F2hc, even after an expression period of 4 days, demonstrating that all results reflected an interaction between the truncated 4F2hc proteins and the co-expressed light chains.

DISCUSSION

In this study we investigated the effect of 4F2hc truncations on two different activities: (i) the trafficking of the 4F2hc–LAT1 complex to the plasma membrane and (ii) the interaction with different light chains. The results indicate that 4F2hc is a protein with domains of different functions. Association of LAT1 with 4F2hc requires only the first 133 amino acids, which include the cytosolic N-terminus and the transmembrane helix. These two elements are most likely involved in the recognition of LAT1. Recognition of all other light chains tested in this study required the complete extracellular domain of the protein. This suggests that the 4F2hc protein has different interaction sites for its associated light chains. The absence of competition between the different light chains further supports this notion. Although only 5 ng of each light-chain cRNA could be injected in these experiments, an increase of the injected amount of either 4F2hc or LAT1 above 5 ng did not result in a significant increase of the transport activity (results not shown). This suggests that the amounts of injected cRNA were still saturating. The independent interaction of 4F2hc with its associated light chains was also observed when 4F2hc was expressed alone in oocytes, causing the induction of several distinguishable transport activities [13]. In contrast to LAT1 that we detected by immunoblotting, the transport activity of the other light chains was taken as a measure of surface expression. Two observations suggest that transport activity is a reliable parameter for surface expression: (i) LAT1 is almost fully active even when the extracellular domain of 4F2hc is completely removed and retains its antiport mechanism (results not shown) and (ii) 4F2hc interacts with a number of light chains forming different transport activities, rendering it unlikely that truncations cause the formation of inactive heterodimers.

The results suggest that recognition of LAT2 and y^+LAT2 is mediated by the C-terminal domain and does not require the transmembrane region or the N-terminus, which interact with LAT1. All deletions caused a delay in the trafficking of LAT1 to the plasma membrane, suggesting that the extracellular domain is required for proper trafficking. This is in agreement with a mutant in the homologous heavy chain rBAT where the lack of glycosylation of the extracellular domain delayed trafficking [14]. Removal of only a few amino acids from the C-terminus appears to disturb trafficking more dramatically than a complete removal of the extracellular domain. The severe trafficking defect of T3 and T4 might be related to the formation of large aggregates, as observed in the Western blot.

The most severe defects in 4F2hc function were observed when the last 70 amino acids were removed. This region shows weak homology to the C-terminal part of the rBAT protein. It has recently been suggested that Cys-664, which belongs to this homologous region, contributes to the functional properties of rBAT/$b^{0,+}$AT heterodimer [15]. In the case of 4F2hc such a participation seems less likely as different light chains display different transport properties while being attached to 4F2hc. Rather, the Western blot indicates that short truncations cause aggregation of heterodimers to larger complexes.

Although 4F2hc interacts with a $b^{0,+}$AT-like endogenous light chain in oocytes [13], and also interacts with $b^{0,+}$AT when overexpressed in mammalian cells [16,17], no increased association of the oocyte endogenous $b^{0,+}$AT-like light chain with any of the truncated 4F2hc forms was observed. The measured transport activities were therefore clearly caused by surface expression of the co-expressed exogenous light chains.

The results gained with deletions of 4F2hc differ from those derived from similar studies with truncated rBAT protein [7]. A short truncation (25 C-terminal amino acids) resulted in a complete loss of activity. However, activity was completely recovered when the deletion was extended to 95 amino acids. Further deletions again caused a loss of activity. Although the latter observation points to functional differences between rBAT and 4F2hc, it appears as a recurrent theme that activity that is lost when only small parts of a heavy chain are removed can be regained. Deora et al. [7] interpreted this result in the frame of a four transmembrane-helix model. Given the low hydrophobicity of the C-terminal part of protein this explanation seems less likely in the case of 4F2hc. As an alternative we would like to propose that the C-terminal ends of both rBAT and 4F2hc proteins loop back and are in contact with the light chains. This would explain the effects of the Cys-664 mutation [15] and would explain the differences in light-chain recognition shown in this study.

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