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The SLC36 family: proton-coupled transporters for the absorption of selected amino acids from extracellular and intracellular proteolysis

Abstract Whilst Na$^+$ has replaced H$^+$ as a major transport driving force at the plasma membrane of animal cells, the evolutionarily older H$^+$-driven systems persist on endomembranes and at the plasma membrane of specialized cells. The first member of the SLC36 family, present in both intracellular and plasma membranes, was identified independently as a lysosomal amino acid transporter (LYAAT1) responsible for the export of lysosomal proteolysis products into the cytosol and as a proton/amino acid transporter (PAT1) responsible for the absorption of amino acids in the gut. In addition to LYAAT1/PAT1, the family comprises another characterized member, PAT2, and two orphan transporters. Both PAT1 and PAT2 mediate 1:1 symport of protons and small neutral amino acids such as glycine, alanine, and proline. Their mRNAs are broadly and differentially expressed in mammalian tissues. The PAT1 protein localizes to lysosomes in brain neurons, but is also found in the apical membrane of intestinal epithelial cells with a role in the absorption of amino acids from luminal protein digestion. In both cases, protons supplied by the lysosomal H$^+$-ATPase or by the acidic microclimate of the brush border membrane drive transport of the amino acids into the cytosol. The subcellular localization and physiological role of PAT2 have still to be determined. SLC36 transporters are related distantly to other proton-coupled amino acid transporters, such as the vesicular neurotransmitter transporter VIAAT/VGAT (SLC32) and system N transporters (SLC38 family).

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Brief history of discovery

SLC36 transporters were identified by homology-based cloning [3, 16], using either the vesicular neurotransmitter transporter VIAAT/VGAT [11, 15] or the yeast amino acid vacuolar transporter AVT3 [14] to search for novel mammalian members of the amino acid/auxin permease (AAAP) superfamily [21, 23]. This superfamily, initially discovered in plants [8, 10] but also present in yeast [14] and animals [4, 11, 15], comprises diverse amino acid transporters coupled, or sensitive, to protons. In 2001 Sagné et al. identified the lysosomal amino acid transporter 1 (LYAAT1) in rat brain [16] and Boll et al. identified PAT1 (orthologous to LYAAT1) and PAT2 cDNAs from mouse intestine and embryos, respectively in 2002 [3]. Most recently, a human PAT1 cDNA has been cloned from the intestinal cell line Caco-2 [5].

Typical characteristics of the SLC36 members

The *SLC36* genes encode ~500-amino acid proteins with 11 transmembrane domains, as predicted by hydropathy analysis [2, 3, 16], but the presence of transmembrane domains 1 and 9 in PAT1 is still being debated [5, 22]. The SLC36 proteins, including the orphan transporters PAT3 and PAT4 (Table 1), exhibit at least 48% identity to each other. The human *SLC36A1–3* and *SLC36A4* genes are located on chromosomes 5q33.1 and 11q14.3, respectively (Table 1). Functionally, these transporters represent the first proton/amino acid symporters found in mammals. Transport activity is independent of Na$^+$ and Cl$^-$, but is pH dependent, and leads to a pronounced intracellular acidification [3, 5, 16]. Amino acid/proton symport occurs with a 1:1 stoichiometry and is thus electrogenic. Conversely, the transport rate is altered by

Table 1 SLC36—the proton-coupled amino acid transporter family [MeAIB2-(methylamino)-isobutyrate]

Human gene name	Protein name	Aliases	Predominant substrates	Transport type/coupling ions*	Tissue distribution and cellular/subcellular expression	Link to disease	Human gene locus	Sequence accession ID	Splice variants and their specific features
SLC36A1	PAT1	LYAAT1	Gly, Ala, Pro, GABA, D-Ala, MeAIB	C/H$^+$	Brain (neurons), intestine, colon, kidney, lung, liver, spleen/Lysosomes (neurons), apical membrane (Caco-2 cells)	–	5q33.1	AF516142	–
SLC36A2	PAT2	Tramdorin1	Gly, Ala, Pro	C/H$^+$	Lung, heart, kidney, muscle, testis, spleen, adrenal gland, thymus, sciatic nerve (Schwann cells)	–	5q33.1	AY162214	–
SLC36A3	PAT3	–	–	O	–	–	5q33.1	AY162215	–
SLC36A4	PAT4	LYAAT2	–	O	–	–	11q14.3	XM_058449 (predicted cDNA)	–

* C Cotransporter, O orphan

changes in membrane potential [3, 22]. Typical substrates are small neutral amino acids like glycine, L-alanine and L-proline.

Specific characteristics of each member

The SLC36A1 and SLC36A2 mRNAs show tissue-specific expression and subcellular distribution in mammalian organisms (Table 1). In situ hybridization and immunohistochemical studies show PAT1 to be expressed in neurons in several regions of the rat brain. The protein localizes to lysosomes in brain neurons [16, 22], but also to the apical membrane in intestinal cells [5], in agreement with earlier studies showing the existence of a H$^+$-driven uptake of small neutral amino acids in this cell line [17, 18, 19]. The localization to neuronal lysosomes has been confirmed at optical and ultrastructural levels in diverse brain areas [1]. Interestingly, the presence of PAT1 at axonal exocysts and at the plasma membrane of cultured neurons, in addition to somatodendritic lysosomes, has also been shown recently and, on the basis of the sensitivity of the protein to exogenously added trypsin in intact neurons, it has been suggested that the plasma membrane PAT1 predominates over PAT1 in the intracellular pool [22]. However, since the synaptic vesicle marker synaptophysin was also degraded in these experiments, the quantitative, and physiological, importance of the plasmalemmal PAT1 protein deserves further investigation. In contrast to PAT1, PAT2 does not localize to lysosomes in transfected HeLa cells [3] and its subcellular localization has not yet been determined.

There are also functional differences between the two proteins. PAT1 not only transports short-chain α-amino acids, but also GABA and β-alanine. It is also an efficient carrier for the D-enantiomers of Ala, Pro and Ser [3, 5, 16, 22]. The apparent K_m values for its substrates are in the millimolar range (1–10 mM). On the other hand, PAT2 generally displays higher selectivity, transporting small L-α-amino acids with a higher affinity (K_m ~100–600 μM) than PAT1. On the other hand, it accepts GABA, β-alanine and the D-amino acids tested so far only poorly. PAT2 activity is also less sensitive to changes in extracellular pH and membrane potential [3].

Physiological roles

PAT1 seems to play a dual role in mammals, depending on its cell-specific subcellular localization. In brain neurons, its localization to lysosomes implies a role in the export of small amino acids generated by lysosomal proteolysis (Fig. 1A) [16]. On the other hand, in small intestinal epithelial cells, it is involved in the absorption of small amino acids and their derivatives at the apical membrane (Fig. 1B). In both cases, an electrochemical H$^+$ gradient drives amino acids into the cytosol (Fig. 1). In the case of neurons, there is no significant pH gradient across the plasma membrane, implying that the driving
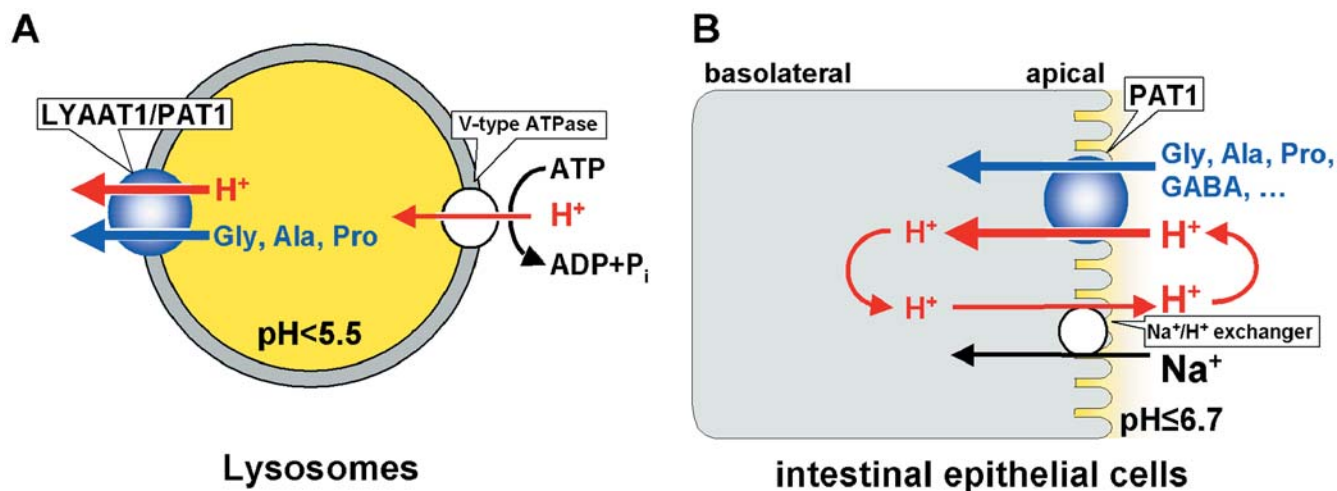
Fig. 1A, B Proposed physiological role of proton/amino acid transporter-1 (*PAT1*). **A** The PAT1/lysosomal amino acid transporter-1 (*LYAAT1*) transporter functions in brain neurons as an export system of small amino acids from lysosomes. **B** In small intestinal epithelial cells, the transporter is involved in the absorption of small amino acids across the apical membrane. In both cases, the acidification of the lysosomal lumen by the V-type H+-ATPase (*A*, *yellow colour*) or the acidic microclimate generated by a Na+/H+ exchanger (*B*, *yellow shading*) provide the electrochemical proton gradient that drives amino acids to the cytosol

force originates solely from the membrane potential. However, under pathological conditions such as inflammation or ischaemia, acidosis may result in a higher contribution of plasmalemmal PAT1 to overall amino acid uptake. At the apical surface of small intestinal epithelial cells, the existence of an acidic microclimate generated by the Na+/H+ exchanger NHE3 has been demonstrated [6, 13]. This proton motive force is also utilized by other nutrient transporters, such as the peptide transporter PEPT1 or the divalent metal ion transporter DMT1 [7, 9]. PAT1 is thought to represent the "classic" imino system described in rat intestine [5], supported by the high similarity in its substrate specificity and its only partially Na+-dependent transport activity [12]. The weak dependence on Na+ can be explained by the reduced NHE3 activity after the removal of extracellular Na+, leading to a decrease in the transmembrane pH gradient and consequently to a reduced driving force for the H+-coupled PAT1 carrier [5].

The significance of PAT1 in overall intestinal absorption of small neutral amino acid relative to the contribution of the Na+-coupled transporters, such as systems B^{o} and B$^{o,+}$, also present in the intestinal brush border membrane, needs to be determined. The physiological significance of PAT2 is not yet known, although a recent study has suggested a role in the myelination of peripheral nerves, based on its regulation by the transcription factor pou3f1 and on its localization at the paranodes and Schmidt-Lanterman incisures of myelinating Schwann cells [2]. However, the specific role of PAT2 in this process needs further investigation.

Possible pathological implications of PAT1

The imino system is considered to be affected in iminoglycinuria [Online Mendelian Inheritance in Man (OMIM) No. 242600], a benign autosomal recessive disease in humans characterized by a markedly reduced renal reabsorption of proline, hydroxyproline and glycine [20]. Since PAT1 is thought to represent the imino system [5] or at least be part of it, defects in the human *SLC36A1* gene might be associated with the genetic cause of iminoglycinuria. However, the localization of the PAT1 protein within the kidney has not been determined and therefore this hypothesis has not yet a molecular basis.

Pharmacological relevance of PAT1

PAT1 is thought to be responsible for the intestinal absorption of orally administered D-serine and D-cycloserine [5], which are used in the treatment of affective disorders and cancers, respectively. If this proves to be valid, the consequence of PAT1 genetic polymorphism on its transport activity might become an important issue for the intestinal absorption and bioavailability of these drugs but also their action in the central nervous system.

References

1. Agulhon C, Rostaing P, Ravassard P, Sagné C, Triller A, Giros B (2003) The lysosomal amino acid transporter LYAAT-1 in the rat central nervous system: an in situ hybridization and immunohistochemical study. J Comp Neurol (in press)
2. Bermingham JR Jr, Shumas S, Whisenhunt T, Sirkowski EE, O'Connell S, Scherer SS, Rosenfeld MG (2002) Identification of genes that are downregulated in the absence of the POU

domain transcription factor pou3f1 (Oct-6, Tst-1, SCIP) in sciatic nerve. J Neurosci 22:10217–10231

3. Boll M, Foltz M, Rubio-Aliaga I, Kottra G, Daniel H (2002) Functional characterization of two novel mammalian electrogenic proton dependent amino acid cotransporters. J Biol Chem 277:22966–22973

4. Chaudhry FA, Reimer RJ, Krizaj D, Barber D, Storm-Mathisen J, Copenhagen DR, Edwards RH (1999) Molecular analysis of system N suggests novel physiological roles in nitrogen metabolism and synaptic transmission Cell 99:769–780

5. Chen Z, Fei YJ, Anderson CMH, Wake KA, Miyauchi S, Huang W, Thwaites DT, Ganapathy V (2003) Structure, function and immunolocalization of a proton-coupled amino acid transporter (hPAT1) in the human intestinal cell line Caco-2. J Physiol (Lond) 546:349–361

6. Daniel H, Fett C, Kratz A (1989) Demonstration and modification of intervillous pH profiles in rat small intestine in vitro. Am J Physiol 257:G489–G495

7. Fei YJ, Kanai Y, Nussberger S, Ganapathy V, Leibach FH, Romero MF, Singh SK, Boron WF, Hediger MA (1994) Expression cloning of a mammalian proton-coupled oligopeptide transporter. Nature 368:563–566

8. Frommer WB, Hummel S, Riesmeier JW (1993) Expression cloning in yeast of a cDNA encoding a broad specificity amino acid permease from *Arabidopsis thaliana*. Proc Natl Acad Sci USA 90:5944–5948

9. Gunshin H, Mackenzie B, Berger UV, Gunshin Y, Romero MF, Boron WF, Nussberger S, Golan JL, Hediger MA (1997) Cloning and characterization of a mammalian proton-coupled metal ion transporter. Nature 388:482–488

10. Hsu LC, Chiou TJ, Chen L, Bush DR (1993) Cloning a plant amino acid transporter by functional complementation of a yeast amino acid transport mutant. Proc Natl Acad Sci USA 90:7441–7445

11. McIntire SL, Reimer RJ, Schuske K, Edwards RH, Jorgensen EM (1997) Identification and characterization of the vesicular GABA transporter. Nature 389:870–876

12. Munck BG, Munck LK, Rasmussen SN, Polache A (1994) Specificity of the imino carrier in rat small intestine. Am J Physiol 266:R1154–R1161

13. Rawlings JM, Lucas ML, Russell RI (1987) Measurement of jejunal surface pH in situ by plastic pH electrode in patients with celiac disease. Scand J Gastroenterol 22:377–384

14. Russnak R, Konczal D, McIntire SL (2001) A family of yeast proteins mediating bidirectional vacuolar amino acid transport. J Biol Chem 276:23849–23857

15. Sagné C, El Mestikawy S, Isambert MF, Hamon M, Henry JP, Giros B, Gasnier B (1997) Cloning of a functional vesicular GABA and glycine transporter by screening of genome databases. FEBS Lett 417:177–183

16. Sagné C, Agulhon C, Ravassard P, Darmon M, Hamon M, El Mestikawy S, Gasnier B, Giros B (2001) Identification and characterization of a lysosomal transporter for small neutral amino acids. Proc Natl Acad Sci USA 98:7206–7211

17. Thwaites DT, McEwan GT, Brown CD, Hirst BH, Simmons NL (1993) Na$^+$-independent, H$^+$-coupled transepithelial beta-alanine absorption by human intestinal Caco-2 cell monolayers. J Biol Chem 268:18438–18441

18. Thwaites DT, McEwan GT, Brown CD, Hirst BH, Simmons NL (1994) L-alanine absorption in human intestinal Caco-2 cells driven by the proton electrochemical gradient. J Membr Biol 140:143–151

19. Thwaites DT, McEwan GT, Simmons NL (1995) The role of the proton electrochemical gradient in the transepithelial absorption of amino acids by human intestinal Caco-2 cell monolayers. J Membr Biol 145:245–256

20. Wellner D, Meister A (1981) A survey of inborn errors of amino acid metabolism and transport in man. Annu Rev Biochem 50:911–968

21. Wipf D, Ludewig U, Tegeder M, Rentsch D, Koch W, Frommer WB (2002) Conservation of amino acid transporters in fungi, plants and animals. Trends Biochem Sci 27:139–147

22. Wreden CC, Johnson J, Tran C, Seal RP, Copenhagen DR, Reimer RJ, Edwards RH (2003) The H$^+$-coupled electrogenic lysosomal amino acid transporter LYAAT1 localizes to the axon and plasma membrane of hippocampal neurons. J Neurosci 2003 23:1265–1275

23. Young GB, Jack DL, Smith DW, Saier MH Jr (1999) The amino acid/auxin:proton symport permease family. Biochim Biophys Acta 1415:306–322