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INVITED REVIEW

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Adaptation of plasma membrane amino acid transport mechanisms to physiological demands

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Abstract The molecular identification of almost all physiologically characterized amino acid transporters in recent years has facilitated the functional analysis of this important class of transport proteins. The picture that emerges from these studies is that antiport is the prevalent mode of amino acid transport rather than a combination of uniporters and cotransporters. Mainly neurotransmitters and osmolytes are transported by complex cotransport mechanisms that allow a high intracellular accumulation. Antiport mechanisms almost invariably include the nonessential amino acids alanine and glutamine, which are used as exchange substrates. The intracellular level of both amino acids is well regulated by Na^+/amino acid cotransporters. Transport mechanisms are not conserved within families and may change with mutation of even a single amino acid residue in the transport protein. Thus transport mechanisms are easily adapted to physiological demands during evolution.

Keywords Amino acid transport · Antiporter · Cloning · Symporter · Transport mechanism

Classical concepts revisited

In the pre-cloning era, studies on amino acid transport were dominated by three different concepts. The first concept, mainly developed for epithelial cells by Richard Crane [17], hypothesized that metabolites (including amino acids) are taken up by mechanisms exploiting the Na^+-electrochemical gradient on the apical side of reabsorbing epithelia and are released on the basolateral side by electroneutral uniport mechanisms (at least in the case of neutral solutes). The second concept, developed by Halvor Christensen mainly for nonepithelial cells [16], postulated that amino acid transporters are substrate-class transporters recognizing specific groups of amino acids, such as neutral, anionic or cationic amino acids. Finally, the third concept, developed by Alton Meister, was that of an ATP-driven amino acid uptake that occurred in the context of the γ-glutamyl-cycle [40]. The first two concepts have now to be regarded as seminal ideas, whereas after many years of research it is clear that, as a mediator of amino acid transport, the γ-glutamyl-cycle either does not exist at all or only plays a negligible role. At least 95% of amino acid uptake activity in well investigated cell types can be attributed to transporters fitting into concepts one or two, whereas the latter 5% remain undefined due to experimental problems. As they were developed in different model systems, concepts one and two are not exclusive. Crane's concept focused on the energetic coupling of solute transport, whereas Christensen's concept focused on substrate specificity and substrate recognition.

Further characterization and in particular cloning of plasma membrane amino acid transporters subsequently have led to modifications of both concepts. It has now become clear that, firstly, antiporters are the prevalent mechanism of amino acid transport, and are even essential for vectorial transport across epithelia, and secondly that electrochemical gradients other than that of sodium are frequently used to drive amino acid transport or at least contribute to the driving force. Similarly, amino acid transporters have been identified crossing substrate class borders that transport neutral and cationic amino acids or neutral and anionic amino acids, for example.

It is now generally accepted to subdivide physiologically characterized amino acid transporters into systems that have an acronym that roughly indicates the substrate specificity. The cloned amino acid transporters have been allocated to these systems but the nomenclature unfortunately remains inconsistent (Table 1).

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Table 1 Mammalian amino acid transporters, designations, mechanisms and substrate specificities

Amino acid transport system	Isoforms (cloned cDNAs)	Other names	Mechanism	Physiological substrates[a]
System A	ATA1 ATA2 ATA3	GlnT, SAT1, NAT2, SA2 SA1, SAT2 NAT3	$1Na^+$/AA cotransport	G, A, S, C, Q, N, H, M G, P, A, S, C, Q, N, H, M G, P, A, S, C, N, M
System asc	4F2hc/asc1 ?hc/asc2		Antiporter	G, A, S, C, T (D and L isoforms) G, A, S, T
System ASC	ASCT1 ASCT2	SAAT1 ATB^0	Na^+-dependent antiporter	A, S, C A, S, C, T, Q
System $b^{0,+}$	rBAT/$b^{0,+}$AT	NBAT/lc6	Antiporter	K, R, A, S, C, T, N, Q, H, M, I, L, V, F, Y, W, C^-
System B^0	–	–	Na^+/AA cotransporter	A, S, C, T, Q, F, W, Y
System $B^{0,+}$	$ATB^{0,+}$	–	$2Na^+/1Cl^-$/AA cotransporter	(K, R), A, S, C, (T, N, Q), H, M, I, L, V, F, Y, W
System β	GAT1 GAT2 GAT3 GAT4	GAT1 BGT1 GAT2 GAT3	$2–3Na^+/1Cl^-$/AA cotransporter	GABA GABA, betaine, P, β-Ala GABA, betaine, taurine GABA, betaine
System Gly	GlyT1 GlyT2		$2–3Na^+$/1Cl/AA cotransporter	G G
System IMINO	PAT1?[b]		$1H^+$/AA-cotransporter	P, G, A, β-Ala
System L	4F2hc/LAT1 4F2hc/LAT2	 4F2hc-lc5	Antiporter	(Q), H, M, L, I, V, F, Y, W A, S, C, T, N, Q, H, M, L, I, V, F, Y, W
System N	SN1 SN2	NAT1	Na^+/AA-cotransport/H^+-antiport	Q, N, H Q, N, H, S, G
System T	TAT1		Uniport	F, Y, W
System X^{AG}	EAAT1 EAAT2 EAAT3 EAAT4 EAAT5	GLAST GLT-1 EAAC1	$3Na^+/1H^+$/AA-cotransport/ $1K^+$-antiport	E, D E, D E, D, C E, D E, D
System x^{-c}	4F2hc/xCT		Antiporter	E, C^- (no D)
System y^+	CAT-1 CAT-2A/B CAT-3 (CAT-4)		Uniporter	R, K, H R, K, H R, K ?
System y^+L	4F2hc/y^+LAT1 4F2hc/y^+LAT2		Na^+-dependent antiporter	K, R, Q, H, M, L K, R, Q, H, M, L, A, C

[a] Amino acids are given in the one-letter code (C^- Cystine), weak substrates are shown in parantheses
[b] Assignment not yet clarified

Mechanisms of plasma membrane amino acid transport: an overview

Plasma membrane amino acid transporters fall into five families when analysed by sequence homology (for substrate specificities see Table 1; SLC denotes the solute carrier family):

a. The Na^+- and Cl^--coupled neurotransmitter/amino acid transporter family (SLC6).
b. The EAAT/ASCT transporter family (SLC1).
c. The CAT transporter family (SLC7).
d. The 4F2heavy chain/light chain and rBATheavy chain/light chain transporter family (SLC3+SLC7).
e. The ATA/SN family of AAAP-related transporters (amino acid and auxin permeases, SLC38).
f. The TAT transporter related to the monocarboxylate transporter family (MCT, SLC16).

The transport mechanisms inside these families differ substantially. The family (b) members ASCT1/2, for example, are obligatory antiporters, whereas EAAT1–5 have a complex mechanism, involving the cotransport of Na^+ ions and H^+ and the antiport of K^+ ions that results in the highly accumulative net uptake of glutamate (see below). Family (e) comprises electrogenic Na^+/amino acid cotransporters and electroneutral Na^+/amino acid cotransporters/H^+ antiporters. Amino acid transporters also have different structures. Group (b) transporters have eight transmembrane helices and irregularly folded elements between helix seven and eight [18]. Groups (a, c and e) are comprised of polytopic membrane proteins

with 11–14 transmembrane helices [44]. Group (d) are heterodimeric proteins that are constituted by a heavy and a light chain. The heavy chain is a highly glycosylated type II membrane protein (intracellular amino-terminus) with only one transmembrane helix. The light chain, in contrast, is a nonglycosylated typical polytopic membrane protein, weakly related to the CAT transporters [15, 65, 66]. In respect to physiological functions, transporters may be grouped in a different way:

1. Highly accumulating transporters (EAAT, Na^+- and Cl^--coupled amino acid transporters).
2. Weakly accumulating transporters (the ATA/SN family, the CAT transporters and TAT).
3. Antiporters or metabolite exchangers (4F2hc-lc/rBAT-lc and ASCT-transporters).

Not surprisingly, neurotransmitter and osmolyte transporters are found in group (1). Group (2) comprises transporters that allow a net uptake or efflux of substrate depending on the prevalent transmembrane concentration gradient. Group (3) is a versatile group of antiporters, some of which are tailored for resorptive functions and others for secretory function. Antiporters, in general, offer the advantage that loss of amino acids is avoided in the absence of extracellular substrates and even while transporting the total concentration of amino acids remains constant. This could be why the reabsorption of essential cationic amino acids in the kidney is mediated by an antiporter (rBAT associated light chain) rather than by weakly accumulating CAT transporters [42].

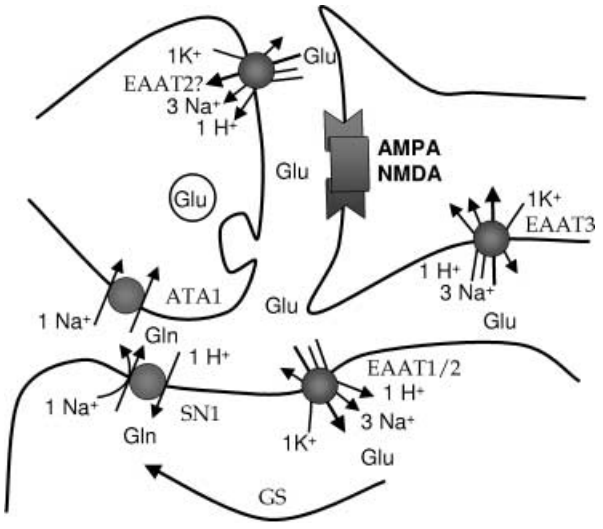

Fig. 1 Recycling of neurotransmitter glutamate. Glutamate is released as a neurotransmitter and binds to post-synaptic NMDA and AMPA receptors. Glutamate is subsequently removed largely by the glial glutamate transporters EAAT1 and EAAT2. The post-synaptic neuronal glutamate transporter EAAT3 contributes to a lesser extent to glutamate uptake. The presynaptic glutamate transporter has not yet been identified but appears to be similar to EAAT2. Inside astrocytes, glutamate is converted into glutamine by the action of glutamine synthetase (*GS*) and is subsequently released via the glutamine transporter SN1. The Na^+ cotransporter ATA1 appears to be the major uptake site for glutamine in neurons

Highly accumulating transporters with a complex mechanism

Glutamate is the major excitatory neurotransmitter in the brain. Its intracellular concentration in the brain is high, rising up to several millimolar. By contrast, extracellular concentrations have to be maintained at a few micromolar to avoid excitotoxicity. Thus, the concentration gradient that is constantly maintained is about 10,000-fold [18]. In addition to high affinity, a high capacity is needed in the brain, as glutamate is continuously released from neurons and glial cells by different routes (Fig. 1). The analysis of glutamate transporters in a variety of expression systems has now established that glutamate is cotransported with three Na^+ ions and one H^+ ion [71]. Return of the unloaded carrier to the extracellular face is facilitated by the binding of one intracellular K^+ ion [30]. The stoichiometry appears to be the same for all isoforms of the transporter. The antiport of potassium does not contribute to the driving force because the potassium electrochemical gradient in mammalian cells is close to equilibrium. The transporter has the potential to mediate both substrate exchange and net transport [30]. In the absence of intracellular potassium, exchange is mandatory, because the empty carrier cannot return. Mutations of residues Y403 and E404 lock the glutamate transporter in the exchange mode and also cause the ion selectivity and affinity of the Na^+-binding site to change [72]. The exchange function of the glutamate transporter, however, is also observed in the presence of potassium and may protect cells against the osmotic effects of glutamate accumulation. Under optimal conditions, glutamate transporters accumulate substrates more than a million-fold. Even at micromolar extracellular concentration this results in molar intracellular concentrations. A switch to exchange would reduce accumulation and at the same time prevent loss of substrate. During glutamate transport an acidification of the cytosol is observed, indicating the cotransport not only of Na^+ ions but also of protons [71]. Kinetic analysis reveals that protons (rather than OH^- ions) bind to the transporter before the substrate, and that glutamate is transported as an anion [67]. Although the different isoforms apparently have identical stoichiometries, differences were observed in the rate constants of the individual steps in the transport cycle. It appears that the substrate translocation step in EAAT2 and EAAT3 is very fast and that the complete transport cycle is limited by the return of the potassium-loaded carrier. This ensures that glutamate, released during neurotransmission, is rapidly cleared by a single turnover of the substrate-loaded transporter [23, 41]. Such a scenario is feasible because of the high density of glutamate transporters around the synapse (estimates between 2000 and 12,000 molecules per μm^2) matching the number of released glutamate molecules [18]. The cerebellar EAAT1, in contrast, has a slow transport–translo-

Table 2 Variations of mechanisms in the family of Na^+- and Cl^--dependent amino acid/amine transporters

Transporter molecule	Substrate	Cotransported ions	Antiported ions	Net charges translocated	Ref
GAT1	GABA	2 Na^+, 1 Cl^-	1 Cl^-	2	[35]
TAUT	Taurine	2 Na^+, 1 Cl^-		2	[49]
BGT1 (GAT-2)	Betaine	3 Na^+, 1 Cl^-		2	[36]
GlyT1b	Glycine	2 Na^+, 1 Cl^-		1	[53]
GlyT2a	Glycine	3 Na^+, 1 Cl^-		2	[53]
SERT	Serotonin (+)	1 Na^+, 1 Cl^-	1 K^+	0	[24]
NET	Norepinephrine (+)	1 Na^+, 1Cl^-		1	[24]
DAT	Dopamine (+)	2 Na^+, 1Cl^-		2	[58]
$B^{0,+}AT$	Neutral+cationic AA	2 Na^+, 1Cl^-		1–2	[56]
KAAT1	Neutral AA	2 K^+, 1Cl^-		1	[13]
CAATCH1	Neutral AA	2 K^+		2	[21]

cation step and might be less effective in clearing the synapse. The affinity of EAAT3 in addition is regulated by the ancillary protein GTRAP3–18 [33]. When GTRAP3–18 is coexpressed with EAAT3, its affinity is decreased from 9 µM to 40 µM. The physiological relevance of this modulation remains to be established.

As mentioned above, transport mechanisms are not well conserved within families. Remarkable examples of such variations are the Na^+/Cl^--coupled neurotransmitter/amino acid transporters. Initially it was thought that members of this family invariably have a Na^+:Cl^-:substrate cotransport stoichiometry of 2:1:1. Closer examination of the transport mechanisms, however, revealed a high variability. A partial overview over the different stoichiometries is shown in Table 2.

Roux and Supplison [53], for example, showed in a very detailed study that neuronal and glial glycine transporters have different stoichiometries. The neuronal GlyT2a transported two net charges per glycine, whereas a transfer of only one net charge was detected in the glial GlyT1b. Both transporters depended on the presence of chloride and a cotransport of 1 mol ^{36}Cl per mol of substrate was demonstrated in both cases. A thermodynamic analysis of the reversal potential of glycine transport supported the two observed stoichiometries. Again in agreement with the different coupling ratios it was found that elevating the intracellular glycine concentration could indeed reverse glycine transport via GlyT1b, whereas in the case of GlyT2a both the intracellular glycine and sodium concentration had to be elevated in order to reverse the flux of glycine. The authors proposed that both transporters initially contributed to the rapid clearing of glycine from the synaptic cleft, thereby transporting the neurotransmitter into both neurons and astrocytes. However, over a period of time the greater accumulation provided by the neuronal transporter will force glycine out of the glial cells by the reversal of GlyT1b, to be subsequently transported uphill into the neurons via the GlyT2a transporter [53].

The Na^+-cotransport stoichiometry is not the only mechanistic parameter that varies in this family. GlyT1b and GlyT2a mediate a net cotransport of chloride, whereas an exchange of chloride ions was observed in the case of the GABA transporter GAT-1 [35]. Although transport depends on Cl^-, Cl^- does not contribute to the driving force of the transporter because it is exchanged. The 2 Na^+/1GABA cotransport is thus accompanied by the net transfer of two charges per cycle rather than one. The closely related betaine transporter BGT-1, by contrast, also transfers two net charges per transport cycle, but by using the same $3Na^+/1Cl^-$/substrate cotransport mechanism as GlyT2a [36].

The general amino acid permease $ATB^{0,+}$ complies to the rule in this family with a Na^+:Cl^-:substrate stoichiometry of 2:1:1 [56]. Despite its accumulative capacity, the transporter does not appear to be involved in the general absorption of amino acids in the intestine. It might, however, be involved in the transport of D-amino acids that are generated by bacteria in the distal parts of the intestine where expression of $ATB^{0,+}$ is high [26]. Further indication of a more specialized role of this transporter is its strong expression in the lung and salivary gland.

Further mechanistic variations are displayed by the monoamine transporters in this family. The serotonin transporter [24] mediates $1Na^+/1Cl^-$/serotonin$^+$ cotransport in which import of a single positive charge is balanced by the export of one K^+ ion. Similar to the glutamate transporters, the K^+ ion is used in the serotonin transporter to allow the return of the unloaded transporter. This restricts the accumulative power of the serotonin transporter to little more than the sodium chemical gradient. An even less accumulative mode of transport has been described for the norepinephrine transporter [22]. Electrochemical detection of norepinephrine fluxes suggested that large numbers of substrate molecules pass through transiently formed substrate-gated channels in the norepinephrine transporter. This transport mode might overcome the slow turnover rate of amine transporters of only about 1/s [47]. The low accumulative power of this and other monoamine transporters appears surprising at first glance because all substrates are potent neurotransmitters. However, in contrast to glutamate, which is recycled as a neurotransmitter, amines are rapidly degraded by the action of monoamine oxidase and catechol-*O*-methyl-transferase [55]. Thus, intracellular monoamine concentrations are continuously maintained

at low levels. It is likely that the transporters only have to supply sufficient capacity for monoamine removal. The high affinity of monoamine transporters (in the nanomolar range) ensures that the transporters are fully activated even at low concentrations. As the turnover rate of monoamine transporters is rather low, the channel-like transport mode aids in the rapid clearance of the neurotransmitter. The substrate-induced channel is also permeable to ions (reviewed in [20, 57]) thereby obscuring the cotransport stoichiometries.

The family of Na^+- and Cl^--dependent amino acid transporters has evolved in a separate way in certain insect species such as lepidoptera. In contrast to the Na^+ gradient that prevails in mammalian and other eukaryotic tissues, a K^+ gradient is exploited for intestinal reabsorption of amino acids in these insects [68]. Two cell types, goblet cells and columnar cells, mainly constitute the midgut of these insects. Goblet cells express a H^+-ATPase that generates a proton-motive force in the first place, which in turn is converted into a potassium electrochemical gradient by the action of a $K^+/2H^+$ antiporter. The potassium is thereby transported from the hemolymph into the lumen. The combined action of H^+-ATPase and $K^+/2H^+$ antiporter generates a large potassium electrochemical gradient that is used to drive the uptake of amino acids by K^+-amino acid symporters, which are allocated to columnar cells in the midgut. The first cloned member of this family, KAAT1, mediates the electrogenic uptake of neutral amino acids [13]. Potassium can be replaced by other cations, such as Na^+ and Li^+, thereby demonstrating the relationship of KAAT1 to the Na^+-dependent transporters of this family. In agreement with its similarity to the Na^+- and Cl^--dependent amino acid transporters, amino acid uptake via KAAT1 was also found to be chloride dependent. A cotransport stoichiometry of 2:1:1 (K^+:Cl^-:amino acid) was proposed [13].

The transport mechanisms in this family thus comprise highly accumulative mechanisms on the one hand; such as those of osmolyte transporters, which maintain very high substrate gradients in the kidney (betaine, taurine), those of neurotransmitter transporters, that capture neurotransmitters that are not metabolically inactivated (glycine and GABA), or those of apical transporters that mediate the reabsorption of amino acids in epithelia ($B^{0,+}$ and potassium driven transporters). On the other hand, the family also includes transporters with low accumulative power, transporting neurotransmitters that are metabolically inactivated.

Weakly accumulating transporters

Surprisingly, there are only very few simple Na^+-coupled amino acid transporters. Members of the recently identified family of system A transporter-isoforms display such a mechanism. Electrophysiological recordings and flux data are in accordance with a $1Na^+$/amino acid cotransport mechanism catalysed by system A isoforms ATA1, 2 and 3 [1, 59, 69]. Although they are prototypical Na^+-dependent transporters, it appears rather unlikely that the resorption of amino acids across the apical membrane of epithelia is mediated by system A. First, only low levels of system A (isoform ATA2) are expressed in the kidney [59]. Secondly, substrate affinities of system A variants are usually low. Thirdly, accumulation ratios achieved by system A are relatively low (<100-fold) encompassing the risk of metabolite loss. The primary function of system A appears to maintain elevated cytosolic levels of nonessential amino acids mainly in nonpolarized cells. These amino acids are either synthesized in abundance by the cell itself or are taken from nutritional sources. The nonessential amino acids, particularly glutamine and alanine, are subsequently used to accumulate essential amino acids via the plethora of amino acid exchangers (see below).

The sequence-related isoforms of the system N family (SN1 and SN2) display a more complex mechanism. Transport of substrates via SN1 is accompanied by the cotransport of one Na^+ ion and the antiport of one H^+ [5, 14]. The preferential direction of transport is thus sensitive to changes of the extracellular pH. At pH 6.0, for example, the transporter releases glutamine, while at pH 8.0 glutamine is accumulated [5]. Similarly to the monoamine transporters, the stoichiometry has been obscured by a cation conductance, which is induced by the expression of the transporter in *Xenopus* oocytes [5, 14]. It has been proposed that the cation conductance is mainly permeable to protons, which could counterbalance the efflux of protons occurring as part of the transport mechanism [14]. Both system A and system N have a similar allosteric pH dependence that increases the transport activity five- to tenfold between pH 6 and pH 8. System N in addition has a catalytic pH dependence that is detectable at pH>8.0 [5]. Due to the overall electroneutral mechanism, the accumulative power of system N is much lower than that of the related system A. Thus, glutamine transport via system N is readily reversible, whereas system A transport reverses only when the membrane is depolarized or when the intracellular Na^+ concentration is elevated [1, 50]. The participation of protons in the transport mechanism and the narrow substrate specificity clearly discriminates system A isoforms from system N isoforms (Table 1) [1, 50]. The recent assignment of ATA1 as a system-N-like transporter is, thus, not supported by functional data [25]. In agreement with the reversible transport mechanism of SN1, the protein is found in tissues and cells that release glutamine. In the brain neurotransmitter glutamate is recycled via the glutamate-glutamine cycle (Fig. 1) [9]. After being released during neurotransmission, glutamate is taken up largely by astrocytes. There it is converted into glutamine and subsequently released into the extracellular space. Glutamine is then taken up by neurons and converted into glutamate. Similarly, perivenous scavenger cells in the liver take up glutamate and use glutamine synthetase to convert it into glutamine, which is then released into the blood [27]. Striated muscle cells take up

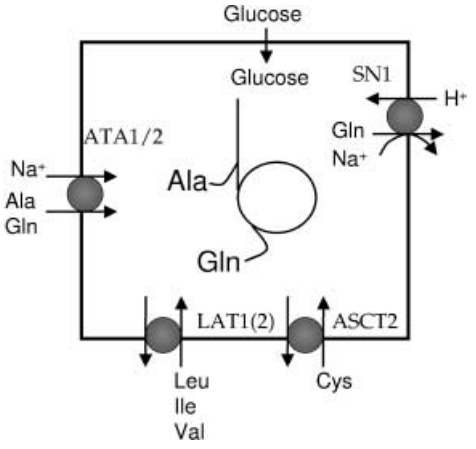

Fig. 2 Interaction between antiporters and cotransporters in non-epithelial cells. Cells are able to synthesize alanine and glutamine from metabolic intermediates. Glycolysis and the tricarboxylic acid (TCA) cycle are schematically indicated by a *straight line* and a *circle*, respectively. Levels of glutamine and alanine are kept elevated by the action of Na^+ cotransporters. Alanine and glutamine act as exchange substrates for the uptake of essential amino acids. In glutamine-releasing cells expression of SN1 is observed

or release glutamine, depending on whether they reside in an anabolic or catabolic state, respectively [51]. All three cell types have been shown to express SN1. The related isoform SN2 [38] prefers small neutral amino acids but is likely to have a similar mechanism. Accumulation of glutamine in nonpolarized mammalian cells appears to be regulated by the interaction of three types of transporters: system A, system N and system ASC (Fig. 2). System A can accumulate glutamine up to 100-fold and may actually play a major role in the maintenance of an elevated intracellular glutamine concentration. The presence of system N in addition to system A probably decides whether a cell releases glutamine or accumulates it. System A is known to be regulated by osmolarity, amino acid availability and hormonal stimuli [2, 28, 34]. System A isoforms could also form together with the antiporter ASCT2 (see below) a tertiary active amino acid uptake system as follows. Glutamine synthesized inside cells is maintained by the accumulative power of system A. The high intracellular glutamine concentration makes it the prevalent substrate for amino acid exchange by ASCT2. Thus, uptake of small neutral amino acids could be coupled to the efflux of glutamine. Such a mechanism might play a role in alanine and cysteine transport in the brain, and also may contribute to the resorption of small neutral amino acids in the intestine [3].

Proton-coupled transport mechanisms are frequently found in organisms that produce a proton electrochemical gradient across their plasma membrane, such as plants, yeast and bacteria. The proton electrochemical gradient in mammalian cells is significantly lower than the Na^+ electrochemical gradient, but larger than the electrochemical gradients of chloride and potassium. In the renal nephron and in the intestine, however, a significant pH gradient is built up due to an acidic microclimate in the lumen. This gradient is used to drive the uptake of peptides [19] and also of amino acids [61]. Thwaites and Stevens [62] have described a transporter with striking similarity to the IMINO system, which cotransports proline, glycine, alanine, β-alanine, MeAIB and sarcosine with protons. The pH dependence is similar to that of monocarboxylate transporters [10], showing maximal transport at pH 5.0 but negligible transport at pH 8.0. The transport system has now been identified on a molecular basis and named PAT1 (H. Daniel and M. Boll, Technical University Munich, personal communication).

Many of the transport systems that were expected to be uniporters, particularly basolateral transport systems for neutral amino acids, appear to be tightly coupled antiporters. The number of true uniport systems is surprisingly low. Potential explanations for the low abundance of uniporters are that this mechanism does not protect the cell from the loss of valuable metabolites. Nutritional changes in the plasma amino acid content are converted into similar changes in intracellular amino acid levels. Prototypical uniport mechanisms have, however, been demonstrated in the case of the cationic amino acid transporters. These transporters are able to mediate the uptake and release of arginine. The concentration gradient is well balanced with the membrane potential. A depolarization of the membrane potential thus results in a release of arginine from the cells, whereas hyperpolarization drives the influx of arginine. However, isoforms of the cationic amino acid transporter with low K_m values show high exchange activity of the substrate, thereby limiting net transport. The high-affinity isoform hCAT-2B, for example, although mediating arginine uniport, does not display the voltage dependence expected for a cation uniporter when cells are preloaded with arginine [39]. This result suggests that the arginine binding site is easily saturated on both sides of the membrane, rendering the transporter almost incapable of performing uniport. It may be speculated that hCAT-2B plays a role in the equilibration of arginine, ornithine and lysine pools rather than being involved in net uptake. The low-affinity isoform hCAT-2A, by contrast, displays strong substrate-induced inward currents at negative holding potentials eventually ceasing at positive holding potentials [39]. Cationic amino acids are the only class of essential amino acids that are transported by uniporters rather than by using antiport mechanisms. The intracellular arginine concentration thus reflects the nutritional status and could present a metabolic signal. In support of this notion it was recently found that the neutral amino acid antiporter LAT1 is regulated by arginine availability, although arginine is not a substrate of the transporter [12].

Recently, a uniporter for aromatic amino acids has been identified and named TAT1 [31]. Surprisingly, its cDNA sequence is related to the family of monocarboxylate transporters rather than to any amino acid transporter family. The family of monocarboxylate transporters mediates the cotransport of protons with monocarboxylate anions, such as lactate or pyruvate [10]. The

transport activity increases with decreasing pH. In contrast to this behaviour, the transport of aromatic amino acids by TAT1 was pH independent, suggesting that the coupling to proton cotransport was lost. The transporter also accepted carboxymethylesters of amino acids as substrates. This was taken as an indication that the protonated forms of amino acids may serve as substrates of the transporter [31]. The hypothesis bears the problem that transport should be strongly pH dependent, because the amount of protonated amino acids increases with decreasing pH. Moreover, the amount of protonated amino acids at physiological pH is exceedingly low, as the p*K* of the α-carboxyl-group is close to 2.0. It is rather likely that other features of aromatic amino acids are recognized by the transporter. In contrast to those members of the monocarboxylate transporter family that mediate monocarboxylate-proton cotransport, a critical aspartate residue in helix eight is not conserved in TAT1 and its closest homologue MCT8. This residue has been implicated in proton transport and might thus have become redundant [48]. The main physiological task of this transporter appears to be the release of aromatic amino acids on the basolateral side of epithelia.

Antiporters

Antiporters constitute the majority of amino acid transporters in mammalian tissues. This reflects the fact that most tissues or cells have excessive amounts of nonessential amino acids but may lack essential amino acids. Almost any cell can synthesize alanine, glutamine, glutamate, asparagine and aspartate from metabolic intermediates, whereas branched-chain amino acids, aromatic amino acids and most cationic amino acids are essential. Antiporters, thus, preferentially mediate an exchange of essential amino acids against nonessential amino acids without causing a net loss or increase of the total amount of amino acids (Fig. 2). This notion is supported by the fact that all amino acid exchangers (see Table 1) accept alanine, glutamine or glutamate as a substrate. The only exception to this rule is 4F2hc/LAT1, which does not mediate efficient transport of glutamine (alanine and glutamate are not substrates of LAT1) in the presence of other amino acids (own unpublished observations and [37]). Its role in cell physiology may be the equilibration of pools of branched-chain and aromatic amino acids. For cell growth, however, a net uptake of nitrogen has to take place. This net uptake is mediated by ubiquitously expressed transporters that allow the net uptake of alanine or glutamine (system A). Cationic amino acids do not take part in this exchange process. As outlined above, cationic amino acid transporters are electrogenic uniporters. Surprisingly, it has now been firmly established that antiporters play a major role in the transfer of cationic amino acid across epithelia [15, 65, 66]. Two isoforms of exchangers, system $b^{0,+}$ and y^+L, mediate the transfer of cationic amino acids across the apical and basolateral membrane, respectively (see below).

All antiporters, apart from ASCT type antiporters, belong to the family of heterodimeric amino acid transporters. These transporters are constituted by a type II transmembrane protein that has only one transmembrane helix (also called the heavy chain) and an associated polytopic transporter-like membrane protein of 12 transmembrane helices (also called the light chain) [15, 65, 66]. Depending on the heavy chain, transporters are either expressed on the apical side (rBAT heavy chain) or the basolateral side (4F2 heavy chain) in epithelial cells or in nonepithelial cells (4F2 heavy chain). The main function of the heavy chain is the trafficking of the complex to the plasma membrane. The light chain, by contrast, determines the functional properties of the transporter [15, 64, 66].

Although substrate translocation by antiporters is symmetrical, antiporters can display remarkable asymmetry in substrate binding. It has recently been demonstrated that neither 4F2hc/LAT1 nor 4F2hc/LAT2 is saturated by millimolar concentrations of intracellular amino acids, whereas extracellular affinities are in the micromolar range [37]. The rBAT/$b^{0,+}$AT antiporter, when expressed in mammalian cells, accumulates cationic amino acids but not neutral amino acids out of the amino acid mixture provided by the cell culture medium (M. Palacin, University of Barcelona, personal communication), although it transports both types of amino acid with equal efficacy when expressed in oocytes [11]. The 4F2hc/y^+LAT2 antiporter on the other hand is designed to preferentially mediate the efflux of arginine (see below and [8]).

An example of a complex interplay between antiporters and their strategies to generate asymmetry is the resorption of cationic amino acids in the kidney [15, 65, 66]. In the apical brush border an obligatory antiporter mediates membrane resorption of cationic amino acids. It is constituted by the heavy chain rBAT and the light chain $b^{0,+}$AT. Mutations in both proteins, rBAT and $b^{0,+}$AT, cause a failure of reabsorption of cystine and arginine/lysine from the urine [43]. The rBAT/$b^{0,+}$AT heterodimer mediates the Na^+-independent exchange of its substrates. The prevalent membrane potential and differences in intracellular binding cause the transporter to accumulate arginine/lysine and ornithine, while releasing neutral amino acids. The only neutral amino acid that is reabsorbed by rBAT/$b^{0,+}$AT to a significant extent appears to be cystine. The released amino acids have to be reabsorbed by a transport system mediating the net transport of amino acids. It is assumed that system B^0 constitutes the second component of this "tertiary" active transport mechanism [15, 65, 66]. On the basolateral side, both cationic and neutral amino acids have to be released into the blood. This transport is carried out by two other heterodimeric transporters, 4F2hc/y^+LAT1(and perhaps y^+LAT2) and 4F2hc/LAT2 [45, 63], and in the case of aromatic amino acids the TAT1 transporter (see above). Kinetic characterization of 4F2hc/y^+LAT1 and y^+LAT2 revealed that it is specifically designed for the release of cationic amino acids [8, 29]. Neutral amino

acids are high-affinity substrates for uptake, but not for release. Asymmetry is achieved by coupling the transport of neutral amino acids to Na^+. Thus efflux of cationic in exchange for neutral amino acids is not opposed by the membrane potential. The Na^+ concentration difference across the membrane further supports a release of cationic amino acids. In contrast to this notion it has been proposed that y^+LAT1 is involved in the release of tryptophan on the basolateral (fetal) membrane of the placenta [32]. The release of nonaromatic neutral amino acids has not been resolved completely yet. Although the heterodimer 4F2hc/LAT2 is expressed in the basolateral membrane [52] it appears to be an obligatory antiporter [46, 52]. One report, however, suggests a uniport mechanism for LAT2 [54]. It remains to be established whether an as yet unknown transporter mediates the efflux of neutral amino acids on the basolateral membrane as suggested recently [15, 65] or whether LAT2 can switch between uniport and antiport mechanisms.

A largely symmetrical antiport is carried out by ASCT-type transporters. The ASCT1/2 transporters form a family together with the EAAT-glutamate transporters. The antiport activity is not as remote from the function of the glutamate transporters as it seems. First, flux experiments indicated that two to three Na^+ ions are taken up together with the substrate, but that at the same time two to three Na^+ ions are transported in the opposite direction, overall constituting an electroneutral antiport mechanism. Thus, both substrate and Na^+ undergo antiport [7, 70]. Secondly, there is some overlap in substrate specificity. The neuronal glutamate transporter EAAC1 (EAAT3) transports cysteine with a K_m of 190 µM, while the ASCT transporters accept glutamate and other anionic amino acids as substrates at reduced pH [6, 60, 70]. When arginine 447 in EAAT3 is replaced by a cysteine, the transport of glutamate and aspartate is abolished, whereas Na^+-dependent cysteine transport is retained [4]. Thirdly, the ASCT transporters, like the EAAT transporters, exhibit a substrate-modulated chloride conductance that is not coupled thermodynamically to substrate translocation [7, 70]. Finally, a point mutation (E404D) in the sequence of the glutamate transporter GLT1 (EAAT2) abolishes both potassium countertransport and the concentrative net transfer of glutamate, limiting the activity of the mutant to Na^+-dependent obligate exchange [30]. Appropriately enough, E404, which is conserved in all five of the cloned mammalian glutamate transporters (EAAT1–5), is *not* conserved in ASCT1/2. ASCT1/2 are good examples for demonstrating that the dependence of any transport on a certain ion species does not necessarily indicate that this species is used as a driving force.

General principles

Primary structure

The comparison of the different amino acid transporters (and families) reveals that neither transport mechanisms nor substrate specificity is conserved within families. Thus no firm conclusion can be made about the mechanism of related transporters without experimental evidence. This is reflected by the fact that only a limited number of residues have to be exchanged to alter substrate specificity or transport mechanism. A sequence identity of 90% between two transporters of usually 400–500 amino acids allows the exchange of 40–50 amino acids, much more than is required to change the mentioned properties. The most conserved property inside an amino acid transporter family is the structure of the transporter. In agreement with this notion hydropathy plots of members within one family are virtually superimposable.

Mechanism

Antiporters are the prevalent mode of amino acid transport. Antiporter substrate specificity always includes abundant nonessential amino acids that can be exchanged against essential amino acids. Alanine and glutamine appear to be the cellular currency for exchange. Na^+-dependent cotransporters of the system A family control the elevated intracellular level of these amino acids.

Antiporters can display marked asymmetry, particularly when involved in vectorial transport. Factors that contribute to asymmetry are: electrogenic antiport mechanisms, inclusion of ions in the antiport mechanism and asymmetrical binding of substrates on the two faces of the membrane.

One strategy that is frequently observed in the adaptation of amino acid transporters is that the cotransport of ions is converted into exchange, thereby annihilating the driving force of the respective ion.

The driving force of a transporter can be fine-tuned by the inclusion of ions in the transport mechanism that are close to electrochemical equilibrium, such as protons, potassium ions or chloride ions.

Physiological function

A number of amino acid transporters are considered as housekeeping transporters and appear to be ubiquitously expressed, such as the system L variant LAT1. The expression of other transporters, however, is indicative of specialized functions. The antiporters 4F2hc/y^+LAT1/2, for example, are tailored to mediate the efflux of arginine or lysine, rather than being involved in the general exchange of amino acids. The occurrence of these transporters, thus, marks a cell as an arginine release site. Similarly, the expression of system N appears to indicate a glutamine releasing site.

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