Chapter 2
Amino Acid Transporters and Glutamine

Catherine Zander, Zhou Zhang, Thomas Albers, and Christof Grewer

Key Points

• Glutamine, a nonessential but physiologically important amino acid, is transported across cell membranes by a variety of amino acid transport systems.
• Cloned glutamine transporters have been assigned to classically characterized transport systems by their substrate and inhibitor specificity, as well as cation dependence and mechanistic properties.
• The molecular identification of the relevant genes has allowed the determination of physical transport mechanisms, as well as the identification of tissue distribution and regulatory pathways that affect expression levels and transport rates.
• This review focuses on the current knowledge of glutamine transport by the Na+ -dependent transport systems ASC (ASCT2), N (SNATs 3, 5, and 7), and A (SNATs 1, 2, and 4).
• Mechanistic properties and regulatory pathways are described, as well as the involvement of glutamine transporters in the glutamate-glutamine cycle in the brain.
• These transport systems are targets of ongoing research that will further our understanding of glutamine transfer between organs and cells.

Keywords
Amino acid transport • Glutamine transport • ASCT2 • SNAT • System A • System N • System ASC • Glutamate-glutamine cycle

Abbreviations

APC Amino acid polyamine and organocation transporter
ASC/ASCT Alanine serine cysteine transporter
ATB0+ Sodium- and chloride-dependent neutral and basic amino acid transporter
BetP Sodium betaine symporter

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Introduction

Glutamine transporters play important roles in metabolism and amino acid homeostasis of cells in most tissues [1]. While glutamine has been classified to be a nonessential amino acid, it has been shown that glutamine supply may become limiting for metabolism under conditions of stress and illness [2]. Furthermore, glutamine is a critical nutrient for rapidly proliferating cells, such as dividing cancer cells [3, 4]. On a cellular level, glutamine is imported into cells, or exported from cells, by plasma membrane glutamine transporters (see [1] for a review). Many glutamine transporters have been characterized traditionally by their specificity profile for substrates and inhibitors, their cation dependence, and mechanistic properties. Most of these transport systems have now been cloned, enabling the detailed investigation of their functional properties, tissue distribution, regulation, and pharmacology. In this review, we focus mainly on the description of the functional properties of the physiologically important and Na\(^{+}\)-dependent glutamine transporters belonging to the solute carrier 1 (SLC1, [5]) and SLC38 families [6]. These transporters were assigned to the classically-identified transport systems ASC (neutral amino acid transporters from the SLC1 family [5]), and systems A and N (SLC38 members [6]), summarized in Table 2.1. ATB\(^{0,+}\) is another Na\(^{+}\)-dependent amino acid transporter with broad specificity and below mM affinity for glutamine. It belongs to the SLC6 family of transporters and is also Cl\(^{-}\) dependent [7]. Due to the low activity of this transport system in most non-proliferating cells, it will not be discussed in this review. One of the major Na\(^{+}\)-independent transport systems is system L, which exchanges glutamine for other amino acids and may use the
Table 2.1  Basic characteristics of the transporters of the SLC1 and SLC38 families

<table>
<thead>
<tr>
<th>Human gene name</th>
<th>Protein name</th>
<th>Alias</th>
<th>Predominant substrate</th>
<th>Transporter type/coupling ions</th>
<th>Tissue distribution</th>
<th>Sequence accession ID</th>
<th>Human gene locus</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLC1A4</td>
<td>ASCT1, SATT</td>
<td>System ASC</td>
<td>A, S, C, T</td>
<td>C/Na⁺, E/amino acids</td>
<td>Widespread</td>
<td>Q76GL9</td>
<td>2p15-p13</td>
</tr>
<tr>
<td>SLC1A5</td>
<td>ASCT2, AAAT</td>
<td>System ASC</td>
<td>A, S, C, T, Q, N</td>
<td>C/Na⁺, E/amino acids</td>
<td>Lung, skeletal muscle, large intestine, kidney, testis, adipose tissue</td>
<td>Q15758</td>
<td>19q13.3</td>
</tr>
<tr>
<td>SLC38A1</td>
<td>SNAT1</td>
<td>ATA1,NAT2,SAT1</td>
<td>Q,A,N,C,H,S</td>
<td>C/Na⁺</td>
<td>Brain, retina, heart, placenta, adrenal gland</td>
<td>Q9H2H9.1</td>
<td>12q13.11</td>
</tr>
<tr>
<td>SLC38A2</td>
<td>SNAT2</td>
<td>ATA2,SAT2</td>
<td>A,N,C,Q,G,H,M,P,S</td>
<td>C/Na⁺</td>
<td>Ubiquitous</td>
<td>CAG33548.1</td>
<td>12q13.11</td>
</tr>
<tr>
<td>SLC38A3</td>
<td>SNAT3</td>
<td>SN1</td>
<td>Q,H,A,N</td>
<td>C/Na⁺, E/H⁺</td>
<td>Liver, skeletal muscle, kidney, pancreas</td>
<td>CAG33251.1</td>
<td>3p21.31</td>
</tr>
<tr>
<td>SLC38A4</td>
<td>SNAT4</td>
<td>ATA3,NAT3,PAAT</td>
<td>A,N,C,G,S,T</td>
<td>C/Na⁺</td>
<td>Brain, retina, liver, kidney, adipose tissue</td>
<td>Q969I6.1</td>
<td>12q13.11</td>
</tr>
<tr>
<td>SLC38A5</td>
<td>SNAT5</td>
<td>SN2</td>
<td>Q,N,H,S</td>
<td>C/Na⁺, E/H⁺</td>
<td>Stomach, brain, liver, lung, small intestine, spleen, colon, kidney</td>
<td>Q8WUX1.1</td>
<td>Xp11.23</td>
</tr>
<tr>
<td>SLC38A6</td>
<td>SNAT6</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Brain, eye, heart, liver, kidney</td>
<td>Q8IZM9.2</td>
<td>14q23.1</td>
</tr>
<tr>
<td>SLC38A7</td>
<td>SNAT7</td>
<td>–</td>
<td>Q,H,S,A,N</td>
<td>Na⁺/Na⁺</td>
<td>Brain, liver, skeletal muscle, uterus, pituitary</td>
<td>Q9NVC3.1</td>
<td>16q21</td>
</tr>
</tbody>
</table>

For detailed information about the SLC gene tables, please visit: [http://www.bioparadigms.org](http://www.bioparadigms.org). SNAT8–11 are omitted from the table.

Abbreviations for transport type: C cotransporter, E exchanger.
transmembrane [glutamine] gradient as a driving force for the import of leucine [8]. System L has been described classically through leucine transport [9], as well its preference of amino acids with bulky, hydrophobic side chains. This system will not be described in this review in detail.

System ASC Transporters of the SLC1 Family

System ASC has traditionally been characterized by its preference for short-chain amino acids, such as alanine, serine, and cysteine, from which its name was derived [10]. However, system ASC also transports glutamine with an affinity that is comparable to that for the prototypical substrate alanine [5], although glutamine is transported with a lower \( v_{\text{max}} \). System ASC was distinguished from other glutamine transport systems by its insensitivity to N-methylated amino acids, such as MeAIB ((2-(methylamino) isobutyric acid)).

cDNA cloning resulted in the identification of two sequences that are now accepted to code for system ASC transporters, namely ASCT1 and ASCT2 [5] (ASCT = alanine serine cysteine transporter). Both transporters belong to the SLC1 family of amino acid transporters. The cloning of the transporters revealed an interesting transmembrane topology with 6 N-terminal transmembrane domains followed by a C-terminal domain, which has a difficulty to interpret hydrophobicity profile. The crystal structure of the homologous bacterial glutamate transporter GltPh revealed that the C-terminal transport domain consists of a repeat of a reentrant-loop-helix motif, which is inserted into the membrane in opposing orientation [11]. Although the crystal structure of ASCTs is not known, homology models predict that this C-terminal domain harbors the binding site for the amino acid [12].

**Substrate Selectivity and Pharmacology**

While ASCT1 showed no measurable transport activity for glutamine, ASCT2 is able to transport glutamine with reasonably high affinity (\( K_\text{m} \) in the range of 20–70 μM, [5, 13, 14]). This affinity is in the same range as that of the other preferred substrates, alanine, serine, and cysteine. Because this review focuses on glutamine transporters, we will only discuss ASCT2. Asparagine is also transported by ASCT2, as are methionine, glycine, leucine, and valine, to some extent, although with low affinities [5]. Glutamate is a transported substrate only at acidic pH, in contrast to transport of neutral amino acids, which is pH independent [13], indicating that glutamate is protonated at the γ-carboxylate when interacting with ASCT2.

The pharmacology of ASCT2 is not well established. Inhibitors were developed on the basis of structural homology with glutamate transporter inhibitors [12, 14] (Fig. 2.1a). Benzylserine (Fig. 2.1b (1)), which is structurally related to a glutamate transporter inhibitor blocks ASCT2 function, although with low apparent affinity (\( K_i = 0.8 \) mM). Inhibitory potency was increased by adding hydrophobic bulk to the benzyl group. The resulting inhibitor serine biphenyl-4-carboxylate blocked ASCT2 activity with a \( K_i \) of 30 μM [12]. Interestingly, it was found that the amount of hydrophobic bulk of the substituent not only correlated with affinity, but also with the ability of compounds to be transported [12] (Fig. 2.1b (2) shows a potent inhibitor with large hydrophobic bulk). In another report, compounds were identified based on \( N-\gamma \)-substituted glutamine [15] (Fig. 2.1b (3)). Several aromatic substituents were used, leading to the characterization of an amide that inhibits with an apparent \( K_i \) in the 250 μM range (at 100 μM glutamine). Based on the analysis of electron-withdrawing properties of the substituent, the authors predict the existence of an important hydrogen bond formed between the transporter-binding site and the amide hydrogen [15].
Northern blot analysis detected mRNA coding for rat ASCT2 in most tissues, except for brain, liver, and heart tissue [5]. However, it was later found that ASCT2 is expressed in the brain as well [13]. In the intestine and kidney, ASCT2 is expressed mainly in epithelial cells [4].

In the brain, ASCT2 expression was found in dendrites of neurons, whereas the cell bodies showed little detectable ASCT2 expression [16]. Functional evidence for ASCT2 presence in nerve terminals was obtained by measuring D-serine uptake [16]. The functional significance of ASCT2 expression in neurons is not clear, although it has been implicated in the uptake of glutamine and D-serine, which is an endogenous NMDA (N-methyl-D-aspartate) receptor ligand. A connection to regulation of oxidative stress may also be possible, since L-cysteine, as a regulator of glutathione levels, is a transported ASCT2 substrate [5].

Expression of ASCT2 in astrocytes is more controversial. While several reports have detected mRNA and protein expression in neonatal glial cell cultures [17], no immunostaining was found in adult brain astrocytes [16]. The reasons behind this discrepancy are not clear. It is possible that ASCT2 is only expressed in developing astrocytes, or that results from cell culture expression cannot be transferred to native tissue.

ASCT2 has been found to be highly overexpressed in many cancer cells [3, 4], as well as rapidly growing cell lines, and has been particularly studied in hepatoma cells [18]. While ASCT2 is not expressed in the normal liver, it is expressed in rapidly growing and aggressive human hepatomas [18]. Glutamine uptake in these cells can be enhanced up to 20-fold compared to normal cells.
Glutamine uptake by ASCT2 appears to be essential for the survival of this rapidly-growing tissue, due to its high nitrogen demand. In fact, ASCT2 antisense RNA expression resulted in apoptotic cell death, a response that is similar to that of glutamine deprivation [3]. These findings indicate that ASCT2 may be a useful target for pharmacological intervention to prevent rapid growth of cancerous cells.

**Functional and Predicted Structural Properties**

The transport mechanism of natively expressed system ASC and ASCT2 has been analyzed using radiotracer flux studies and electrophysiology [5, 13, 14, 19]. Most importantly, it was observed that ASCT2 is unable to support inward transport of amino acids in the absence of cytosolic amino acids. However, transport activity was found when internal amino acid was present [19]. This has led to the conclusion that ASCT2 catalyzes obligate amino acid exchange across the cell membrane (Fig. 2.2a). Native system ASC as well as ASCT1 and ASCT2 are electroneutral transporters [19, 20], consistent with the obligate exchange hypothesis. Consistently, no steady-state transport currents can be detected in ASCT2 upon substrate application [14].

ASCT2 is, however, not fully electrically silent. Upon substitution of chloride with more hydrophobic anions, such as NO$_3^-$ or SCN$^-$, amino acid-induced currents were observed that were dependent on the electrochemical gradient of the anion across the membrane [14, 19] (Fig. 2.2a). Interestingly, the anion current was dependent on the extracellular Na$^+$ concentration [14, 19], similar to observations made previously for the related glutamate transporters. It was concluded that the substrate-induced anion conductance is conserved between the acidic and neutral amino acid transporters of the SLC1 family.

Amino acid transport by ASCT2 is strongly dependent on the Na$^+$ concentration [19] (Fig. 2.2a), on the extracellular and intracellular sides of the membrane, indicating that Na$^+$ must be bound to the transporter for amino acid translocation. However, ASCT2 is independent of K$^+$ [14]. Furthermore, proton cotransport is not required for ASCT2 function.

The effect of Na$^+$ has been incorporated into proposed transport mechanisms. Here, Na$^+$ binds to the transporter after amino acid association is complete, resulting in a Na$^+$-amino acid-bound transporter [19]. Recent evidence, however, points to association of Na$^+$ with the empty transporter before amino acid binds [14]. The “fully loaded” transporter then undergoes conformational change resulting in amino acid translocation. In this model, Na$^+$ binding has a regulatory effect, as it has been found that the affinity for Na$^+$ is high (low mM range). Thus, Na$^+$ would never dissociate from the transporter, unless its concentrations became unphysiologically low [21].

![Fig. 2.2 ASCT2 coupling stoichiometry and anion fluxes (a) and importance for cell growth in rapidly growing cells (b, adapted from [8])](image-url)
**Regulation**

Mechanisms for regulation of ASCT2 expression have been identified. First, ASCT2 expression levels in tumor cell lines were proposed to be stimulated by glutamine and inhibited by glutamine deprivation [4]. A transcriptional mechanism was identified as the cause for this stimulation. Second, regulation of membrane expression occurs through growth factors, such as epidermal growth factor (EGF), as well as insulin and insulin growth factor (IGF) [22]. Signaling pathways involve mitogen-activated kinase (MAPK), protein kinase C (PKC), as well as PI3 kinase [22]. The downstream targets in the latter signaling pathway are serum and glucocorticoid inducible kinase (SGK) isoforms. Consistently, expression of constitutively active SGK1 and SGK3, as well as protein kinase B (PKB), increased ASCT2 transport activity in *Xenopus* oocytes [22].

The function of ASCT2 as a glutamine importer was proposed to be connected to mammalian target of rapamycin (mTOR) [3]. TOR is a serine/threonine kinase, which is involved in signaling related to energy status and nutrient supply. ASCT2 activates the mTOR pathway by importing glutamine into the cell, which in turn leads to leucine influx through transporters of the LAT family, in exchange with intracellular glutamine [8] (illustrated in Fig. 2.2b). Glutamine and leucine then activate mTOR by mechanisms that are currently not well understood.

**System N Transporters of the SLC38 Gene Family**

According to regulation and the functional properties, the Sodium-Coupled Neutral Amino acid Transporters (SNATs) of the SLC38 gene family are classically described as system A and system N transport activities [6, 23]. Unlike system A subtypes (described in the next section), which transport small, aliphatic amino acids and are rheogenic and pH sensitive, the system N subtypes (SNAT3, SNAT5, and SNAT7) counter transport H\(^+\), which may be a key property to allow their operation in reverse and catalyze glutamine efflux from cells [24] (Fig. 2.3a). The system N subtypes have narrower substrate profiles than do the system A subtypes [6]. The system A transporters show substrate selectivity for the amino acid analog MeAIB, as well as a broad range of amino acids such as glycine, L-alanine, L-cysteine, and L-glutamine, whereas, as its name suggests, system N preferentially

![Fig. 2.3](image-url) Proposed mechanism of amino acid transport by SNAT3 (a, adapted from [48]) and SNAT1/2 (b, adapted from [24])
transports amino acids containing nitrogen in the side chain, such as l-glutamine, l-histidine, and l-asparagine [6].

So far, three isoforms of system N transporters have been identified, namely, SNAT3, SNAT5, and SNAT7 [6, 25]. Amino acid transport is coupled to the cotransport of Na\(^+\). Activities classified as system N-like have been studied in neurons. Since l-glutamine is preferred as a substrate, the system N glutamine transporters of the SLC38 family have been suggested to be part of the synthesis of the neurotransmitter glutamate, and are therefore likely involved in altering both \(\gamma\)-aminobutyric acid (GABA) and glutamate levels as well as the cycling of glutamate [26] (detailed below).

SNAT7 is a recently cloned system N amino acid transporter [25]. SLC38A7 bears the hallmarks of system N, with preference for l-glutamine, l-histidine, and l-asparagine, and is insensitive to MeAIB. However, the preferred substrate profile of SLC38A7 is unusually broad compared with other known system N transporters, SNAT3 and SNAT5. Therefore, it was suggested that SLC38A7 is a new system N amino acid transporter on the basis of the expression profile, showing high expression in liver, and substrate selectivity [25].

**Tissue Distribution and Subcellular Expression**

Most of the system N SLC38 family members have relatively broad expression profiles. In contrast to SNAT1 and SNAT2, which are present in neurons, SNAT3 (also referred to as SN1 or NAT) expression in the brain is largely confined to astrocytes. Abundant expression of the SNAT3 protein is detected in astrocytes throughout the brain and the retina, but SNAT3 is absent from neurons and oligodendrocytes. SNAT3 mRNA is also abundant in liver, kidney, heart, skeletal muscle, and adipose tissue [27]. SNAT5 mRNA transcript was detected in multiple brain regions, lung, colon, small intestine, and spleen, whereas three other transcripts are observed in other tissues, including a 2.6-kb transcript in liver and kidney and a 1.4-kb transcript that is dramatically expressed in stomach [26].

Functional data showed that system N transporters are expressed in retinal Müller cells and that system N is the principal mediator of glutamine transport in these cells. SNAT3 and SNAT5 are also expressed robustly in retinal ganglion cells.

**Functional and Predicted Structural Properties**

SNAT3 (SLC38A3) cotransports glutamine together with one Na\(^+\) ion into the cell, in exchange for H\(^+\) (Fig. 2.3a). H\(^+\) countertransport was identified by the ability of SNAT3 to make the cell interior more alkaline upon extracellular glutamine application, a principal feature distinguishing system N subtypes from the system A subfamily [24]. Due to the coupling stoichiometry, glutamine transport by SNAT3 is unaffected by changes of the membrane potential and is, therefore, thought to be electroneutral [24]. Despite the proposed electroneutral nature, currents could be measured in response to application of glutamine to SNAT3-expressing *Xenopus* oocytes [28]. It is not clear whether these currents are caused purely by uncoupled, glutamine induced ion fluxes across the membrane, or if there is also electrogenic function associated with SNAT3. It should be noted that in another study the coupling stoichiometry was found to be 1 glutamine to 2 Na\(^+\) ions, suggesting electrogenic nature of transport [27].

The three-dimensional structure of the system N transporters is not known. However, the SLC38 family is distantly related to the amino acid/polyamine/organocation transporter family APC [29]. Crystal structures of members of the APC family have been determined and the SNAT3 structure was modeled by using the Mhp1 transporter structure as a template [28]. According to this homology
model, predicted transmembrane segment 1 contributes to a conserved Na\textsuperscript{+} binding site. Interestingly, asparagine 76 of SNAT3, which is a conserved residue in transmembrane domain 1 (TM1), is critical for the substrate-induced ion conductance of SNAT3 and mutations to N76 affect binding of the cosubstrate Na\textsuperscript{+}. Therefore, it was hypothesized that this residue is likely to be localized in the translocation pore in the center of the membrane [28].

SNAT5 mediates Na\textsuperscript{+}/amino acid cotransport and counter transport of H\textsuperscript{+}, but differs from SNAT3 in its substrate profile. Human SNAT5 favors serine along with the classic system N substrates glutamine, asparagine and histidine. Assigning SNAT5 to the system N subfamily is therefore based on its countertransport of H\textsuperscript{+} and its 61\% sequence identity to SNAT3, and not on its substrate profile [6]. Amino acid transport activity of SNAT5 exhibited marked pH sensitivity, with influx of substrate increasing with pH in the range of 7.0–8.0 [30].

It has been demonstrated that SNAT5 is capable of mediating bidirectional fluxes of amino acid substrate. The ability of SNAT5 to mediate both accumulation of glutamine from an external supply and efflux of glutamine into amino acid-free medium demonstrates the important capability of system N to facilitate net movement of amino acid across the plasma membrane both into and out of cells, a property that is shared between SNAT3 and SNAT5 [23].

**Regulation**

The primary sequence of SNAT3 contains a number of putative phosphorylation sites [31]. PKC isoforms are activated by a variety of pathways that are involved in cell growth, migration, and differentiation. It has been shown that SNAT3 is rapidly downregulated by activating PKC through the treatment of *Xenopus laevis* oocytes with phorbol-12-myristate-13-acetate (PMA). This downregulation occurs in a caveolin-dependent, dynamin-independent manner and was suggested to be independent of the direct phosphorylation of the transporter [32], although phosphorylation at a particular serine residue has later been shown [31]. SNAT3 is also regulated by pathways involving serum and glucocorticoid inducible kinase SGK and PKB [33], by protein degradation through the ubiquitin ligase Nedd4-2, an effect which is reversed by coexpression of SGK1 [33]. Regulation of SNAT3 expression by insulin and serum starvation was found in the liver [34]. Dietary restriction increased plasma membrane expression, whereas chronic insulin application resulted in downregulation [34], likely involving PI3 kinase.

In the kidney, it has been demonstrated that diets and conditions that stimulate renal ammoniagenesis and urinary ammonium excretion, such as potassium restriction, high protein intake, and metabolic acidosis, lead to higher expression of the SNAT3 transporter. This correlation and the localization of SNAT3 to the basolateral membrane of the late proximal tubule strongly suggest that SNAT3 plays a pivotal role in supplying the proximal tubule with glutamine for ammoniagenesis. Some of these proposed regulatory processes are illustrated in Fig. 2.4.

Not much is known regarding the regulatory processes affecting SNAT5 function. It has been proposed that the C-terminal histidine residue (H471) of SNAT5 is a pH-sensing residue which regulates substrate (Na\textsuperscript{+} plus amino acid) transport activity, at least partly by allosteric effects on Na\textsuperscript{+} binding and which may, therefore, be important for physiological functioning of the transporters [30].

**System A Transporters of the SLC38 Family**

Within the SLC38 family, three distinct isoforms with hallmarks of system A activity were identified through cDNA cloning: SNAT1, SNAT2, and SNAT4 [35, 36]. Although no crystal structure is available, useful information of the fold is provided by distantly related transporter families.
At present, sequence homology has been established with transporters of the mammalian SLC32 and SLC36 families, as well as with the more distantly related plant auxin carriers and the bacterial APC family [29]. Considering the established structural relationship between the APC-family transporters and those of the SLC6 family, it is reasonable to assume that the system A transporters adopt a fold that is similar to LeuT(Aa) and Mhp1, for which crystal structures are known (see for example [37]). LeuT(Aa) has been one of the most useful templates for SNAT2 structural homology modeling [38]. The inverted repeat topology is proposed to be shared with many different families of transporters despite low sequence homology.

Based on hydropathy analysis, SNAT1 and SNAT2 are predicted to have 11 transmembrane domains; with an intracellular N terminus and an extracellular C terminus and a large glycosylated loop between TM5 and TM6 [6]. The intracellular location of the N-terminus is consistent with the absence of an N-terminal signaling sequence for membrane insertion.

Substrate Selectivity and Pharmacology

All three system A isoforms are secondary active transporters, which cotransport one aliphatic, zwitterionic amino acid with one Na\(^+\) ion down the Na\(^+\) concentration gradient [39]. The preference for amino acid substrates between isoforms varies. SNAT1 and 2 have similar substrate specificity, transporting most hydrophilic or small neutral amino acids. System A is so named because of its affinity for alanine. SNAT1 has a much higher affinity for glutamine than SNAT2, $K_m = 230\ \mu M$ and $K_m = 1.65\ mM$, respectively. The reverse is found for alanine; SNAT1’s $K_m$ for alanine is $520\pm 80\ \mu M$, and SNAT2’s $200\pm 17\ \mu M$ [38, 39]. SNAT4’s $K_m$ for alanine however, is significantly lower, $3.52\pm 0.62\ mM$.

MeAIB, a non-metabolizable amino acid analogue, is thought to be a specific transportable substrate for system A transporters. It has long been considered the paradigm system A substrate, used to differentiate system A activity in native cells from that of other amino acid transporters [9]. Although MeAIB is specific to system A transporters, it originally caused mistaken family assignment of SNAT4. At low concentrations, MeAIB works poorly as an inhibitor. It has been strongly recommended to use caution when using MeAIB as an inhibitor to characterize system A transporters [6, 41].
**Tissue Distribution and Subcellular Expression**

SNAT2 is ubiquitously expressed within mammalian and avian cells [6]. SNAT2 mRNA has been found in every tissue analyzed by using Northern blotting. SNAT2’s mRNA and protein levels are increased by amino acid deprivation in many different cell types [42, 43]. Hypertonic stress also increases the abundance of SNAT2 mRNA in most studied cell types. In the brain, SNAT2 is expressed in excitatory neurons in the hippocampus [44], but is not found in astrocyte-enriched cultures. In the pancreas, SNAT2 is responsible for the majority of glutamine uptake within α-cells of the islets of Langerhans [45].

The placenta requires expression of SNAT4 for proper mammalian fetal development and healthy birth weight. SNAT4 is also found in large concentrations within the liver. The full function of SNAT4 within the liver is not yet fully understood. When liver cells are incubated in alanine and glutamine they have a higher gluconeogenesis activity [46]; insulin, which up regulates SNAT4 mRNA inhibits gluconeogenesis [9].

In the brain, SNAT1 is expressed mainly in GABAergic and glutamatergic neurons [6]. It contributes to the plasticity of inhibitory synapses. SNAT1 transports glutamine as a precursor of synaptic GABA within hippocampal cells and synaptosomes. SNAT1 is expressed within the larger microvesicles within the cortex [47]. The cortical expression aids in shuffling glutamine from the astrocytes into the neurons [6]. SNAT1 is also expressed in the heart, placenta and the adrenal gland.

**Functional and Predicted Structural Properties**

For SNAT1, it was proposed that binding of Na\(^+\) and the substrate is sequential: Sodium binding occurs before amino acid binding (Fig. 2.3b). Once both Na\(^+\) and the amino acid are bound, the complex is translocated across the membrane in the same step [40] (Fig. 2.3b). In both SNAT1 and 2, when alanine was rapidly applied in the presence of Na\(^+\), an “instantaneous,” rapidly decaying inward transient current was observed [40]. If the alanine binding step is electroneutral, because at neutral pH alanine has no net charge, then either movement of Na\(^+\) and/or movement of charges of the binding sites cause this rapid charge movement. The bimolecular rate constant of alanine binding to SNAT1 and 2 was estimated as >2×10^7 M\(^{-1}\) s\(^{-1}\) [40], indicating close to diffusion-controlled substrate binding.

SNAT2 has a large anion leak that is not stoichiometrically coupled to the transport current. The presence of extracellular Na\(^+\) increases the size of the leak current [48]. The binding of amino acid in SNAT2 inhibits this anion leak [48]. The SNATs function poorly at low pH. They have the largest activity at a pH of 7.4 for SNAT1, pH 8.0 for SNAT2, and 8.5 for SNAT4 [35, 49]. Protons regulate the transporters by raising the \(K_m\) for Na\(^+\) through allosteric changes. System A transporters are electrogenic, and display voltage-dependent inward substrate transport currents, which increase in size at more negative voltage [35, 39, 41].

Like other transporters related to the APC family, SNAT2 was proposed to adopt a fold based on an inverted repeat topology [38], likely resulting from gene-duplication and fusion events, with 11 transmembrane domains (topology illustrated in Fig. 2.5a). TM1-5 and TM 6-10 are placed in the membrane mirrored with opposite vertical orientations. These two TM sections have similar structural characteristics although they do not have high sequence homology. The inverted topology motif was proposed to be instrumental for the cotransport of the substrates through a slight tilting of the 5-TM bundle motifs, or a “rocking” bundle mechanism, as hypothesized for the SLC6 transporters [50].

Analysis of the SNAT2 Na\(^+\)-binding site through site-directed mutagenesis experimentally supports the hypothesis that the LeuT(Aa) fold is shared with the SLC38 system A family. Threonine 384 on TM8 was identified as being part of the Na\(^+\) binding site. Asparagine 82, located on TM1, was also hypothesized to be involved in the Na\(^+\) binding site [38], in analogy to studies of the homologous N76
residue in SNAT3 [28] (Fig. 2.5b). The Na$^+$ binding site for SNAT2 is situated in the region where predicted TM1 and 8 intersect one another, creating a v-motif similar to that of LeuT(Aa), BetP, vSGLT and Mhp1, as predicted by X-ray crystallography (see for example [37]).

The deletion of TM11 in SNAT2 produced an inactive transporter. This is hypothesized to be due to poor cell surface expression and not a change in transport ability. The C-terminus in SNAT2 is important for voltage regulation and necessary for transport at physiological potentials. The pH dependence was only partially retained with the truncation of the C-terminus. A mutation of histidine 504 to alanine in the c-terminus of SNAT2 reduces pH sensitivity without changing the $K_m$ for Na$^+$ [30].

**Regulation**

Because SNAT2 is assumed to be the prototypical, ubiquitously-expressed system A transporter, much of what is known about the regulation of system A transporters is thought to be related to regulation of SNAT2 function/expression levels. Several reviews have summarized regulatory pathways in more detail (see for example [6]). Therefore, we present here only a brief summary.

When cells are amino acid starved or in hypertonic conditions they undergo a regulatory volume increase (RVI). SNAT2 is upregulated in response to these situations of deprivation and is responsible for short term RVI. The upregulation of SNAT2 is both short term through the redistribution of SNAT2 proteins from intracellular stores into the membrane, and long term by increased transcription. SNAT2 is also upregulated in response to insulin [51]. In some cell types, such as oligodendrocytes, where SNAT2 expression is not seen under basal conditions, SNAT2 is upregulated under systemic hypertonic conditions. When any system A substrate is available, SNAT2’s mRNA is downregulated, and if the hypertonic conditions are corrected, the speed of downregulation is increased through a reduction in mRNA stability and changes in gene expression [52].

SNAT1 and 4 likely play very specific roles in specialized cell types to maintain pools of glutamine as metabolic precursors. SNAT1 is regulated intrinsically within inhibitory hippocampal cells through transporter activity and not substrate availability, via depolarization and developmental cues, in order to maintain a supply of glutamine to serve as a GABA precursor. Within these cells, SNAT2 is...
Amino Acid Transporters and the Glutamate Glutamine Cycle

A physiologically important function of glutamine is to serve as a precursor for the neurotransmitter glutamate in the glutamate-glutamine cycle in the mammalian brain. In this cycle (Fig. 2.6), glutamate, released into the synapse during neurotransmission, is taken up into adjacent glia cells. Within glia cells, glutamate is converted to glutamine by glutamine synthase. Glutamine is then shuttled back into neurons, in which glutamate is regenerated by glutaminase. Shuttling of glutamine from glia cells to neurons requires release through transporters in the glia cell membrane, followed by neuronal glutamine uptake (see for example [54, 55]).

Amino acid transporters are critically involved in many of these steps. Uptake of glutamate into glia cells is performed by high-capacity glutamate transporters of the excitatory amino acid transporter (EAAT) family. Subtype EAAT2 is mainly responsible for this uptake. Glutamine release from glia cells requires a transporter that can catalyze efflux. Although the exact nature of the transport systems involved in efflux is still discussed, SNAT3 is a potential candidate that has the functional pre-requisites to serve as an efflux system [56]. Most importantly, glutamine transport by SNAT3 is thought to be electroneutral, rendering efflux possible even at negative transmembrane potentials [24]. However, another report suggests electrogenic glutamine transport by SNAT3 [27], caused by the cotransport of two Na⁺ ions. If this was the case, glutamine efflux would be hindered at negative voltage. In addition to system N, ASCT2 and the system L transporter LAT2 may contribute to glutamine release, in particular in cultured astrocytes [57], in exchange for other extracellular amino acids. However, the lack of ASCT2 expression in mature brain astrocytes makes its physiological importance for glutamine efflux less clear [16]. Furthermore, an unidentified transporter has been implicated in the efflux mechanism [57].

Once glutamine is released from glia cells, it is taken up into neurons by amino acid transporters. The identity of the transport systems involved is also not well established, although evidence points to SNAT2 and SNAT1 (system A) as a major contributor to neuronal uptake. SNAT2 is strongly expressed in neurons and the inhibition of SNAT2 by the specific transported substrate MeAIB results in an elevation of extracellular glutamine.

Fig. 2.6  Proposed involvement of several amino acid transporters in the glutamate-glutamine cycle
After entering the presynaptic terminal, glutamine is converted to glutamate by glutaminase, and subsequently repackaged into synaptic vesicles. Transport into the vesicles is accomplished by the vesicular glutamate transporters (VGLUTs) [58].

Conclusions

The preceding sections have highlighted the function of glutamine transporters in many physiological processes, as well as the molecular properties and regulatory processes that determine transporter activity. Understanding of the biophysical transport mechanism, structure function relationships, as well as development of specific pharmacological tools for manipulation of glutamine transporter function remain challenges that require future investigation. These issues are particularly important with respect to recent reports that implicate glutamine transporters in the growth of rapidly dividing cells, such as tumor cells. Our ability to manipulate glutamine uptake by these cells, either through pharmacological intervention, or through modulation of transporter expression or activity by targeting regulatory pathways, at either the transcriptional or trafficking level, will be paramount to the identification of new avenues to inhibit cell growth.

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