Glutamine Metabolism: Nutritional and Clinical Significance

Recent Molecular Advances in Mammalian Glutamine Transport[1,2]

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ABSTRACT Much has been learned about plasma membrane glutamine transporter activities in health and disease over the past 30 years, including their potential regulatory role in metabolism. Since the 1960s, discrimination among individual glutamine transporters was based on functional characteristics such as substrate specificity, ion dependence, and kinetic and regulatory properties. Within the past two years, several genes encoding for proteins with these defined activities (termed "systems") have been isolated from human and rodent cDNA libraries and found to be distributed among four distinct gene families. The Na^+-dependent glutamine transporter genes isolated thus far are System N (*SN1*), System A (*ATA1, ATA2*), System ASC/B^0 (*ASCT2 or* ATB^0), System $B^{0,+}$ ($ATB^{0,+}$) and System y^+L (y^+LAT1, y^+LAT2). Na^+-independent glutamine transporter genes encoding for System L (*LAT1, LAT2*) and System $b^{0,+}$ ($b^{0,+}AT$) have also been recently isolated, and similar to y^+L, have been shown to function as disulfide-linked heterodimers with the 4F2 heavy chain (CD98) or rBAT (*r*elated to $b^{0,+}$ *a*mino acid *t*ransporter). In this review, the molecular features, catalytic mechanisms and tissue distributions of each are addressed. Although most of these transporters mediate the transmembrane movement of several other amino acids, their potential roles in regulating interorgan glutamine flux are discussed. Most importantly, these newly isolated transporter genes provide the long awaited tools necessary to study their molecular regulation during the catabolic states in which glutamine is considered to be "conditionally essential." J. Nutr. 131: 2475S–2485S, 2001.

KEY WORDS: • *glutamine* • *transporters* • ATB^0 • *ASCT2* • *SN1* • *ATA* • $ATB^{0,+}$ • 4F2hc • rBAT, y^+LAT • $b^{0,+}AT$ • LAT

Introduction to glutamine and amino acid transporters

The amino acid glutamine has been the focus of many studies in physiology and medicine due to its important pleiotropic roles in metabolism and tissue homeostasis. As discussed in detail in other articles of this issue of the *Journal of Nutrition*, glutamine serves as an essential metabolic precursor in nucleotide, glucose and amino sugar biosynthesis, glutathione homeostasis and protein synthesis (Neu et al. 1996). Moreover, the growth of proliferating cells such as fibroblasts, lymphocytes and enterocytes relies heavily on glutamine as an oxidative energy source. Although classified as "nonessential" in most biochemistry text books, glutamine appears essential for the viability and growth of cells maintained in tissue culture, as first demonstrated by the seminal work of Harry Eagle in the 1950s (Eagle 1955). The nitrogen-rich character and unique metabolism of this amino acid allow it to serve as the major interorgan ammonia shuttle. Glutamine also supports tissue homeostasis by participation in intercellular substrate cycles in the brain and liver and in fetal-placental nutrient exchange. Indeed, the fact that glutamine is the most abundant amino acid in the plasma (at 600–800 μmol) and exhibits extremely rapid cellular turnover rates (Darmaun et al. 1986) is a testament to its diverse utility in mammalian physiology. It is the expression of specific transporters in the plasma membrane, however, that affords the prodigious intercellular exchange and metabolism of glutamine. Although transporters in intracellular organelles also regulate glutamine metabolism (Lenzen et al. 1987), this review will focus exclusively on those in the plasma membrane and the considerable progress that has been made in the past two years on the identification of the genes that encode for them.

For the past 30 years, the field of mammalian amino acid transporter biology has relied on functional characteristics to distinguish between specific transporters, an effort largely initiated and advanced by Christensen and colleagues in the 1960s using radiolabeled amino acids and amino acid analogs (Christensen 1990). Among these functional characteristics are substrate specificity, kinetic and regulatory properties, ion dependence and pH sensitivity. On the basis of these characteristics, a classification of the discriminated activities termed "systems" was developed. For example, System A and System L were the first to be so-designated, and stood for "alanine-preferring" and "leucine-preferring," respectively (Oxender

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[3] Abbreviations used: ATA1, amino acid transporter System A-1; EAAT, excitatory amino acid transporter; gluT, glutamine transporter; gpa-at, glycoprotein-associated amino acid transporter; hATB°, human amino acid transporter B°; LAT1, System L amino acid transporter 1; MeAIB, α-(methylamino)isobutyric acid; PKC, protein kinase C; rBAT, related to $b^{o,+}$ amino acid transporter; VGAT, vesicular γ-aminobutyric acid amino acid transporter.

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and Christensen 1963). Over the subsequent three decades, more "systems" were characterized and named in different cells and tissues. Broadly speaking, amino acid transporters fall into two categories, i.e., Na^+ dependent and Na^+ independent. The former utilizes the potential energy present in the transmembrane Na^+ electrochemical gradient, maintained largely by the Na^+/K^+-ATPase, to drive the uptake of amino acids against their concentration gradient. It is by this mechanism that cytoplasmic amino acid levels are ultimately maintained above their transmembrane equilibrium distribution. In contrast, the latter transporter type facilitates the selective movement of amino acids across the plasma membrane independent of Na^+. The nomenclature that has been adopted for mammalian amino acid transporters designates Na^+-dependent systems in uppercase letters and Na^+-independent systems in lowercase letters. The notable exception to this rule is the Na^+-independent transporter System L which has retained its uppercase designation for historical purposes.

Glutamine transporter genes

Na^+-dependent glutamine transporters include Systems ASC (or B^0), $B^{0,+}$, y^+L, A and N, and Na^+-independent transporters include Systems L, $b^{0,+}$ and n. It should be noted that although the focus of this review is on transmembrane glutamine movement, each of these transporters mediates the movement of several other amino acids as well, to varying degrees. Here, the isolation of Na^+-dependent, followed by Na^+-independent glutamine transporter genes is catalogued, including molecular features, catalytic mechanism and tissue distribution. As with most fields of molecular biology, the initial naming of these genes was often inconsistent with attempted normalization of the nomenclature **(Table 1)**. To this end, many of the researchers have since agreed to try to follow the suggestions of Christensen et al. (1994) when naming newly discovered transporter genes, although the field is still evolving in this respect. In the context of this special issue of the *Journal of Nutrition*, most of the emphasis is placed on System ASC/B^0 and System N because these two transporters have been studied more extensively with respect to glutamine transport and metabolism than have the others. Similarly, emphasis is placed on the human transporter wherever possible.

Na^+-dependent transporters

System ASC or B^0 ($ASCT2$, ATB^0). System ASC was originally named for three of its preferred substrates (*a*lanine, *s*erine, *c*ysteine) to distinguish it from System A activity (discussed below) (Christensen et al. 1967). A transport activity similar to ASC, formerly know as "neutral brush border" (Stevens et al. 1982), was previously described in intestinal and kidney epithelia (Doyle and McGivan 1992, Souba et al. 1992), and later designated B^0 to distinguish it from $B^{0,+}$, a transporter originally described in mouse blastocysts (thus, the designation B) that shared an identical zwitterionic substrate selectivity (the superscript 0), but also took up cationic amino acids (the superscript +) in a Na^+-dependent manner (Van Winkle et al. 1985). Earlier studies stated that these two activities (ASC vs. B^0) could be distinguished by criteria such as threonine selectivity or the uptake of anionic amino acids at acidic pH values, but more recent expression studies with their isolated cDNAs have not supported these assumptions.

Molecular cloning and features. The first mammalian glutamine transporter gene was isolated in 1996 from a mouse testis cDNA library, encoding for a 553 amino acid protein with functional properties of System ASC (Utsunomiya-Tate et al. 1996). It was termed *ASCT2* to distinguish it from ASCT1, a System ASC isoform isolated in 1993 that does not transport glutamine (Arriza et al. 1993, Shafqat et al. 1993). At the same time, Kekuda et al. (1996) isolated a cDNA from human choriocarcinoma and colon carcinoma (Kekuda et al. 1997) cell lines with functional properties nearly identical to ASCT2, and named it *hATB*^{0} for *h*uman *a*mino acid *t*ransporter B^0. Rabbit intestinal *ATB*^{0} was isolated in 1997 and like *hATB*^{0}, was found to encode for a 541 amino acid protein

TABLE 1

Guide to the isolation of glutamine transporter genes[1]

Transporter gene (original designation)	Year	Reference
Excitatory amino acid transporter family		
ASCT2 or ATB0		
mASCT2	1996	Utsonomiya-Tate et al.
hATB0	1996	Kekuda et al.
rbATB0	1997	Kekuda et al.
rATB0 (rASCT2)	1999	Bröer et al.
Na^+/Cl^--dependent amino acid transporter family		
ATB0,+		
hATB0,+	1999	Sloan et al.
Vesicular γ-amino butyric acid amino acid transporter family		
SN1		
rSN1	1999	Chaudhry et al.
mSN1 (mNAT)	2000	Gu et al.
hSN1	2000	Fei et al.
ATA		
rATA1 (glnT)	2000	Varoqui et al.
rATA2 (SAT2)	2000	Yao et al.
rATA2	2000	Sugawara et al.
rATA2 (SA1)	2000	Reimer et al.
hATA2	2000	Hatanaka et al.
gpa-AT family of heterodimeric transporters (with 4F2hc/CD98 or rBAT)		
y+L		
hy+LAT1	1998	Torrents et al.
hy+LAT2 (KIAA0245)	1998	Torrents et al.
my+LAT1	1999	Pfeiffer et al.
hy+LAT1	1999	Pfeiffer et al.
ry+LAT1	2000	Kanai et al.
LAT		
hLAT1 (E16, hAmAt-L-lc)	1998	Mastroberardino et al.
rLAT1	1998	Kanai et al.
hLAT1	1999	Prasad et al.
bLAT1	1999	Boado et al.
rLAT2	1999	Segawa et al.
hLAT2	1999	Pineda et al.
mLAT2	1999	Rossier et al.
hLAT2	1999	Rossier et al.
rbLAT2	2000b	Rajan et al.
b0,+AT		
rb0,+AT (BAT1)	1999	Chairoungdua et al.
rbb0,+AT (4F2-1c6)	1999	Rajan et al.
mb0,+AT	2000a	Rajan et al.
hb0,+AT	2000a	Rajan et al.

[1] The genes isolated from each of the four transporter families are listed, along with the reference and the original designations in parentheses. The lowercase letter preceding each of the genes indicates the species from which the cDNA was isolated, as follows: h = human, r = rat, m = mouse, b = bovine, rb = rabbit. The designations shown are the ones most frequently used. Presently, only the gpa-AT transporters have adhered to the suggestions for normalizing amino acid transporter gene names proposed by Christensen et al. (1994).

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(Kekuda et al. 1997). In 1999, isolation of the rat *ASCT2* gene was reported from a brain astroglia-enriched cDNA library, encoding for a 539 amino acid protein (Bröer et al. 1999). After several cross-species cDNA library screenings that failed to produce distinct *ASCT2* and *ATB*^{0} cDNAs from the same species, it was concluded that ATB^0 and ASCT2 are orthologous isoforms from different species, and not different transporters (Bröer et al. 2000). At the amino acid level, rat ASCT2 is 83% identical to mouse ASCT2 and 76% identical to hATB^0 (Bröer et al. 1999); mouse ASCT2 shares 79% identity with hATB^0, whereas rabbit and human ATB^0 are 85% identical (Kekuda et al. 1997).

The ATB^0 and ASCT2 transporters are part of the excitatory amino acid transporter (EAAT) family composed thus far of ASCT1 and several glutamate transporters (Kanai 1997). Interestingly, both ASCT1 and ASCT2 have recently been shown to be retrovirus receptors (Marin et al. 2000, Rasko et al. 1999). The human ATB^0 gene is located on chromosome 19 (Kekuda et al. 1996). These transporters share structural features of 8–10 transmembrane-spanning domains (depending on the algorithm used), a long hydrophobic carboxy terminus and putative intracellular protein kinase C (PKC) phosphorylation sites. Treatment of human hepatoma and colon carcinoma cells with phorbol esters has been shown to rapidly attenuate ATB^0-mediated glutamine uptake by a post-translational mechanism, but it is unclear whether phosphorylation of the putative PKC sites underlies this regulation (Bode et al. 1998, Pawlik et al. 2000).

Catalytic mechanism. Both ASCT2 and ATB^0 take up glutamine with high affinity and transport a wide panel of other zwitterionic amino acids such as serine, threonine, cysteine, alanine and asparagine, as well as bulky/branch-chain amino acids (leucine, valine, methionine) to a lesser degree (Kekuda et al. 1996, Utsunomiya-Tate et al. 1996). The catalytic mechanism of ATB^0/ASCT2 involves a Na^+-dependent exchange of intracellular for extracellular amino acids, effectively serving to equilibrate cytoplasmic amino acid pools (Torres-Zamorano et al. 1998). This transporter can therefore mediate either glutamine uptake or release (Bröer et al. 1999). Indeed, the long-established enhancement of System ASC activity by intracellular substrates, termed "*trans*-stimulation" (Gazzola et al. 1980), is probably attributable to this transport exchange mechanism. Glutamine uptake via hATB^0 is electroneutral, with one amino acid and Na^+ ion transported inwardly and with no evidence for the countercurrent movement of K^+ or H^+ as opposed to other members of the EAAT family (Kanai 1997). The electroneutrality of amino acid transport through ASCT2 instead has been proposed to involve the bidirectional movement of Na^+ (Bröer et al. 2000), but these studies did not control for Na^+ efflux mediated by the Na^+/K^+-ATPase. The possible involvement of K^+ efflux as part of the electroneutral exchange mechanism, although proposed (Utsunomiya-Tate et al. 1996), remains to be addressed more specifically.

The K_m for glutamine uptake and glutamine-evoked threonine release via hATB^0 was reported to be 23 and 45 μmol, respectively, when heterologously expressed in *Xenopus* oocytes (Torres-Zamorano et al. 1998). Studies in human hepatoma cells reported K_m values for hATB^0-mediated glutamine uptake from 90 to 147 μmol (Bode et al. 1995); in human breast carcinoma cells values ranged from 115 to 388 μmol (Collins et al. 1998) and in the human Caco-2 colon carcinoma cell line, a K_m of 247 μmol was reported (Souba et al. 1992). Why are there such wide ranges of hATB^0 affinities for glutamine in different cells? The answer probably resides in the factors that affect the measured affinity for glutamine such as intracellular substrate levels and transmembrane electrical potentials. For example, electrophysiologic studies with heterologously expressed rat ASCT2 in *Xenopus* oocytes revealed that the K_m for glutamine was 43 and 90 μmol at voltage-clamped transmembrane potentials of -60 and -20 mV, respectively, indicating that substrate binding was voltage dependent (Bröer et al. 2000). Net glutamine movement through this transporter will therefore be dictated by the summation of several variables, including transmembrane electrical potentials and glutamine gradients.

Tissue distribution. Northern blot analysis revealed that the hATB^0 mRNA (2.9 kb) was expressed in placenta, lung, kidney, pancreas, skeletal muscle and human colon carcinoma cell lines (Kekuda et al. 1996). Further studies by Northern blot analysis showed its expression in a human kidney proximal tubule cell line and reverse transcriptase-polymerase chain reaction analysis showed its expression in human intestinal epithelia (Kekuda et al. 1997). Previous functional studies of this transporter suggest that it is localized to the brush border (apical surface) of intestinal and renal cells (Doyle and McGivan 1992, Stevens et al. 1982), but its basolateral presence cannot be discounted. In rabbit intestine (ileum), the ATB^0 mRNA is expressed much more strongly in villous than in crypt cells (Sundaram et al. 1998). The hATB^0 gene is expressed and mediates the majority of glutamine uptake in human hepatoma (Bode et al. 1995 and 1998, Pawlik et al. 2000), breast carcinoma (Collins et al. 1998) and colon carcinoma cell lines (Kekuda et al. 1996 and 1997, Pawlik et al. 2000, Souba et al. 1992). Glutamine uptake in human liver-derived fibroblasts is mediated almost exclusively by hATB^0, (B. Bode, unpublished data), which supports previous studies implicating this role for System ASC in human foreskin fibroblasts (Dall'Asta et al. 1990), but contradicts the supposition that hATB^0 expression is restricted to epithelial cells (Kekuda et al. 1996).

Studies in rats indicate that ASCT2/ATB^0 is expressed in glia, but not neurons and that it may participate in the well-characterized brain glutamine-glutamate cycle by mediating glutamine release (Bröer et al. 1999). Similarly, ATB^0 is expressed in rat liver endothelial cells in which it mediates the majority of glutamine uptake, but not in rat hepatocytes, which utilize System N for this purpose (Lohmann et al. 1999). The hATB^0 transporter has also been proposed to play a role in mediating glutamine release from the placenta by virtue of its expression in the fetal-facing basolateral membrane of the syncytiotrophoblast as discussed by Ganapathy (Torres-Zamorano et al. 1998). In this capacity, it may help to drive the well-characterized glutamine-glutamate exchange between fetal liver and placenta during development (Battaglia 2000). Elucidation of the plasma membrane domain and cellular distribution of this transporter in the intestine will yield important information given the highly glutamine-dependent nature of this tissue (Windmueller and Spaeth 1974). One recent report indicates that hATB^0 mRNA levels do not change during chronic intestinal inflammation despite a decrease in alanine uptake rates (Sundaram et al. 1998). Given its seemingly ubiquitous expression in human tumor-derived cells (Collins et al. 1998, Kekuda et al. 1996 and 1997), its proposed role in human hepatocellular transformation (Bode and Souba 1999) and its utility in mediating glutamine-dependent growth (Bode et al. 1998, Pawlik et al. 2000), the study of hATB^0 in tumor biology will also be of great interest.

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System N (SN1)

System N was first described in rat hepatocytes by Kilberg, who demonstrated that this unique transport activity had a rather narrow substrate specificity of glutamine, histidine and asparagine only—all substrates containing *N*itrogen in their side chain (Kilberg et al. 1980). Other System N-like activities were since described in skeletal muscle (Hundal et al. 1987) and neurons (Tamarappoo et al. 1997), and designated N^m and N^b, respectively, to distinguish their functional properties from the liver transporter. This transporter has served as the focus of several studies in liver (Bode et al. 1990) and muscle (Rennie et al. 1994), in which it has been shown to represent a potentially rate-limiting step in metabolism (Häussinger et al. 1985, Low et al. 1993). Although space limits discussion on the topic, hepatic and skeletal muscle System N activities exhibit reciprocal regulation during catabolic states in which System N-mediated glutamine uptake is predictably enhanced in the liver and decreased in skeletal muscle. This situation undoubtedly reflects the respective roles of each tissue in glutamine homeostasis, with skeletal muscle serving as the main "bank" for export, and the liver serving as a primary "processing center." The cloning of the *SN1* gene will allow the basis for this differential regulation to be studied more closely in the coming years.

Molecular cloning and features. The System N gene was first isolated from a rat brain cDNA library in 1999 and designated *SN1* (Chaudhry et al. 1999). Three months later, the isolation of the mouse System N gene was reported from a kidney cDNA library and designated *mNAT* (Gu et al. 2000). In August of 2000, the isolation and characterization of the human System N gene, designated *hSN1*, from a human hepatoblastoma cell line (HepG2) cDNA library was reported (Fei et al. 2000). Rat and human SN1 proteins are both 504 amino acids long, and are 90% identical to one another. Mouse SN1 is 505 amino acids long and 89% identical to hSN1. The SN1 transporter from all species has a predicted mass of 56 kDa and 9–12 transmembrane-spanning domains, depending on the algorithm used (Chaudhry et al. 1999, Fei et al. 2000, Gu et al. 2000). Two species of immunoreactive SN1 protein have been reported in the liver with estimated molecular weights of 54 and 67 kDa (Gu et al. 2000). The hSN1 protein has a single putative PKC phosphorylation sequence in the C-terminus. The *hSN1* gene is located on chromosome 3 and contains 16 exons and 15 introns, spanning ~16 kb of nucleotide sequence (Fei et al. 2000). Together with the System A transporters (discussed below), SN1 belongs to a family of transporters that include the *v*esicular γ-aminobutyric acid (GABA) *a*mino acid *t*ransporters (VGAT), with which it shares a 28% similar amino acid sequence (Chaudhry et al. 1999, McIntire et al. 1997).

Catalytic mechanism. Through a series of elegant experiments, Robert Edwards' group (Chaudhry et al. 1999) showed that heterologously expressed SN1 caused an intracellular alkalinization when mediating Na^+-dependent glutamine uptake, suggesting that the catalytic mechanism involved an efflux of H^+ through the transporter. This Na^+/H^+ countertransport mechanism was later confirmed with heterologously expressed hSN1 (Fei et al. 2000) and undoubtedly underlies the long-established pH sensitivity of hepatic System N-mediated glutamine uptake in the physiologic range (Bode et al. 1995, Kilberg et al. 1980, Lenzen et al. 1987). The SN1 catalytic mechanism has led to its description as a *glutamine-driven Na^+/H^+-exchanger* (Fei et al. 2000). Glutamine transport mediated by both rat and human SN1 has been shown to be electrogenic, with a proposed two Na^+ ions and amino acid transported inward coupled to the efflux of one H^+ (Fei et al. 2000). In hSN1 expression studies, the K_m for glutamine was found to be 0.7–1.5 mmol, in agreement with values reported in human hepatocytes (Bode et al. 1995). System N is therefore a lower affinity glutamine transporter than ATB^0. However, similar to ATB^0, the substrate affinity of hSN1 was reported to be highly dependent on the membrane potential (Fei et al. 2000). In contrast, the reported K_m for asparagine was ~16 mmol, confirming values reported recently (Pawlik et al. 2001). One surprising new piece of information on both human and mouse SN1 is that it also mediates alanine uptake (Fei et al. 2000, Gu et al. 2000), albeit with lower affinity than for glutamine and histidine. On the basis of these findings, the description of System N's selectivity should be revised as follows: high affinity substrates glutamine and histidine, and low affinity substrates asparagine and alanine.

Perhaps the most surprising finding that has emerged from the recent isolation of SN1, however, is its demonstrated ability to mediate both glutamine uptake and release. This dual role was proposed for System N^m by Mike Rennie 15 years ago as a hypothesis (Rennie et al. 1986), but contradicted the paradigm that Na^+-dependent transporters could mediate only uptake due to their intimate link to the Na^+ electrochemical gradient. It is now evident that the collective driving forces for SN1 catalysis determine which direction glutamine will move, with the Na^+ gradient being only one of several. For example, SN1-mediated glutamine transport is highly dependent on the membrane electrical potential because progressive depolarization of the membrane results in a switch from glutamine uptake to release at −20 to −30 mV (Fei et al. 2000), well within the physiologic range for hepatocytes (Edmondson et al. 1985). Transmembrane glutamine gradients also affect the direction of transport, with glutamine uptake observed at extracellular concentrations >400 μmol and efflux when levels fall below this value (Chaudhry et al. 1999). It is relevant that changes in all of these driving forces that determine glutamine flux through SN1 occur within the physiologic range, with obvious implications for glutamine homeostasis. Results from future studies on the role of this transporter in catabolic states should be interpreted with this new finding in mind **(Fig. 1)**. Finally, in light of these recent mechanistic findings, it is possible that the previously described Na^+-independent "System n" activity in the liver (Pacitti et al. 1993) was simply SN1 acting in the absence of Na^+.

Tissue distribution. In rodents, SN1 is expressed most strongly in the liver, with skeletal muscle, kidney, heart and brain also exhibiting detectable levels of this mRNA (Chaudhry et al. 1999, Fei et al. 2000, Gu et al. 2000). Detailed tissue-specific expression studies for human SN1 remain to be performed, but it is assumed that they will yield similar results. The first report on SN1 isolation (Chaudhry et al. 1999), which was based on Northern blot analysis, stated that this gene was not expressed in skeletal muscle raising the possibility that System N and System N^m were encoded by different genes. However, SN1 was subsequently isolated from a rat skeletal muscle cDNA library and shown to be identical in sequence and function to the rat brain SN1 (Fei et al. 2000). Expression of SN1 in skeletal muscle was confirmed in a third report by Northern blot analysis, albeit at levels several fold lower than in liver (Gu et al. 2000).

The revelation that SN1 is also expressed in heart and kidney is a recent finding, and should serve as an impetus for more detailed investigations into its role in glutamine metabolism in these tissues. In the rat kidney, SN1 mRNA was reported to be localized to the tubules in the medulla (Chaudhry et al. 1999). In rat brain, both SN1 mRNA and

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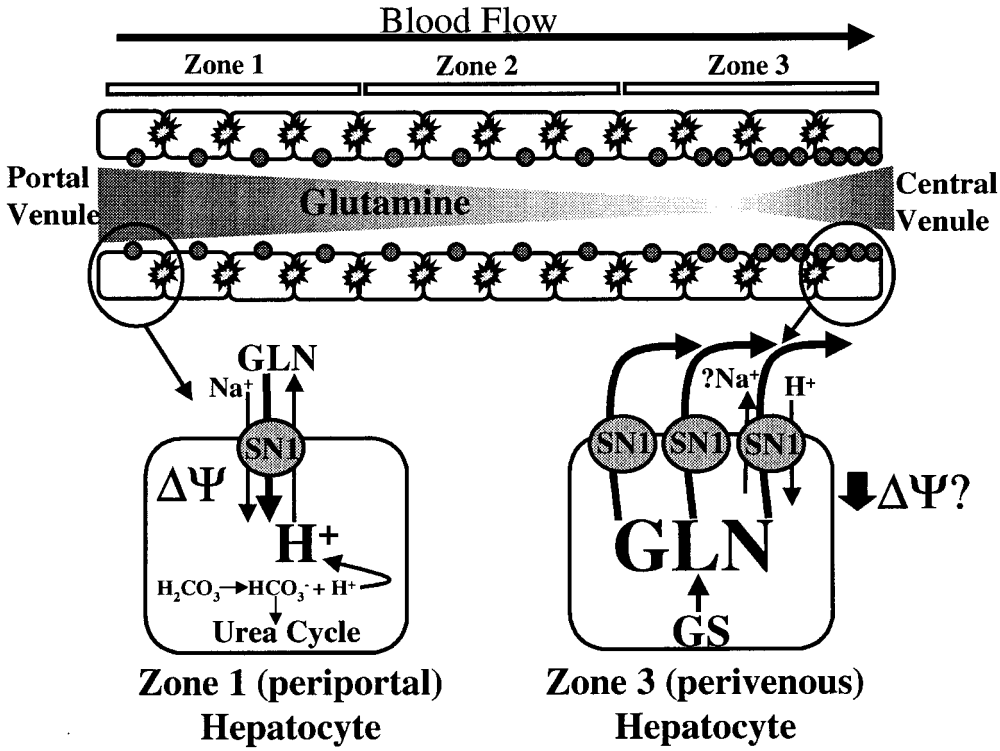

FIGURE 1 Proposed model for role of amino acid transporter SN1 in the hepatic intercellular glutamine cycle. The liver acinus is shown in the context of the intercellular glutamine cycle (Häussinger 1998). Urea synthesis occurs predominantly in zones 1 and 2 (periportal hepatocytes), consuming bicarbonate and creating a more acidic intracellular environment. Together with higher concentrations of glutamine in the incoming portal blood supply, this outwardly directed proton gradient helps drive the inward uptake of glutamine through SN1 via its Na^+/H^+ exchange mechanism (Chaudhry et al. 1999, Fei et al. 2000). In contrast, zone 3 contains a small population (5–7%) of perivenous glutamine synthetase (GS)-positive hepatocytes with enriched plasma membrane SN1 (Easson et al. 2000, Gu et al. 2000). Glutamine effluxes from these cells, aided by diminished plasma glutamine content in zone 3 (due to consumption in zones 1 and 2), less acidic cytoplasm and high cytoplasmic glutamine levels from GS activity. Transmembrane electrical potentials ($\Delta\psi$) also influence net glutamine movement, but it is currently unclear whether different values exist in periportal or perivenous hepatocytes.

protein were restricted to glial cells (Chaudhry et al. 1999), which is interesting given that System N^b was described in rat neurons (Tamarappoo et al. 1997), suggesting that other distinct System N isoforms may exist. The plasma membrane domain-specific expression and role of SN1 and $hATB^0$ in glia and their role(s) in the brain intercellular glutamine cycle are obviously fruitful areas of future research (**Fig. 2**). In the liver acinus, it has recently been shown that SN1 activity (Easson et al. 2000) and protein (Gu et al. 2000) are enriched in the plasma membrane of distal perivenous hepatocytes containing glutamine synthetase. This makes sense given that SN1 is now known to mediate glutamine release from the cells as well, a function that would be markedly enhanced in this cell population (Fig. 2). Finally, the isolation of SN1 will greatly aid in elucidating the mechanism of its rapid activation by amino acid–induced cell swelling in both muscle (Low et al. 1996) and liver (Bode and Kilberg 1991), a topic discussed more globally by Dieter Häussinger in this issue of the *Journal of Nutrition*.

System $B^{0,+}$ ($ATB^{0,+}$)

System $B^{0,+}$ was first described in mouse blastocysts and is capable of transporting zwitterionic and cationic amino acids in a Na^+-dependent manner (Van Winkle et al. 1985). This transporter has been studied most heavily in the area of murine embryogenesis (Van Winkle and Campione 1987, Van Winkle et al. 1990). Its role in mediating glutamine uptake in mammalian cells has been limited, and specifically addressed mainly in this field (Jamshidi and Kaye 1995, Lewis and Kaye 1992).

Molecular cloning and features. The cDNA for this glutamine transporter (termed *$hATB^{0,+}$*) was isolated in 1999 from human mammary gland, and found to code for a protein of 642 amino acids with 12 putative membrane-spanning domains, 7 potential glycosylation sites and two PKC phosphorylation sites (Sloan and Mager 1999). Western blot analysis has yet to be performed on this transporter protein. The gene is located on the X chromosome and contains 14 exons. Typical of membrane transporter genes, the sequences encoding putative transmembrane-spanning domains are uninterrupted by introns (Sloan and Mager 1999). This transporter belongs to a family of Na^+/Cl^--dependent neurotransmitter transporters, including those for glycine (hGLYT1 and hGLYT2) and proline (hPROT).

Catalytic mechanism. The uptake of zwitterionic and cationic amino acids by $hATB^{0,+}$ is highly dependent on both Na^+ and Cl^-. On the basis of electrophysiologic data, the estimated K_m of $hATB^{0,+}$ for glutamine was reported to be 633 μmol (Sloan and Mager 1999), which is close to the affinity of SN1, with the notable exception that $hATB^{0,+}$ transports 17 other amino acids as well. This transporter takes up 2 Na^+ and 1 Cl^- molecule per amino acid; its mechanism is therefore electrogenic and also regulated by the membrane potential (Sloan and Mager 1999).

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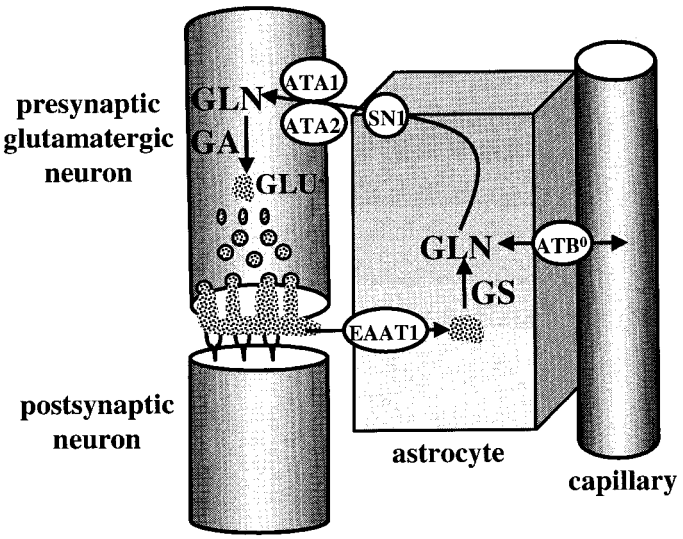

FIGURE 2 Schematic model for role of Na^+-dependent amino acid transporters in the brain intercellular glutamine cycle. Astroglia have been shown to express SN1 and ASCT2 (ATB^0), whereas glutamatergic neurons express both System A isoforms, but not the former two transporters. Based on these data, the following model is proposed. Excitatory amino acid transporter (EAAT)1, a high affinity glutamate transporter in astrocytes, would rapidly remove this neurotransmitter from the synaptic cleft, where it would subsequently be converted to glutamine via glutamine synthetase (GS) present in these cells. The ATB^0 transporter expressed adjacent to the blood-brain barrier would rapidly extract or export astrocyte glutamine, depending on the intracellular concentration of this and other small neutral (zwitterionic) amino acids. Due in part to the extremely low extracellular amino acid concentration, SN1 would efficiently export glutamine from the astrocyte where it would be taken up by the unidirectional System A transporters (ATA1 or ATA2) present in neurons. Inside the neuron, glutamine is converted to glutamate via glutaminase (GA), and repackaged into secretory vesicles, thus completing the cycle.

Tissue distribution. RNA dot blot analysis revealed that $hATB^{0,+}$ is expressed strongly in lung and trachea, consistent with a previous report showing the presence of a Na^+/Cl^--dependent arginine transport activity in primary cultures of human lung epithelia (Galietta et al. 1998). Two mRNA species of 4.5 and 2.0 kb were observed in lung, where it is proposed that this transporter may serve to remove NaCl and amino acids from the airway surface fluid (Sloan and Mager 1999). Given that $hATB^0$ is also expressed in the lung, it will be interesting to determine the cellular and plasma membrane domain distribution of each of these transporters because this tissue has received some attention in whole-body glutamine homeostasis (Lukaszewicz et al. 1997, Welbourne 1988). Strong expression of $hATB^{0,+}$ mRNA was also observed in salivary gland, whereas lower levels of expression were noted in uterus, prostate, stomach, mammary and pituitary gland (Sloan and Mager 1999). The authors also hypothesized that the depolarizing effects of the 2–3 Na^+ molecules transported during $hATB^{0,+}$ catalysis may serve as a secretory signal in the pituitary and underlie the secretagogue properties ascribed to certain amino acids.

System A (ATA1 and ATA2)

System A was originally distinguished from other zwitterionic amino acid transporters through its ability to tolerate *N*-methylated amino acids as substrates (Christensen et al. 1965). The use of α-(methylamino)isobutyric acid (MeAIB) has since become the benchmark for assessing its function in cells. This transporter has been a favorite model for amino acid transporter regulation due to its low basal activity and high degree of inducibility by multiple stimuli (Kilberg et al. 1985). Although not traditionally considered a glutamine transporter per se, it has been noted in rat hepatocytes that under certain conditions, MeAIB-inhibitable glutamine uptake activities are evident (Fischer et al. 1996, Handlogten et al. 1982). System A-mediated glutamine uptake seems to play more of a role in human than in rodent liver (Bode et al. 1995, Mailliard and Kilberg 1990), but not nearly as much as SN1. System A has been reported to take up glutamine on the basolateral surface of intestinal epithelia (Wilde and Kilberg 1991); in human fibroblasts, System A can mediate glutamine uptake, but its contribution becomes appreciable only after prolonged periods of amino acid starvation (Dall'Asta et al. 1990). The ubiquitous expression of System A activity coupled with its differential sensitivity to protein-modifying reagents in specific cells (Dudeck et al. 1987) suggests that multiple isoforms may exist, a hypothesis borne out by recent cloning studies.

Molecular cloning and features. The first System A isoform cDNA was isolated from cultured rat glutamatergic neurons and termed *glnT* for "glutamine transporter," based on the encoded transporter's preference for this amino acid relative to others (Varoqui et al. 2000). It encoded a protein of 485 amino acids with 11 putative transmembrane-spanning domains and a deduced molecular weight of 54 kDa. This transporter has since been renamed ATA1 for *a*mino *a*cid *t*ransporter System *A–1* (Sugawara et al. 2000), because shortly thereafter, three separate groups reported the cloning of a second ubiquitous System A isoform, now know as ATA2, from rat brain (named SA1) (Reimer et al. 2000), rat skeletal muscle (named ATA2) (Sugawara et al. 2000) and rat glutamatergic neurons (named SAT2) (Yao et al. 2000). All encode for a 504 amino acid protein with 11–12 transmembrane-spanning domains and a deduced molecular weight of 56 kDa. The amino acid sequence of rATA2 is 55% identical to both rATA1 and rSN1 (Reimer et al. 2000, Sugawara et al. 2000,Yao et al. 2000). Rat ATA1 appears to be more restricted to the central nervous system, whereas ATA2 is expressed in multiple tissues (see below). The human ATA2 gene has since been isolated, encoding for a 506 amino acid protein that is 88% identical to rATA2 (Hatanaka et al. 2000). ATA1, ATA2 and SN1 belong to the VGAT family.

Catalytic mechanism. The transport mechanism for ATA2 is electrogenic, with 1 Na^+ transported per amino acid; it is progressively inhibited with decreasing pH values, but unlike SN1, does not apparently involve countercurrent H^+ efflux during catalysis (Reimer et al. 2000, Sugawara et al. 2000). The rATA1 (glutamine-preferring) isoform possesses a K_m for glutamine of 498 μmol, whereas the rATA2 (alanine-preferring) isoform exhibits a markedly lower affinity for glutamine (K_m = 1.65 mmol, compared with 530 μmol for alanine and MeAIB) (Yao et al. 2000). Separate studies with rATA2 showed a higher affinity toward glutamine, based on its K_i of 510 μmol for inhibition of MeAIB uptake (Reimer et al. 2000). Similar to ATB^0 and SN1, transport mediated by rATA2 (specifically Na^+ binding) is sensitive to the membrane potential (Yao et al. 2000), but it is unclear whether the same is true for ATA1. It is interesting that one of the hallmark features of System A, i.e., *trans*-inhibition by intracellular amino acids, was never investigated in any of these cloning and characterization studies.

Tissue distribution. Northern blot analysis revealed an 8-kb rATA1 mRNA species in the central nervous system,

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with lighter hybridization in the colon, whereas Western blot analysis revealed a prominent 55-kDa protein band in specific regions of the central nervous system (Varoqui et al. 2000). For rATA2, a major 4.3- to 4.8-kb (Reimer et al. 2000, Sugawara et al. 2000, Yao et al. 2000) and a minor 2.5-kb (Sugawara et al. 2000) mRNA species were noted in several tissues. The hATA2 mRNA (4.5 kb) was expressed in all tissues examined, including brain, liver, heart, kidney, colon, small intestine, lung, muscle, spleen, stomach, testis and placenta, whereas the hATA1 cloned from human placenta showed a 9-kb mRNA in heart as well (Hatanaka et al. 2000). In rat brain, both rATA1 and rATA2 are restricted to neurons and are absent in astrocytes (Varoqui et al. 2000, Yao et al. 2000), thus representing an opposite expression profile to SN1 and ASCT2/ATB^0 (Fig. 2).

The gpa-AT family

The next three glutamine transporters exist as disulfide-linked heterodimers with either the 4F2 heavy chain (4F2hc, also called CD98 in mice) or rBAT (*r*elated to $b^{0,+}$ *a*mino acid *t*ransporter), and seem to function as amino acid exchangers (Verrey et al. 1999). The human 4F2hc gene is located on chromosome 11 and seems to be more ubiquitously expressed than human rBAT, whose gene is on chromosome 2 and is expressed primarily in the kidney and intestine (Palacin et al. 1998a). Although these two proteins share similar structural features, they exhibit only a 30% amino acid identity, and when injected into *Xenopus* oocytes, the mRNAs for 4F2hc and rBAT induce System y^+L and System $b^{0,+}$-like activities, respectively (Palacin et al. 1998a). Through this type of expression cloning in the early 1990s, it was initially thought that these proteins were amino acid transporters. However, sequencing revealed that the 4F2hc and rBAT gene products are type II membrane proteins with a limited number (1–4) of transmembrane domains, and seem incapable of forming an aqueous pore for translocation in the plasma membrane, prompting the hypothesis that they were transporter subunits (Palacin et al. 1998a). This hypothesis was further supported by electrophoresis and Western blot analyses, which revealed in several studies that under nonreducing conditions, a 125-kDa rBAT or 4F2hc complex existed, but under reducing conditions, this complex dissociated into 85- to 90-kDa "heavy chains" (4F2hc or rBAT) and unidentified 40- to 50-kDa "light chains" (Palacin et al. 1998a). Indeed, this hypothesis was confirmed over the last two years, with the cloning of the "light chains," described below, which seem to be responsible for the corresponding transport activities. Although all "light chains" have predicted molecular weights of ~53–59 kDa, all exhibit electrophoretic mobilities closer to 40 kDa. Each light chain requires coexpression with 4F2hc or rBAT for full function, presumably by being "chaperoned" to the plasma membrane by these "heavy chain" proteins (Mastroberardino et al. 1998). Together with System asc [asc-1, facilitative small zwitterionic amino acid exchanger (Fukasawa et al. 2000)] and System x_c^- [xCT, cystine/glutamate exchanger (Sato et al. 1999)], which do not transport glutamine, all belong to a fourth family of mammalian amino acid transporters. This family has been termed gpa-AT for *g*lyco*p*rotein-*a*ssociated *a*mino acid *t*ransporters (Pfeiffer et al. 1999, Rossier et al. 1999). The discovery of these heterodimeric proteins broke the long-held paradigm of "one protein, one activity" in amino acid transporter biology. Such associations for transporters are not unprecedented, however, as exemplified by the Na^+/K^+-ATPase, which functions as a heterodimeric complex (Blanco and Mercer, 1998). A model for the gpa-AT association can be seen in **Figure 3**, based on a recent article (Mastroberardino et al. 1998). For a comprehensive review on the gpa-AT family, the reader is referred to Verrey et al. (1999). These heterodimeric proteins have not been as well studied as Systems N, ASC/B^0 and A in specifically mediating tissue glutamine flux, and their descriptions here will therefore be brief. However, it is likely that we will learn much more about potential roles for these interesting transporters in glutamine homeostasis in the near future.

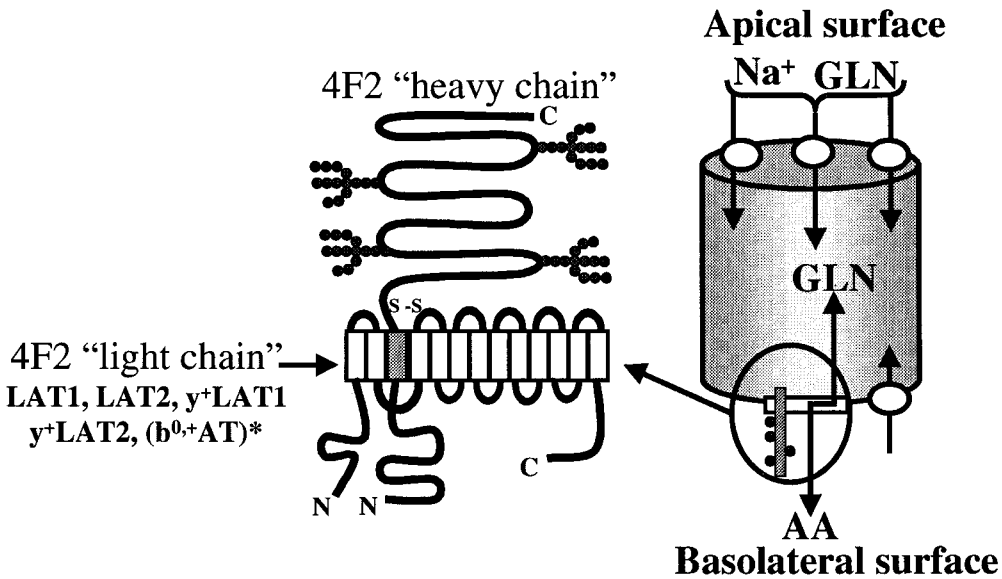

FIGURE 3 Model for heterodimeric glycoprotein-associated amino acid transporters (gpa-AT), and proposed role in glutamine transport in epithelial cells. The 4F2 "heavy chain" presumably chaperones 4F2 "light chains" to the plasma membrane where they function as amino acid exchangers, as discussed in the text. The model depicted is based on the article by Mastroberardino et al.(1998). Most of these transporters are localized to the basolateral surface of polarized epithelia, the notable exception being $b^{0,+}AT^*$, which is a relatively weak glutamine transporter, and associates with rBAT on the apical surface. Na^+-dependent transporters on both the apical and basolateral surfaces pump glutamine into the cell, establishing a cytoplasmic glutamine pool. The gpa-AT can use this glutamine pool to pump other amino acid substrates into the cell, exporting glutamine to the blood. Alternatively, these heterodimers may use plasma glutamine to pump intracellular substrates absorbed from the apical surface into the circulation [e.g., arginine via y^+LAT, or branch chain amino acids via System L amino acid transporter (LAT)]. In other words, the net direction of gpa-AT-mediated glutamine transport in polarized epithelia is dependent upon its transmembrane gradient and the intracellular levels of other amino acids, all of which can be altered in critical illness. [For a comprehensive review of these transporters, the reader is referred to Verrey et al. (1999).]

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System y^+L (y^+LAT1 and y^+LAT2)

This transport activity was first described in the plasma membrane of human erythrocytes (Deves et al. 1992). System y^+L is unique among transporters in that it mediates the uptake of cationic amino acids with high affinity in a Na^+-independent manner, but requires Na^+ to efficiently transport zwitterionic amino acids such as glutamine. This transporter is thought to mediate the basolateral extrusion of dibasic amino acids in kidney and intestinal epithelia (Pfeiffer et al. 1999), and it is currently hypothesized that mutations in this transporter gene underlie lysinuric protein intolerance (Torrents et al. 1999).

Molecular cloning and features. The gene encoding for human System y^+L activity, identified by expression cloning and termed *y^+LAT1*, was one of the first members of the gpa-AT family to be isolated (Torrents et al. 1998). In the same study, human cDNA clone number KIAA0245 (Nagase et al. 1996) was identified as a second isoform of human System y^+L and termed *y^+LAT2*. Shortly thereafter, a second report of both human and mouse y^+LAT1 isolation and characterization appeared (Pfeiffer et al. 1999), followed by rat y^+LAT1 (Kanai et al. 2000). The hy^+LAT1 and hy^+LAT2 proteins require coexpression with the 4F2hc for activity (Kanai et al. 2000, Pfeiffer et al. 1999, Torrents et al. 1998) via a disulfide linkage presumably to cysteine 109 of 4F2hc (Torrents et al. 1998). The *hy^+LAT1* gene, located on chromosome 14, encodes for a protein composed of 511 amino acids with a predicted molecular weight of 56 kDa and 12 putative transmembrane domains (Torrents et al. 1998). The hy^+LAT2 protein also contains 12 putative transmembrane domains, and is composed of 515 amino acids with a 75% amino acid identity to hy^+LAT1 (Torrents et al. 1998). It is proposed that a conserved cysteine residue between transmembrane domains 3 and 4 of hy^+LAT1 participates in the disulfide bridge formation with 4F2hc (Pfeiffer et al. 1999).

Catalytic mechanism. System y^+L primarily mediates the heteroexchange of intracellular cationic amino acids (arginine, lysine) for extracellular Na^+ and zwitterionic amino acids such as glutamine (Kanai et al. 2000, Pfeiffer et al. 1999). This catalytic mechanism is unique among transporters in that the uptake of extracellular zwitterionic amino acids is Na^+ dependent, but is Na^+ independent for dibasic amino acids. It is currently hypothesized that this unique mechanism is attributable to the binding site, which requires a positive charge supplied either by the amino acid side chain or a Na^+ ion in the exchange process (Kanai et al. 2000). The implication of this mechanism is that high intracellular levels of arginine or lysine would help to drive glutamine uptake through this carrier. No kinetic constants were determined for glutamine uptake via y^+L, however, and its role as a substrate of this transporter was inferred by its effective inhibition of arginine uptake in the presence of Na^+ (Torrents et al. 1998). By inference, however, the affinity of y^+L for other zwitterionic amino acids such as leucine is in the low micromolar range [e.g., 16 μmol for y^+LAT1 (Pfeiffer et al. 1999)]; thus, it is probably a high affinity glutamine transporter as well. This is the only glutamine transporter other than SN1 that will accept Li^+ as effectively as Na^+ to drive its catalysis (Pfeiffer et al. 1999), and its activity in tissues will therefore manifest as arginine-inhibitable Li^+-dependent glutamine uptake.

Tissue distribution. The 2.4-kb hy^+LAT1 mRNA is expressed most abundantly in the kidney (Pfeiffer et al. 1999, Torrents et al. 1998), and on the basis of mouse studies, may be enriched in the cortex of this organ (Pfeiffer et al. 1999). It functions in human platelets (Mendes Ribeiro et al. 1999) and is also expressed in peripheral blood leukocytes, lung, placenta, spleen and small intestine (Torrents et al. 1998), and probably in many other tissues if analyzed more closely (e.g., polyA vs. total mRNA, in situ hybridization) (Pfeiffer et al. 1999). A role for this protein in mediating glutamine transport has been described in human placenta (Novak and Beveridge 1997), corroborating the presence of its mRNA in this tissue.

Na^+-independent transporters

System L (LAT1 and LAT2). System L was one of the first transport activities to be described and was designated as such for its *l*eucine-preferring nature (Oxender and Christensen 1963). A role for System L in mediating glutamine transport has been more frequently described than for other Na^+-independent carriers (Brookes 1992, Bussolati et al. 1993, Calvert et al. 1998, Dall'Asta et al. 1990, Lewis and Kaye 1992, Low et al. 1991, Piva et al. 1992, Taylor et al. 1989, Wilde and Kilberg 1991), but its contribution almost always represents a minority of total uptake. Functional precedents for distinct System L isoforms have been established (Weissbach et al. 1982), an observation confirmed by recent cloning studies.

Molecular cloning and features. Human (Mastroberardino et al. 1998) and rat (Kanai et al. 1998) LAT1 (System *L a*mino acid *t*ransporter *1*) share the distinction of being the first "4F2 light chains" isolated and characterized, along with hy^+LAT1 described above. Originally identified as a surface antigen induced in activated lymphocytes and designated E16 (Gaugitsch et al. 1992), and as an oncofetal protein designated TA1 in rat hepatomas (Sang et al. 1995), *hLAT1* was first named *hAmAt-L-lc* (Mastroberardino et al. 1998). A second report of hLAT1 isolation from a human placental cDNA library appeared shortly thereafter (Prasad et al. 1999) with a bovine isoform recently isolated from brain capillary endothelial cells (Boado et al. 1999). The 507 amino acid hLAT1 protein requires coexpression with 4F2hc for activity, contains 12 putative transmembrane-spanning domains and possesses a predicted molecular weight of 55 kDa (Mastroberardino et al. 1998, Prasad et al. 1999). Several months after the isolation of hLAT1, a second System L isoform cDNA was isolated from human (Pineda et al. 1999) and rat (Segawa et al. 1999) sources and termed *LAT2*. A second report of human as well as murine LAT2 isolation appeared shortly thereafter (Rossier et al. 1999), followed by the rabbit LAT2 ortholog (Rajan et al. 2000b). The hLAT2 protein is 535 amino acids long with 12 putative transmembrane-spanning domains and a predicted molecular weight of 58.6 kDa. At the amino acid level, it is 50, 44, 45 and 41% identical to hLAT1, hy^+LAT1, hy^+LAT2 and $hb^{0,+}$AT (see below), respectively (Pineda et al. 1999, Rossier et al. 1999). The chromosomal assignment of *hLAT1* gene has yet to be reported, whereas the *hLAT2* gene has been assigned to chromosome 14 (Pineda et al. 1999).

Catalytic mechanism. Classical System L with a preference for bulky hydrophobic side chains seems to be encoded by hLAT1, whereas hLAT2 encodes for a System L isoform that demonstrates higher affinity for small zwitterionic amino acids such as alanine, serine and threonine. Experiments with rodent and human LAT isoforms revealed that glutamine is transported much more efficiently by LAT2 than by LAT1. The K_m for hLAT1-mediated glutamine uptake is 2.2 mmol (Mastroberardino et al. 1998). Although kinetic parameters for glutamine uptake via hLAT2 remain unknown, studies in rat, mouse and rabbit LAT2-expressing cells showed that the affinity for glutamine was much higher than LAT1, with K_m values of 151, 275 and 316 μmol reported, respectively (Rajan et al. 2000b, Rossier et al. 1999, Segawa et al. 1999). Furthermore, although both System L isoforms function as amino acid

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exchangers (Pineda et al. 1999), LAT2-mediated transport is stimulated by decreasing pH, whereas LAT1 is not influenced by protons (Rajan et al. 2000b). Both require disulfide linkage to 4F2hc, which appears to facilitate their translocation to the plasma membrane from intracellular compartments (Pineda et al. 1999). Despite their obligate linkage, 4F2hc and LAT isoforms exhibit differential regulation by amino acid availability, and it appears that "light chain" expression determines the extent of the resulting transport activity (Campbell et al. 2000). In these same studies, glutamine failed to regulate LAT1 expression in hepatoma cells, which seemed more dependent on arginine.

Tissue distribution. The hLAT1 mRNA appears to be expressed more ubiquitously than the hLAT2 mRNA, which is expressed primarily in the kidney and small intestine. The 5.0-kb hLAT1 mRNA is expressed very strongly in placenta and skeletal muscle, and at lower levels in leukocytes, heart, lung, spleen, thymus, kidney and colon. In the liver, hLAT1 mRNA is only ~4 kb in length, and it is absent in the intestine (Prasad et al. 1999). Conversely, hLAT2 mRNA species of 5 and 3.7 kb are markedly expressed in kidney and placenta, with lower levels in brain, liver, spleen, skeletal muscle, heart, small intestine and lung (Pineda et al. 1999). The hLAT2 protein has been localized to the proximal convoluted tubules in human kidney (Pineda et al. 1999) and to the basolateral domain of kidney and intestinal epithelia in rodents (Rossier et al. 1999). Depending on the cell type, both LAT isoforms could mediate either glutamine uptake or release, based on their exchange mechanism. System L-mediated glutamine uptake has also been reported in human fibroblasts (Bussolati et al. 1993).

System $b^{0,+}$ ($b^{0,+}$ at)

This transport system was first described in preimplantation mouse blastocysts (Van Winkle et al. 1988) and mediates the uptake of both cationic and zwitterionic amino acids. In contrast to System $B^{0,+}$ described above, this transporter is Na^+ independent and is a relatively weak glutamine transport mediator, unless complexed with 4F2hc instead of rBAT as described below. Due to its demonstrated ability to take up cystine in the kidney, it has instead been more heavily studied along with rBAT as serving a potential role in renal cystinuria (Palacin et al. 1998b). Mutations in both have recently been shown to underlie different forms of this disease (Feliubadalo et al. 1999).

Molecular cloning and features. Simultaneous reports appeared on the cloning of $b^{0,+}$ AT from rabbit intestine and rat kidney cDNA libraries based on their sequence similarities to LAT1 (Chairoungdua et al. 1999, Rajan et al. 1999). Shortly thereafter, the human $b^{0,+}$ AT gene was isolated (Feliubadalo et al. 1999) followed by a second report of human and mouse $b^{0,+}$ AT (Rajan et al. 2000a). Similar to other gpa-AT members, $b^{0,+}$ AT has 12 putative membrane-spanning domains, a predicted molecular weight of 53.6 kDa and a 42–44% amino acid identity with other members of the gpa-AT family (Chairoungdua et al. 1999, Rajan et al. 1999).

Catalytic mechanism. The $b^{0,+}$AT transporter has been described as a "tertiary active transporter" because it is able to concentrate substrates such as arginine and cystine several fold intracellularly by dissipating existing amino acid gradients through its heteroexchange mechanism (Palacin et al. 1996). There is interesting new evidence that runs contrary to previous models, namely, that the $b^{0,+}$AT protein can associate with either rBAT or 4F2hc (Rajan et al. 1999) and that its linkage to either of these "heavy chains" affects its substrate affinity (Rajan et al. 2000a). This is particularly interesting for glutamine because the heteroduplex of $b^{0,+}$AT · 4F2hc exhibited high affinity ($K_m \cong 83$ μmol) glutamine uptake, whereas the $b^{0,+}$AT · rBAT complex exhibited no such activity (Rajan et al. 2000b). However, differential and functional heteroduplex formation between $b^{0,+}$AT and 4F2hc has yet to be clearly established in vivo, and such results have been equivocal thus far. Future studies in this field will therefore be required to help to further clarify these issues.

Tissue distribution. A 1.9-kb rat $b^{0,+}$AT mRNA and 2.0-kb rabbit $b^{0,+}$AT were evident only in kidney and small intestine by Northern analysis (Chairoungdua et al. 1999, Rajan et al. 1999). Immunoreactive $b^{0,+}$AT and rBAT localized strongly to proximal convoluted and straight tubules, respectively, in rat kidney and were both apically rather than basolaterally localized, in contrast to other 4F2hc-associated gpa-AT members (Chairoungdua et al. 1999). Given its proposed primary role in the intracellular accumulation of cystine and arginine at the expense of other amino acids, it is likely that if $b^{0,+}$AT plays any role at all in glutamine movement, it probably mediates its apical release from absorptive epithelia in the kidney and intestine and only if complexed with 4F2hc. In this model, Na^+-dependent carriers in the brush border membranes would subsequently take up the released glutamine.

CONCLUSIONS

There is likely to be a dramatic increase in the number of reports on the molecular physiology of glutamine transporters and their tissue-specific regulation over the next several years, spearheaded largely by the molecular cloning studies described in this review. The long-awaited molecular tools afforded by these efforts coupled with newly established catalytic mechanisms will allow the elucidation of roles for individual glutamine transporters during health and critical illness. One theme that is likely to resonate frequently in the glutamine transporter field is that the regulation of these membrane proteins will mostly involve translational or post-translational pathways such as cellular trafficking and changes in driving forces for their catalysis, rather than transcriptional regulation. This theme will apply especially to transporters such as SN1 in the liver, $hATB^0$ in the kidney or $hATB^{0,+}$ in the lung, where the mRNA is already prodigiously expressed. The elucidated effect and role of such regulation on interorgan glutamine flux, when combined with metabolic data, should eventually aid the rational prediction of tissue-specific glutamine handling and the utility of this amino acid in "nutritional pharmacology."

Note Added in Proof: During the editorial process of this publication, two additional glutamine transporters have been isolated; a second System N isoform (hSN2) and a third System A isoform (rATA3). The reader is referred to the following articles:

Sugawara, M., Nakanishi, T., Fei, Y. J., Martindale, R. G., Ganapathy, M. E., Leibach, F. H. & Ganapathy, V. (2000) Structure and function of ATA3, a new subtype of amino acid transport system A, primarily expressed in the liver and skeletal muscle. Biochim. Biophys. Acta 1509: 7–13.

Nakanishi, T., Sugawara, M., Huang, W., Martindale, R. G., Leibach, F. H., Ganapathy, M. E., Prasad, P. D. & Ganapathy, V. (2001) Structure, function, and tissue expression pattern of human SN2, a subtype of the amino acid transport system N. Biochem. Biophys. Res. Commun. 281: 1343–1348.

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