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RECENT ADVANCES IN MAMMALIAN AMINO ACID TRANSPORT

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CONTENTS

AMINO ACID TRANSPORT SYSTEMS

Characterization of mammalian amino acid transport systems began with the pioneering work of Christensen and his coworkers in the early 1950s (37). Many of the features of Systems y^+, A, ASC, and L originally described in the Ehrlich cell also hold for these systems in other cell types as well, but a number of activities, such as Systems Gly, N, and those specific for imino or anionic amino acids, are not expressed by the Ehrlich cell. In hindsight, the relative simplicity of amino acid transport by the Ehrlich cell may have facilitated the original description of the systems, but now it might be

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considered atypical in comparison to other mammalian cells. Most mammalian cells express a common "core" set of amino acid transport activities, but all also exhibit wide variations in the total number, type, and activity of these transport systems. As a result each cell type is unique with regard to the processes available for amino acid accumulation, and has undoubtedly adapted for its particular physiological role and metabolic needs.

A number of reviews have covered, through the early 1980s, some of the more common amino acid transport systems that exist in many cell types and have described individual transport activities and the associated regulation (26–30, 52, 128, 157). The present report focuses primarily on the more recent advances of the last decade. Progress has been made in identifying transporter proteins and cloning the corresponding cDNAs. As expected, this newly acquired information has reaffirmed some of the principles derived from whole cell studies, but it has also introduced unexpected and exciting new insights.

Facilitated Transporters

SYSTEM L One of the first amino acid transport systems described for mammalian cells was a Na^+-independent system termed System L (113). Until recently, most investigators have somewhat arbitrarily assigned all saturable, Na^+-independent uptake to this activity, especially in those cases where the analog 2-aminobicyclo-(2,2,1)-heptane-2-carboxylic acid (BCH) was used because of its System L specificity in the Ehrlich cell (32). However, it is now clear that several distinct systems may contribute to the saturable Na^+-independent transport in any given cell type. Regulation by substrate availability of the System L activity in CHO cells has been studied by Oxender and his colleagues and reviewed elsewhere (39). They have demonstrated that System L activity is increased when leucyl-tRNA becomes limiting either by reduction of leucine content in the medium or by inactivation of leucyl-tRNA synthetase in a temperature-sensitive mutant cell line.

What was initially thought to be a single System L activity in isolated hepatocytes (107) was later shown to exhibit heterogeneity (68, 167). In hepatocyte primary cultures, the kinetics of leucine, histidine, or BCH saturable uptake in the absence of Na^+ are biphasic. Component I (L_1) is a high affinity, low capacity agency with estimated K_m values of less than 200 μM, whereas component II (L_2) is a low affinity, high capacity system with K_m values of 2–5 mM. System L_2 activity is relatively high in freshly isolated cells and declines during the first 24 hr of culture, while uptake by System L_1 is barely detectable immediately after cell isolation, but increases from two- to fivefold after an initial lag period of 12–24 hr. Although the factors controlling these changes are unknown, it is known that the increase in System L_1 requires de novo synthesis of both RNA and protein (167).

System L activity is decreased in B-lymphocytes from patients who have chronic lymphocytic leukemia (CLL) (135). Other amino acid transport activities are normal, despite a 80–90% reduction in System L as measured by BCH uptake. Interestingly, when CLL lymphocytes are treated in vitro with phorbol esters, the cells are converted to a mature immunoglobulin-producing phenotype and the System L activity is restored to rates observed in normal cells treated with phorbol esters (173). Further experimentation is required to distinguish between the contribution of maturational level and transformation to the decreased transport.

SYSTEM $b^{o,+}$ A novel Na^+-independent system that accepts both neutral and cationic amino acids was first described by Van Winkle and his colleagues in mouse blastocysts (157, 158). The substrate specificity of System $b^{o,+}$ overlaps with those of Systems L and y^+ in that amino acids with bulky sidechains, but without branching on either the α or β carbon, are preferred. Interestingly, both organic and inorganic cations, including Na^+, compete for the cationic binding site and inhibit transport (159). The degree to which this effect serves to minimize the contribution of System $b^{o,+}$ in vivo is unknown.

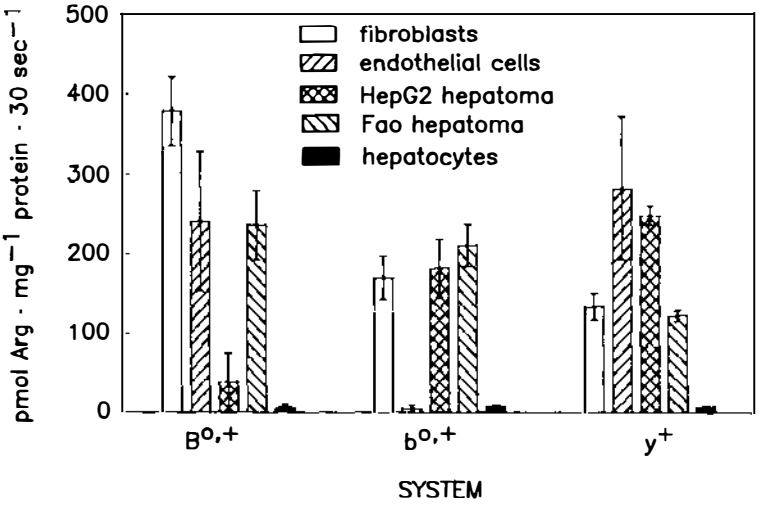

Figure 1 Transport of arginine by multiple pathways in mammalian cells. The uptake of 50 μM ^{3}H-arginine was measured for 30 sec at 37C in cultured cell monolayers as detailed previously (89). System $B^{o,+}$ was defined as the uptake rate in sodium-containing buffer minus the uptake rate in the absence of sodium. The Na^+-independent uptake rate that was inhibited by 10 mM leucine was defined as System $b^{o,+}$. System y^+ was the leucine insensitive Na^+-independent transport inhibited by 10 mM arginine. Each value represents the average ± standard deviations of at least three determinations.

The role of this and other systems during early development has been reviewed by (157). An extensive tissue distribution of System $b^{o,+}$ in the adult mammal has not been reported, but as shown in Figure 1 its presence in a number of cultured cells including human fibroblasts, rat or human hepatoma cells, and porcine endothelial cells suggests widespread occurrence. The existence of System $b^{o,+}$ in a variety of cell types cautions against the former practice of assigning all saturable Na^+-independent arginine uptake to System y^+.

SYSTEM y^+ Uptake of lysine by the Ehrlich cell was shown to be mediated by both neutral and cationic systems, depending on the net charge of the substrate (35). The process mediating uptake of the cationic form was designated System Ly^+ and later was shown to transport the net positively charged forms of lysine, arginine, and histidine. Recently, the term Ly^+ was changed to y^+ to underscore the breadth of acceptance for numerous cationic amino acids and related analogs (6).

An extensive review of System y^+ characteristics is published elsewhere (170). Four of its most interesting properties are as follows. (*i*) Tissue distribution appears to be widespread, but not ubiquitous (Figure 1; 170). (*ii*) Accumulation of amino acid against a concentration gradient occurs despite bidirectional transport because the cationic substrates respond to the membrane potential across the plasma membrane (20). (*iii*) Uptake is subject to trans-stimulation when substrate concentrations are sufficiently high on the opposite side of the membrane (171). (*iv*) A neutral amino acid plus a Na^+ can competitively inhibit System y^+ and participate in exchange reactions with the cationic substrates of this system (33, 170).

The cDNA for the System y^+ transporter was cloned serendipitously when researchers discovered that the corresponding protein, previously shown to be the murine ecotropic retrovirus receptor, exhibited all of the characteristics associated with this transport activity (92, 164). The following defining criteria were demonstrated: (*i*) injection of cRNA into oocytes produced a stimulation of transport activity for cationic amino acids only; (*ii*) certain neutral amino acids could inhibit if Na^+ was present; and (*iii*) among the substrates only histidine showed a sensitivity to pH. Consistent with previous observations that documented the lack of significant y^+ activity in adult rat liver (Figure 1; 170), Northern blot analysis yielded little or no detectable System y^+ mRNA in this tissue (92). Interestingly, System y^+ activity is expressed in both fetal and transformed hepatocytes (170). Figure 2 illustrates the relative amount of System y^+ mRNA for the rat Fao hepatoma cell line as well as newborn and adult rat liver tissue. The relative amounts of mRNA are in agreement with the transport activity within these cells and illustrate that cell-specific expression of System y^+ can occur at the transcriptional level. Given the hepatic catabolism of arginine via the urea cycle, the lack of System y^+ activity

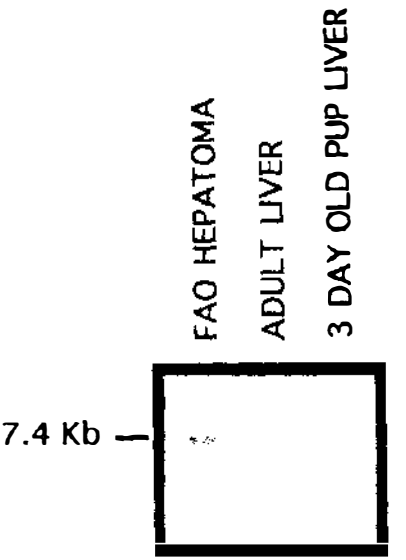

Figure 2 System y^+ mRNA content in adult or newborn rat liver, and a rat hepatoma. Total RNA was isolated from liver tissue or cultured rat Fao hepatoma cells. Size fractionation by agarose gel electrophoresis and subsequent Northern analysis was performed as described previously (136). Each lane contained 20 μg of total RNA and was probed with a cDNA provided by Dr. James Cunningham, Harvard University.

in the hepatocyte may at first seem illogical. However, in this way arginine efflux is prevented (other than by the nonsaturable route) and the cytoplasmic concentration is determined by cellular synthesis/degradation without potential fluctuations due to changes in the plasma levels.

REGULATORY SUBUNITS? The possibility that mammalian amino acid transport activities are composed of multiple subunits or are regulated by associated membrane proteins is suggested by recent cDNA cloning reports from three laboratories (11, 103, 152, 168, 169). As a group these cDNAs code for putative proteins that are integral membrane proteins with a single transmembrane spanning domain. The latter property distinguishes them from many other Na^+-dependent and Na^+-independent organic solute transporters that have been cloned and contain 12–14 transmembrane domains. In vitro synthesis of cRNA from these cDNA clones and subsequent injection into oocytes resulted in stimulation of amino acid transport activities that resemble Systems y^+, $b^{o,+}$, or $B^{o,+}$. Tate et al (152) published the first of these sequences (NAA) and proposed that the corresponding protein mediated a System L-like activity. However, lysine served as an inhibitor of Na^+-inde-

AMINO ACID SEQUENCES OF TRANSPORTER-ASSOCIATED PROTEINS

Figure 3 Alignment of amino acid sequences for transport-associated membrane proteins. The predicted amino acid sequences deduced from the cloned cDNAs for 4F2, D2/NAA, or rBAT were aligned using the CLUSTAL program (77) within PC-GENE (IntelliGenetics, Mountain View, CA). The top two sequences compare 4F2 with D2/NAA, and the bottom two sequences compare D2/NAA and rBAT. Identical residues are marked with an asterisk (*) and conserved substitutions are indicated with a period (.) for each pair of sequences. Gaps (-) are inserted by the computer program to optimize the alignment.

pendent phenylalanine uptake by the expressed NAA activity in *Xenopus* oocytes, an observation consistent with the System $b^{o,+}$ assignment made for the same sequence (termed D2) later reported by Wells & Hediger (168). A cDNA isolated from rabbit kidney (rBAT) by Bertran et al (11) shows a high degree of homology to the rat sequence (NAA/D2) and also results in stimulation of amino acid transport activity similar to System $b^{o,+}$ when expressed in *Xenopus* oocytes (Figure 3). The putative rat proteins are 683 amino acids in length and have a molecular mass of 78 kDa. In vitro translation confirms this core protein size, whereas translation in the presence of microsomal membranes yields a product of 90–100 kDa, suggesting a significant amount of glycosylation (168). The rabbit clone (rBAT) codes for a protein of about 78 kDa as well, based on the deduced sequence of 677 amino acids (11, 103).

Both of these proteins show homology to the heavy chain (85 kDa) of a heterodimer membrane protein complex called the 4F2 cell surface antigen (169). Expression of the human 4F2 protein in oocytes results in stimulation of both Na^+-dependent and Na^+-independent transport of neutral and cationic amino acids. In contrast to the tissue specificity of NAA/D2 and rBAT, 4F2 is widely expressed in both mouse and human tissues. The exact function of these proteins is unclear; they may serve as necessary components for maximal constitutive activity of a given transporter or they may be strictly regulatory so as to alter the basal activity. At least in one example, System y^+, a putative protein that contains 12 transmembrane spanning domains is known to exhibit basal activity (92, 164).

Secondary Active Transporters

SYSTEM A System A, originally described two decades ago in the Ehrlich cell (36, 113), has received a great deal of attention because of the availability of specific nonmetabolizable substrates (AIB or MeAIB) and its regulatory properties. For example, hormone-mediated induction of System A in liver occurs in response to a broad spectrum of hormones (138), and transcriptional control of a System A-associated gene is thought to be responsible for most of the enhanced transport observed.

Glucagon induction of hepatic System A transport activity is typical and will be used as an example. Enhanced System A activity (20–40%) occurs within a few minutes after addition of glucagon, but a much larger increase in activity is seen after a time lag of about 60 min. The increase detected during the first 60 min of exposure to hormone is cycloheximide-insensitive (50) and probably is the result of increased membrane potential (110). The majority of the elevated transport activity (five- to tenfold) is blocked by inhibitors of either RNA or protein synthesis (34, 41, 117). Supportive

evidence for de novo synthesis of a System A-associated glycoprotein comes from Barber et al (7), who showed that tunicamycin blocked the glucagon induction of System A. As shown previously for cycloheximide or puromycin (34, 117), when tunicamycin was added to hepatocytes after the hormone-mediated induction of transport activity had begun, inhibition of further stimulation occurred within 1 hr (7). These results suggest that the System A-associated glycoprotein responsible for the increased uptake must be continuously synthesized. Following glucagon-dependent induction of transport in whole cells, isolated plasma membrane vesicles retain the activity (133) which also can be recovered following solubilization of plasma membrane proteins and reconstitution into artificial proteoliposomes (55).

More recent evidence for glucagon-dependent transcriptional control comes from exogenous RNA expression in *Xenopus* oocytes. Two independent studies have reported that rat liver RNA from glucagon-treated animals, when compared to RNA from control tissue, results in greater expression of System A activity following microinjection of oocytes (116, 151). Size fractionation of mRNA prior to injection into oocytes led both laboratories to conclude that the mRNA responsible was between 1.9 and 2.5 kb in length. The role of this product in initiating and maintaining the glucagon-induced transport is unknown and must await identification and characterization. Given the recent identification of putative transporter-associated regulators, the glucagon-stimulated mRNA may code for the System A transporter itself or for an associated regulatory protein. If it is solely regulatory in function, its action within the Golgi, as described below, must be explained.

Consistent with de novo membrane protein synthesis, Cariappa & Kilberg (22) have shown that glucagon-induced System A activity can be detected in transport-competent Golgi vesicles. Following glucagon treatment, a lag of 30–45 min occurred prior to detection of increased activity in cis Golgi, which then continued through the cellular machinery to include medial and trans Golgi, and finally the plasma membrane. If the newly synthesized protein is a regulator of basal or inactive System A carriers already present, this control must also occur for transporters in transit to the plasma membrane, given the ability to detect the hormone-induced activity in the Golgi compartment. Interestingly, the glucagon-stimulated transport activity present in cis or medial Golgi membranes was insensitive to inactivation by NEM, whereas after transfer to the trans Golgi or plasma membrane the sulfhydryl reagent produced rapid and irreversible inhibition of the carrier (22). This difference in NEM sensitivity was demonstrable even if the protein modification was performed on detergent-solubilized transport activity and then followed by proteoliposome reconstitution for transport measurements. The latter observation argues for an inherent change in the availability of a free sulfhydryl group during processing of a component of the System A transporter.

Studies on the trafficking of hepatic System A activity (23) revealed that the newly synthesized transporter arrives at the basolateral plasma membrane surface of the hepatocyte prior to its transfer, presumably by transcytosis (8), to the canalicular surface. Irreversible inactivation of the glucagon-stimulated basolateral activity with a cell-impermeant maleimide completely prevented transfer of the transport activity to the canalicular membrane.

SYSTEM GLY Isolated rat hepatocytes have been shown to contain a glycine-specific system (31), similar to that originally described in pigeon erythrocytes (48) and rabbit reticulocytes (172). The hepatic System Gly is Na^+-dependent and appears to transport two sodium ions for each glycine. Glycine uptake by normal hepatocytes is not restricted to System Gly, however, as indicated by a sensitivity of a portion of its transport to inhibition by the System A-specific substrate MeAIB (31). Reichberg & Gelehrter have studied glycine transport in HTC cells in some detail, especially the regulation by hormones (121). In this hepatoma cell, glycine transport is mediated by two distinct systems, both of which are inhibited by glucocorticoid treatment. A Na^+-dependent glycine transporter has been described in the central nervous system, and two cDNAs encoding a putative transporter have been reported (63, 140). By Northern analysis, both laboratories demonstrated a high level of expression in spinal cord, brain stem, olfactory bulb, and cerebellum. The presence of significant glycine transport rates in other areas of the central nervous system may be indicative of related transporters.

SYSTEM N The Na^+-dependent uptake of glutamine and histidine by isolated rat hepatocytes is not inhibited competitively by an excess of either the System A-specific substrate MeAIB or the System ASC-specific substrate cysteine (90). These and other results led Kilberg et al (90) to conclude that hepatocytes contained a Na^+-dependent transport system distinct from Systems A or ASC. Additional support for such a system had already been reported by Joseph et al (84), who showed that glutamine uptake did not occur by the same processes as uptake of alanine and serine. A detailed study demonstrated the presence of a previously undetected transport agency, called System N to reflect an apparent affinity for neutral amino acids containing nitrogen-bearing side-chains, which mediated the Na^+-dependent uptake of glutamine, histidine, and asparagine (90). In fact, in cells that contain basal System A activity, glutamine and histidine are specific substrates for System N. Contrary to the original report, glutamine uptake can occur via System A if this carrier's activity is induced by substrate starvation, thus requiring the continued inclusion of MeAIB to restrict glutamine or histidine to System N (67). System N does retain complete specificity for glutamine in the hepatoma cell line

H4-II-EC3 (also called H-35) as well as in hepatocytes isolated from rat fetuses (156).

The importance of System N in relation to glutamine metabolism in the liver has been the subject of a number of investigations (discussed below). The existence of System N-like activity in human placenta (88) and rat muscle (1, 81, 99, 122) has been documented. Dimaline et al (46) have suggested that a System N-like activity is an important component of histidine-induced gastric acid secretion, based on selective inhibition by glutamine and asparagine, and tolerance for lithium substitution for sodium.

Some progress has been made toward identifying the protein(s) responsible for hepatic System N activity. Following development of a procedure to efficiently solubilize the transport activity and subsequently reconstitute it into artificial proteoliposomes, enrichment of the transporter by nearly 600-fold was achieved through selective protein precipitation (150). Using this partially purified fraction as antigen, monoclonal antibodies were prepared that inhibited System N activity in rat hepatocytes and immunoprecipitated solubilized transporter (149). The antibodies revealed a broad band of immunoreactive protein at about 100 kDa on immunoblots of rat liver plasma membrane. A good correlation was observed between enrichment of this immunoreactive protein and System N activity during several steps of purification. Tissues known to lack System N activity do not contain immunoreactive material (B. K. Tamarappoo, unpublished data). Using a different approach, Taylor et al (153) have injected *Xenopus* oocytes with RNA from rat liver and demonstrated enhanced System N transport. Size fractionation of the RNA prior to injection suggested that the size of the mRNA responsible was approximately 1.9 kb. Further progress will require identifying an individual clone from a cDNA library prepared from this RNA fraction.

SYSTEM $B^{o,+}$ System $B^{o,+}$ is a Na^+-dependent activity that mediates uptake of both cationic and neutral amino acids (158). It was first described in mouse blastocysts (160) but also has been characterized in *Xenopus* oocytes (21) and in several other cell types (30). As shown in Figure 1, System $B^{o,+}$ is expressed in human fibroblasts, porcine endothelial cells, and rat Fao hepatoma cells; little or no activity is present, however, in the human hepatoma HepG2 or in normal rat hepatocytes. These data suggest that expression of $B^{o,+}$ activity is tissue and cell specific. The substrate specificity of the $B^{o,+}$ activity is somewhat broader than the Na^+-independent System $b^{o,+}$ because of its tolerance for branching in the α and β carbon positions. Hence, in some cells, a bicyclic analog formerly considered specific for System L, 2-aminobicyclo-(2,2,1)-heptane-2-carboxylic acid (BCH), may be transported by System $B^{o,+}$ in the presence of sodium. The activity of this transport system is developmentally regulated in the blastocyst (157). A

possible variant of System $B^{o,+}$, called System B, exists in the brush border of absorptive epithelial cells (see below). The substrate specificity for System B is remarkably similar to those neutral amino acids accepted by System $B^{o,+}$ (145, 147).

Transporters of the Brain

A number of amino acids and related metabolites serve as neurotransmitters to mediate chemical transmission across the synapse. A hallmark of synaptic transmission is rapid termination of the signal by clearing the synaptic cleft of neurotransmitter. Specific Na^+-dependent transporters on the postsynaptic membrane mediate this process through active extraction of these compounds. A number of cDNAs that code for transport activities thought to function in this role have been cloned recently. One of the earliest advances was the purification of a Na^+-dependent GABA transport activity (120), which led to the subsequent cloning of the corresponding cDNA (64). Using oligonucleotides that have sequence homology to regions of the GABA transporter cDNA (sequences within or near a putative transmembrane domain), other laboratories have obtained clones by cDNA library screening or polymerase chain reaction (PCR) for Na^+-dependent transporters from neuronal tissue for norepinephrine (114), dopamine (62, 91, 137), serotonin (80), proline (59), and glycine (63, 140).

Sequence analysis of these cDNAs shows extensive homology, and they appear to represent a protein family (3, 134, 175). Figure 4 is a computer analysis of amino acid sequences coding for complete transmembrane domains for some of the family members. When each of the twelve putative transmembrane domains for the proline transporter is used to search for sequences of at least 60% identity among the other proteins, a number of relationships are revealed. Note, that the analysis shown in Figure 4 is based on the entire transmembrane sequence of each domain (approximately 20 amino acids); if shorter sequences are considered, as those used to obtain clone-related cDNAs, even stronger regions of identity are present (3, 59, 63, 134, 175). The most highly conserved of these sequences occurs within the first eight transmembrane domains. Within the family of transporters from the brain, the order of the transmembrane sequences appears to be conserved, but if one looks for similar sequences in transporters from other tissues (e.g. Na^+-dependent glucose or nucleotide transporters from brush border), reshuffling of these segments has occurred.

The evolutionary relationship between the proteins will require more extensive analysis, but such a high degree of conservation suggests a common ancestor. Interestingly, tissue-specific conservation may have been a factor in this evolution, because computer analysis using these same sequences shows that they are not as highly conserved within Na^+-dependent transporters

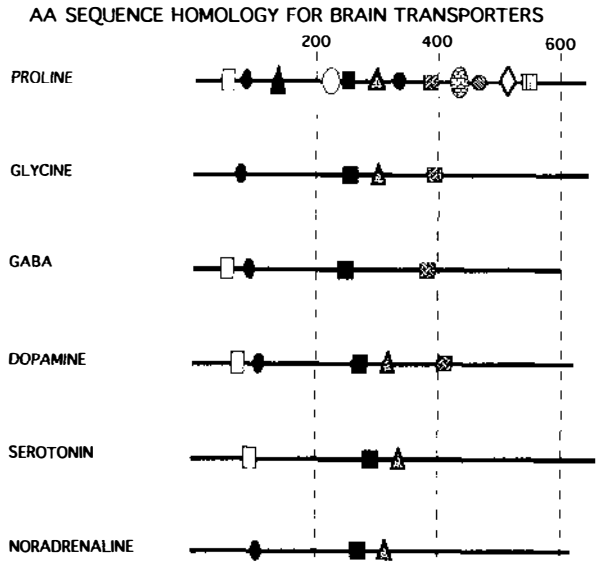

Figure 4 Conservation of transmembrane amino acid sequences for several Na^+-dependent transporters from the central nervous system. The deduced amino acid sequence was obtained for each transporter based on the published cDNA sequence. Using the HELIX MEM program (51) within PC-GENE (IntelliGenetics, Mountain View, CA), the putative 12 transmembrane spanning domains of the proline transporter were predicted in good agreement with those chosen by previous analyses (59). Each of these transmembrane segments was used to search for homologous sequences within the other transporters listed. Only when the amino acid identity was 60% or greater for the entire transmembrane sequence was the segment considered "conserved" and illustrated with a similar symbol.

present in other tissues. Evidence from radiation-inactivation studies suggests that Na^+-dependent organic solute transporters may exist as multiprotein complexes (9, 147). It has been suggested that transmembrane segment 2 for neuronal Na^+-dependent transporters may play a role in oligomerization of these proteins, because it contains a sequence similar to a leucine zipper (3). The leucine zipper motif has been documented within or near the voltage-sensing transmembrane domains of several ion channel proteins (106).

With specific regard to amino acid uptake, cDNAs coding for Na^+-dependent proline (59) and glycine (63, 140) transporters in the brain have been reported recently. At the present time it is unclear whether related glycine transporters are expressed in peripheral tissues. Northern analysis with one of the brain glycine transporter cDNAs revealed no detectable mRNA in spleen, kidney, or aorta, and only a weak signal in liver (140). Hepatocytes contain a glycine-specific Na^+-dependent transporter activity (31). Low

stringency screening of cDNA libraries or PCR amplification using the sequences obtained from the brain may serve as useful strategies to clone cDNAs for homologous transporters in other tissues.

IMPACT OF TRANSPORT ON METABOLISM

Hepatic Zonal Heterogeneity

Division of labor, a basic concept of interorgan biology, is also reflected within the hepatocyte population of the liver through "functional hepatocyte heterogeneity" or "metabolic zonation" (71, 85, 155). With regard to nitrogen metabolism, hepatocytes along the acinus have been divided functionally into two cell populations, periportal and perivenous. Periportal hepatocytes are nearest the portal venules and comprise the vast majority (90%) of the total hepatocyte population. Perivenous cells account for less than 10% of the hepatocytes and are localized as a ring of one to three cells around the terminal central venules (61, 70). Based on a limited number of reports, selective expression of amino acid transporters likely occurs within the two cell populations (19, 74, 148).

Perfusion of rat liver shows significant hepatocyte heterogeneity for glutamate or aspartate uptake (148, 154). These observations are consistent with reports that glutamate uptake is significantly decreased after destruction of perivenous hepatocytes by carbon tetrachloride treatment (72, 154) and that ^{13}N-glutamate administered via the portal vein in vivo is preferentially accumulated by perivenous hepatocytes (40). Although Na^+-independent glutamate uptake was shown to be equal for isolated hepatocyte suspensions enriched in periportal or perivenous cells, a small Na^+-dependent glutamate transport component was 6- to 7-fold higher in perivenous compared to periportal cells (19). Studies using isolated rat liver plasma membrane vesicles from either the sinusoidal or canalicular domain reveal a relatively slow rate of Na^+-dependent glutamate transport by the canalicular membrane only (5, 23). The weak Na^+-dependent glutamate uptake by the plasma membrane vesicles may be the result of localization to the perivenous hepatocytes, which probably contribute proportionally (10% of hepatocytes) to the isolated membrane vesicles. Consistent with the observation of Na^+-independent glutamate transport by the sinusoidal membrane (5), uptake of glutamate by the perfused rat liver appears to occur primarily by an exchange mechanism (74).

Glutamine synthetase is expressed primarily by perivenous hepatocytes, whereas glutaminase activity is greatest in periportal cells (61, 69, 166). This striking change in expression of glutamine-metabolizing enzymes along the liver acinus is consistent with the "Intercellular Glutamine Cycle," described

by Häussinger and his colleagues (74), which proposes net glutamine uptake by periportal cells and net glutamine release by perivenous cells. Apparently, glutamine extraction by the periportal hepatocytes largely involves Na^+-dependent System N (19, 54, 73, 90). In contrast, glutamine efflux from perivenous cells occurs via a facilitated transporter, (54, 127). W. W. Souba and his colleagues have recently obtained evidence for a Na^+-independent transport activity (tentatively termed System n) that exhibits the same narrow substrate specificity as the Na^+-dependent System N (unpublished data). If present in perivenous hepatocytes, this transporter may contribute to glutamine release. Isolated periportal and perivenous rat hepatocytes showed no difference in System N-mediated transport of histidine, but Na^+-independent uptake was higher in perivenous cells (19), consistent with the presence of either System L or System n.

Adaptive Regulation in Intestinal Brush Border

When faced with changing metabolite concentrations, cells can maintain their biochemical and physiological states by adjusting the rate of plasma membrane transport. This process was termed "adaptive regulation" and was originally used to describe increased activity of a specific transporter in response to starvation of substrates for that transporter (65, 138). However, adaptive regulation must be considered as a process capable of either up- or down-regulation in response to substrate availability, given the distinct regulatory differences among epithelial and nonepithelial membrane transporters (43, 87, 145). In general, a decreased extracellular concentration of certain amino acids leads to adaptive up-regulation of transporters in internal organs that maintain their own homeostasis and participate in interorgan nutrient flows. This process is exemplified by the induction of System A following substrate starvation. On the other hand, raising luminal amino acid concentrations induces adaptive up-regulation in epithelial cell apical membranes, as found in the small intestine or kidney. This control mechanism has been demonstrated for System y^+ and System B in enterocytes (M. Pan & B. R. Stevens, unpublished data). System B (formerly referred to as System NBB) is related to System $B^{o,+}$, as discussed above, and transports neutral amino acids. System B is expressed uniquely within the brush border membrane (Table 1; 102, 104, 146, 147).

In addition to absorbing organic nutrients for use by the whole organism, the intestinal mucosa utilizes its ready supply of amino acids for the tasks of Na^+ and water absorption (174), enterocyte volume regulation (100), and enterocyte nutrition. From the vantage point of the whole animal, intestinal transport represents the net vectorial transfer of amino acids from the environment to the body internal milieu, as coordinated by the asymmetric distribution of transporter species in the apical and basolateral membranes.

Table 1 Amino acid transport systems of the intestine

System	Membrane domain	Na^+-dependent	Typical substrates
B	Apical	Yes	Threonine, alanine
ASC	Apical	Yes	Alanine, glycine
IMINO	Apical	Yes	Proline, pipecolic acid
β	Apical	Yes, with Cl	β-Alanine
X^-_{AG}	Apical	Yes, with Cl	Glutamate, aspartate
A	Apical & basolateral	Yes	MeAIB
y^+	Apical & basolateral	No	Arginine, lysine
$b^{o,+}$	—	No	Lysine, leucine
L	Apical & basolateral	No	BCH, phenylalanine, leucine

Table 1 describes some of the well-studied membrane transport systems in enterocytes. Note that the basolateral membrane transporters are the same as those found in internal organ cells, while several of the apical membrane transporters are unique to this epithelial cell membrane. Kinetic analysis of intestinal transport illustrates that K_m values for a given amino acid are matched to those for transport by internal organs (18, 97). More detailed discussions of the kinetic mechanism and distribution of intestinal amino acid transporters are published elsewhere (45, 146).

The small intestine maintains an ontogenetically controlled baseline capacity to absorb amino acids in the starved state, and for many species this baseline value irreversibly declines over the course of the animal's development and maturity (17, 18). It is not clear whether constitutive expression of transporter proteins within the brush border is autonomously controlled or is maintained by localized regulatory signals from nutrients, growth factors, or hormones acting in a paracrine/autocrine manner. Nonetheless, when adult or infant epithelium is exposed to augmented amino acid concentrations, the absorptive capacity of the mucosa reversibly increases above the constitutive level, both acutely and chronically (87, 145). Physiologically, this adapative up-regulation prevents a precarious situation in which intestinal absorption would be the rate-limiting factor governing internal organ intermediary metabolism and the interorgan flow of amino acids. Thus, the intestine responds to increased luminal amino acid by maintaining a reserve absorptive capacity that exceeds the current dietary intake. Diamond (43) notes that this "safety margin" reserve for absorptive capacity only slightly exceeds dietary intake, as constrained by factors shaped by evolution, ecology, and cell energetics.

Down-regulation of intestinal amino acid transport is a return to the baseline absorptive capacity in the absence of a stimulating luminal nutrient. The decay

of transport activity occurs over a period of days as absorptive villus enterocytes are sloughed off and replaced by differentiating stem cells migrating up from the crypts. The transport activity in the membranes of these maturing replacement cells represents the future transport capacity (18).

Both acute trans-effects and de novo protein synthesis of transporters contribute to increasing the intestinal absorptive capacity. Amino acids can acutely alter intestinal transport by the cycloheximide-insensitive process of trans-stimulation (145, 146). For Systems B, ASC, and L, acute increased uptake occurs as the result of an exchange reaction across the membrane with substrates sharing the same transporter. Interestingly, System X^-_{AG} is stimulated by all of the neutral amino acids except glycine (101). On the other hand, the intestinal basolateral membrane presents a unique phenomenon in which low concentrations of leucine stimulate dibasic amino acid transport (94). Cheeseman (26) recently explained this as an "allosteric" property of System y^+.

Alternatively, enterocytes up-regulate transport capacity by inducing de novo synthesis of specific amino acid transporters or transporter-associated regulatory proteins such as those discussed above for System $b^{o,+}$. A single amino acid substrate can increase the expression of its transport activity by 2- to 10-fold, as demonstrated in vivo or in vitro (43, 130, 131, 146). Using cultured enterocytes, B. R. Stevens and colleagues (unpublished data) have shown that substrate-dependent up-regulation of specific membrane transporter systems is cycloheximide-sensitive and follows a 10–24 hr lag period. Interestingly, reports indicate that individual amino acids can induce transporter systems that do not mediate the uptake of that substrate (12). For example, in mice fed a diet supplemented with either aspartate or aginine, both Systems X^-_{AG} and y^+ were induced (44, 56).

CELL PROLIFERATION AND TRANSPORT

Growth Factors, Cytokines, and Transformation

A number of growth factors modulate amino acid transport. Boerner et al (13) used rat kidney cells to demonstrate that epidermal growth factor (EGF) stimulated Systems A and L, but not System ASC. EGF also causes a rapid, but transient 30–40% stimulation of transport in isolated hepatocytes through plasma membrane hyperpolarization (111). Longer exposure to EGF increases basal AIB uptake by hepatocytes (10) and suppresses the glucagon-dependent (cAMP-mediated) stimulation of hepatic AIB transport (109). Visciano & Fehlmann (162) reported that de novo protein synthesis was required for this EGF-dependent antagonism of glucagon action.

In vivo treatment with either interleukin-1 (IL-1) or tumor necrosis factor

α (TNFα) increased amino acid uptake by the liver (4, 115, 123), but neither IL-1 nor TNFα was effective with isolated hepatocytes in culture (123, 165). However, IL-6 alone does stimulate AIB uptake by isolated cells (4, 10). These data imply that the induction by IL-1 or TNFα in vivo is mediated via a secondary response, possibly involving release of other cytokines from nonepithelial cells within the liver. This conclusion is in agreement with studies of the regulation of acute phase protein synthesis by hepatocytes, which demonstrate that IL-6 acts directly (142), whereas TNFα and IL-1 do not (76). TNF administration in vivo may be accompanied by the release of hormonal mediators such as glucagon and glucocorticoid hormones that are known to stimulate Systems A and N. Pretreatment of rats with a glucocorticoid antagonist attenuates the TNF-stimulated increase in Na^+-dependent amino acid transport by approximately 50% (W. W. Souba et al, unpublished data).

Souba et al (144) have shown that, in contrast to hepatocytes, both TNF and IL-1 stimulate System ASC-mediated glutamine uptake by cultured porcine endothelial cells. The lag of 8–12 hr prior to induction of transport in the endothelial cells may reflect the time required for release of an autocrine-acting factor or, alternatively, for signal processing and de novo synthesis of transporter. The induction, one of the few examples of System ASC regulation, was prevented by blocking either RNA or protein synthesis. Although hepatocytes and endothelial cells exhibit cytokine-mediated increases in amino acid transport, the brush border of the intestine responds in an opposite manner. Studies in confluent monolayers of the human intestinal Caco-2 cell line indicate that interferon-γ decreases apical Na^+-dependent glutamine transport by about 30% (143). In contrast, TNF and IL-1 did not alter Na^+-dependent glutamine transport alone or in combination.

Transforming growth factor-β (TGF-β) suppresses the induction by IL-6 and inhibits basal hepatocyte AIB transport when it is administered alone (10). In contrast, TGF-β stimulates both Systems A and L activity in rat kidney cells (13). Extension of these studies on the regulation by cytokines and growth factors should provide considerable insight into the role that transport plays in cell and tissue growth.

The tumor-promoting compounds termed phorbol esters also alter System A–mediated transport. For example, Kitagawa et al (93) used 3T3 fibroblasts to demonstrate stimulation of AIB uptake following a 3-hr exposure to phorbol esters. Down-regulation of protein kinase C prior to tests for hormone induction of transport resulted in a decreased response to phorbol ester, platelet-derived growth factor (PDGF), or the calcium ionophore A23187 (93). These results suggest a common step in the mechanism of action, possibly protein kinase C activity. Boerner & Saier (15) showed that phorbol esters stimulate System A activity in Madin Darby Canine Kidney (MDCK) cells,

whereas other investigators have reported no effect of either phorbol esters or diacylglycerol on uptake by primary cultures of renal proximal tubular cells (60). Dawson & Cook (42) reported stimulation of System A transport activity and redistribution of protein kinase C to the plasma membrane following treatment of LLC-PK_1 cells with phorbol esters. Both of these processes were prevented by the actin filament disruptor cytochalasin B, but only the induction of System A activity was prevented by inhibiting protein synthesis with cycloheximide. These results suggest that activation of protein kinase C is only one step in a sequence of events by which phorbol esters increase amino acid transport.

Induction of System A–mediated amino acid transport in response to transformation has been reported by a number of laboratories. The activity of System A–mediated transport is increased following chemical transformation of MDCK cells (14), despite the fact that the parent and transformed MDCK cell lines grow at similar rates (53). These results permit distinction between the effect of transport on growth rate and transformation per se. One potential step of the transformation mechanism was illustrated by Leister et al (96), who showed that turnover of System A activity in chemically transformed C3H-10T1/2 cells was significantly slower than in the parental line. This change in stability might account for elevated transport activity in the transformed cells, if the synthesis rates are similar for both cell types. Unfortunately, absolute rates of turnover and synthesis must await identification of protein(s) responsible for System A transport.

Beginning with the pioneering work of Foster & Pardee (57) many laboratories have demonstrated that viral transformation of mammalian cells increases Na^+-dependent AIB uptake. Plasma membrane vesicles isolated from virally transformed cells retain the increased transport activity observed in whole cells (98, 118). Borghetti et al (16) demonstrated that the enhanced System A and ASC activities in SV40-transformed 3T3 cells remained elevated in revertant cells that had regained density-dependent inhibition of growth. Like the results of Erlinger & Saier (53) mentioned above, these data illustrate that one can separate the changes in transport activity due to transformation from those due to cell growth.

A temperature-sensitive SV40 mutant used to transform rat hepatocytes has permitted investigators to shift cells from the "normal" to the "transformed" state simply by changing the incubation temperature (26a). At the permissive temperature of 33C, SV40-transformed hepatocytes exhibited rapid cell division and reduced synthesis of liver-specific proteins, but at the restrictive-temperature of 40C, functional T-antigen was no longer expressed, cell division was considerably reduced, and liver-specific proteins once again were produced. System A transport activity was expressed at high levels in rapidly growing cells maintained at 33C, but transport was considerably reduced in

the slowly dividing cells (40C) (66). Transfer of the cells maintained at 40 to 33C resulted in both increased growth rate and transport activity over the following 72 hr. This induction of transport was prevented by protein synthesis inhibitors, and the elevated transport activity present in whole cells at 33C was retained when plasma membrane vesicles or reconstituted proteoliposomes were prepared (66). Collectively, the data suggest that transfer from 40 to 33C results in an increased de novo biosynthesis of a System A–associated membrane protein.

Cell Cycle and Regeneration

Sander & Pardee (132) monitored AIB uptake throughout the cell cycle in cultured cells. In early G phase a twofold increase in the transport rate, which preceded DNA synthesis by approximately 5–10 hr, was observed. The transport was elevated during the remainder of the cell cycle and returned to basal levels during mitosis. The relationship between System A transport and the cell cycle in vivo is suggested by monitoring AIB uptake following partial hepatectomy in the rat. A transient rise in AIB transport by whole tissue (163) or isolated hepatocytes (95) is observed 6–12 hr following 70% hepatectomy. This spike of System A activity following hepatectomy coincides with the initiation of cell replication and may reflect the increased transport observed during the cell cycle. It is likely that the normally quiescent hepatocytes in the liver remnant are initially synchronized by the signals that trigger regeneration. A second smaller peak in System A activity is usually detected 18–24 hr after the first one, and the diminished level of this induction probably reflects the gradual loss of cell synchronization. The return within 24 hr to a low basal rate of transport rather than the elevated levels typical of rapidly dividing hepatoma cells, despite continued liver growth, suggests that the transient elevation observed immediately following hepatectomy is not the same as the adaptation to an elevated growth rate that occurs following transformation. The increased transport activity following hepatectomy appears to reflect synthesis of a required System A-associated protein (47). Isolated plasma membrane vesicles from regenerating livers retained the increase in System A transport, and the specificity of the response was documented by the lack of any change in the activity of Systems ASC and N during the several days of liver regeneration (58).

AMINO ACID DELIVERY TO THE FETUS

Substrate Flux in Whole Animals

The placenta plays an integral role in the supply of nutrients to the developing fetus and serves as the interface between the maternal and fetal circulations

(139). Changes in the activity of specific placental amino acid transporters during the course of normal gestation remain largely unexplored (124). A number of studies have examined maternal and fetal serum amino acid levels at varying times throughout gestation (86, 108, 141), yet it is difficult to understand these data in terms of specific transporter activities. Placenta-specific transport systems have been the subject of a recent review (140), and therefore the present discussion focuses primarily on current concepts of transport regulation during normal gestation and in pathologic states. Fetal/maternal concentration ratios for most amino acids are greater than one, illustrating the concentrative transfer of specific amino acids (119).

The degree to which individual amino acids are transferred to the fetus depends on the level of expression and substrate competition for the numerous amino acid transport systems in basal and microvillous placental membranes. This supposition is supported by the classic observations of Christensen & Streicher (38), who noted in the guinea pig that high dietary intake of histidine, methionine, and proline resulted in a lower fetal/maternal ratio for glycine. These data imply competition between these amino acids for placental transporters. Recent reports showing competitive (proline, methionine) and noncompetitive (histidine) inhibition of System A (83) at the microvillous membrane, and competitive inhibition of System L (79) at the basal membrane, may account for the observed decrease in fetal glycine levels. Certainly, both placental and fetal metabolism also contribute to the steady state fetal/maternal ratios. Battaglia and coworkers utilized a fetal lamb model to demonstrate that glycine is avidly transferred from the maternal to the fetal circulation, where it is taken up by the fetal liver (105) and metabolized to serine (24). Interestingly, serine is then actively extracted from the fetal circulation. This observation is explained presumably by the presence of Na^+-dependent System ASC on the placental basal membrane (79).

The same group of investigators has similarly established the presence of a glutamine-glutamate cycle that transfers glutamine from the maternal to fetal circulation and subsequently to the fetal liver (105). Glutamine is metabolized to glutamate in the fetal liver and glutamate is then released into the fetal circulation to be transferred back to the placenta. The presence of Na^+-dependent System N in both placental microvillous membranes (88) and fetal hepatocytes (156) probably mediates the net transfer of glutamine to the fetus, whereas basal membrane localization of Na^+-dependent System X_{AG}^- activity allows transport of glutamate from the fetal circulation to the placenta (78). This glutamine/glutamate cycle represents a mechanism to shuttle nitrogen into the rapidly growing fetus, whereas the importance of the serine/glycine pathway is less obvious.

Impact on Fetal Development

Placental amino acid transfer during intrauterine growth retardation (IUGR) has been studied extensively. Several authors (25, 49, 75) have compared serum amino acid levels in IUGR infants to those from normal pregnancies; in general, levels of gluconeogenic amino acids are increased in IUGR fetuses, compared to those for "essential" amino acids. Rosso (125), and more recently Varma & Ramakrishnan (161), demonstrated decreased rates of AIB transfer to the fetus during IUGR in the malnourished rat dam. Similar findings have been reported in IUGR guinea pigs (82, 129). Ahokas et al (2) reported that placental transfer of AIB to the fetus was reduced in rat dams fed a diet that contained 50% less calories. Given that only a portion of these changes can be attributed to diminished placental blood flow (112, 126), they imply altered rates of intrinsic placental amino acid transport. Dicke & Henderson (45) prepared placental microvillous membranes derived from human IUGR pregnancies in which no prior risk factors for IUGR were known to exist. Rates of AIB transport were significantly decreased in this group, as compared with vesicles derived from normal pregnancies, and provided direct evidence for a reduction in amino acid transport during IUGR pregnancies. Collectively, the available data point to an inherent defect in placentofetal transfer in many examples of IUGR, but further work is required to delineate the molecular basis for these observations.

SUMMARY

During the last four decades, mammalian amino acid transport systems have been described at the cellular level through general properties such as ion-dependence, kinetics, substrate specificity, regulation of activity, and numerous other characteristics. These studies have allowed the definition of multiple transport systems for neutral, anionic, and cationic amino acids. Each system is distinct but exhibits overlapping substrate specificity. Direct measurement of transport has permitted a wealth of information to be accumulated regarding the regulation of overall activity, but the underlying molecular mechanisms have not been investigated because of a lack of the appropriate tools. Recent research designed to obtain these tools has proven fruitful, and the field of amino acid transport clearly is entering a new era. In the immediate future, transporter properties such as hormonal regulation, adaptive control, ion-dependence, and trans-effects will be studied at the molecular level by assaying mRNA or protein content and by analyzing results obtained with altered protein structures following site-directed mutagenesis.

Identification of specific proteins associated with activities already well

described will provide answers to heretofore untestable questions. For example, is Na^+-independent transport mediated by the same proteins that mediate Na^+-dependent uptake except that their function in this mode does not require sodium binding? What is the protein composition of amino acid transporters? As discussed above, emerging evidence suggests that transporter proteins have different molecular structure, 12 versus 1 transmembrane domains, or that they exist as heterodimers or heterotetramers. Identification of certain transporter proteins and cloning of the respective genes also will provide valuable information about a number of inheritable diseases that are thought to be caused by defects in transporter synthesis or function. The opportunity to ask these questions will certainly generate renewed interest in the field of amino acid transport and lead to exciting advances in our knowledge.

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