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MECHANISMS OF TRANSPORT OF AMINO ACIDS ACROSS MEMBRANES

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INTRODUCTION

The cellular requirements for amino acids for protein synthesis and metabolism must be met by a combination of the biosynthesis of some of the amino acids and the transport of amino acids into the cell. The transport process is considered a step in amino acid metabolism, and may be subject to regulation to meet the nutritional needs of the cell. The work of Sips et al (61) has demonstrated that the transport of alanine, at physiological concentrations, is the rate-limiting step in alanine metabolism in hepatocytes. Through a quan-

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titative analysis, Salter et al (53) have shown that the transport activity of tryptophan and other aromatic amino acids participates in controlling the metabolism of these amino acids in hepatocytes. In such cases, regulation of transport processes can serve to regulate amino acid metabolism. Alterations in nutrient transport often accompany various cellular changes such as malignant transformation, differentiation, and the proliferative state of the cell, possibly responding to and participating in the control of the altered nutritional and metabolic needs of these cells.

There are numerous agencies for the transport of amino acids across cell membranes. Oxender & Christensen (48) first identified distinct systems for the transport of neutral amino acids into Ehrlich mouse ascites tumor cells. The isolation of transport components for amino acid transport in mammalian cells has been limited by the difficulties of working with integral membrane proteins and the apparently low expression of the transport proteins. Another limitation has been the lack of high-affinity specific substrates that can be used to label and identify specifically the amino acid transport components. Consequently, the transport systems have primarily been identified, and the regulation of these systems studied, by kinetic means. Through these approaches, a number of distinct systems have been identified. In this review, we discuss the current status of the characterization of amino acid transport systems and the regulation of their activity.

Some amino acids, particularly the neutral amino acids, are transported by more than one system. The overlapping substrate specificities and the multiplicity of systems complicates the study of transport and its regulation. A complete understanding of transport will require the reconstitution of purified transport components. Recent advances have been made in labeling and identifying transport components to achieve this end. Genetic approaches to the study of amino acid transport have also been applied in attempts to obtain mutants with altered transport properties. In this review, the recent biochemical and genetic advances are also discussed.

CHARACTERIZATION OF AMINO ACID TRANSPORT SYSTEMS

Characterization and discrimination of amino acid transport systems began with studies on the Ehrlich cell (48). Since then, systems for neutral, cationic, and anionic amino acids have been described in various cell types. Several of these systems are able to regulate activity in response to cellular nutritional needs.

Neutral Amino Acid Systems: Na^+ Dependent

Based on competition experiments, Oxender & Christensen (48) first demonstrated the existence of two distinct neutral amino acid transport systems in

the Ehrlich cell. These were designated System A and System L. System A serves mainly for the uptake of amino acids with short, polar, or linear side chains, although many of the other amino acids have a small component of their total uptake provided by System A. System A is characterized by its Na^+ dependence, its reduced activity at low extracellular pH (48), and its tolerance of N-methylated substrates such as the nonmetabolizable substrate, 2-methylaminoisobutyric acid (MeAIB) (15). The presence of intracellular substrates of System A slows the uptake of amino acids by this system, a kinetic phenomenon of the transporter known as *trans*-inhibition (9). System A has been described in a wide variety of cells (31). System A is subject to significant regulation by amino acid availability and by hormones.

A second Na^+-dependent system for the uptake of neutral amino acids was discovered in the Ehrlich cell when MeAIB failed to inhibit alanine uptake completely (13). This agency, named System ASC, is reactive with alanine, serine, cysteine, and homologous amino acids up to five carbons, and the prolines (13). It is distinguished from System A by its intolerance to N-methylated substrates and its different pH sensitivity. Intracellular substrates of System ASC increase the activity of the system (*trans*-stimulation; 9). In most untransformed cell types, System ASC is the major Na^+-dependent system (27, 58).

As the pH of the medium is reduced below 6, System ASC becomes more effective for the transport of anionic amino acids and less effective for zwitterionic amino acids in all cell lines tested (64). This activity corresponds to System x^-_A for anionic amino acids, described below. The increased reactivity of these amino acids with System ASC is not due to an increase in the zwitterionic form of the amino acids; rather, it appears to represent a change in the protonation of the carrier (41), altering its specificity.

In hepatocytes and related cell lines, there is another Na^+-dependent system for the uptake of glutamine, asparagine, histidine, and related analogs (36). This system has been designated System N to indicate the presence of nitrogen in the side chain. System N may be unique to liver cells since it has not been fully characterized for other cell types (35). System N is subject to regulation by amino acid availability, the only Na^+-dependent system apart from System A to show regulation (36).

In hepatocytes and the hepatoma cell line, HTC, glycine uptake is mainly mediated by a Na^+-dependent system distinct from A and ASC, as determined by inhibition experiments (10).

Neutral Amino Acid Systems: Na^+ Independent

This system was one of the first characterized as one of two distinct mediators of neutral amino acids in the Ehrlich cell (48). System L is most reactive with branched-chain and aromatic amino acids, and is subject to *trans*-stimulation. The system can be easily characterized with the nonmetabolizable, system-

specific substrates 2-aminobicyclo-[2,2,1]-heptane-2-carboxylic acid (11) and 3-aminobicyclo-[3,2,1]-octane-3-carboxylic acid (12). System L is present in a wide variety of cell types (31). A recent report demonstrated leucine-proton cotransport, which suggests that System L may be dependent on the proton gradient (43). System L can also regulate its transport activity in response to the availability of System L amino acids (60). In hepatocytes, two Na^+-independent transport systems for neutral amino acids have been identified, designated L1 and L2 (66). L2 is a low-affinity, high-capacity system that is relatively active in freshly isolated cells but declines during 24 hours in cell culture. L1 is a high-affinity, low-capacity system that has minimal activity in freshly isolated cells and increases in activity upon time in culture. This increase in activity can be blocked by cycloheximide or actinomycin D, which suggests that mRNA and protein synthesis are necessary. System L1 appears to serve for a larger number of substrates than System L2 (66).

Human red blood cells have a Na^+-independent system specific for the aromatic amino acids, tryptophan, phenylalanine, and tyrosine (52). This system has been designated System T to indicate its tryptophan preference. A recent report suggests a System-T-like activity may be present in hepatocytes (54).

A Na^+-independent system with ASC-like specificity (designated asc) has also been observed in pigeon nucleated red blood cells and in sheep and horse red blood cells (65). Since the nucleated red blood cell is analogous to the nucleated precursors of mammalian red blood cells, it is speculated that this system may exist early in red blood cell maturation, persisting in the mature blood cell only in some species, such as the horse and sheep (65).

Cationic Amino Acid Systems

The transport of cationic amino acids has recently been reviewed (67). A system has been found in Ehrlich cells, human fibroblasts, and other cell types that recognizes positively charged amino acids; it is referred to as y^+ (68, 70). System y^+ is Na^+ independent, pH insensitive, and subject to *trans*-stimulation. System y^+ is barely detectable in isolated hepatocytes, but is present in hepatoma cells (68). Transport of arginine into hepatocytes is the rate-limiting step for arginine metabolism (68). The near absence of the y^+ system in normal hepatocytes serves as a barrier between intracellular arginine and plasma arginine, since intracellular arginine is completely hydrolyzed by arginase to allow the urea cycle to function. This separation may be necessary to assure proper arginine flow to other tissues. The appearance of System y^+ and the loss of arginase in the hepatoma cell may be critical steps in successful transformation of hepatocytes (66).

Anionic Amino Acid Systems

Fetal hepatocytes and hepatoma cells have a Na^+-dependent transport system specific for short anionic amino acids, such as aspartate and cysteate. This system has been designated x^-_A to indicate the preference for asparate (40). These cells also possess a Na^+-independent route for glutamate and cystine (14). This system has been designated x^-_C for its cystine preference (40). System x^-_C can also be seen in fibroblasts (1).

There appears to be a change in the major anionic acid transport systems upon differentiation of hepatocytes. In mature hepatocytes, the Na^+-independent x^-_C system is minimal; only upon insulin treatment does it become the main route for cystine entry (62). A system that accepts short anionic amino acids and glutamate is the major Na^+-dependent transport component in mature hepatocytes, replacing x^-_A. This system has been designated x^-_{AG} for its preference for aspartate and glutamate (40). System x^-_{AG} has also been observed in fibroblasts (17). It is not known if x^-_A and x^-_{AG} are the same system, having altered activity depending upon the differentiated state of the cell (14). In the transformed hepatoma cell line, the fetal pattern of transport systems reappears (14).

As indicated previously, anionic amino acids are taken up by System ASC at reduced pH. It has been suggested that System ASC and System x^-_A are really one system, existing in different protonated forms (14, 41).

REGULATION OF AMINO ACID TRANSPORT

System A

Neutral amino acid transport System A is regulated by the availability of amino acids in the extracellular environment. In 1972, it was first observed by Riggs & Pan (51) in an immature uterus preparation and by Gazzola et al (28) in chick embryo heart cells that starvation for all amino acids by incubation in amino-acid-free medium increased neutral amino acid transport activity. The increase in activity was shown to be specific for System A by its Na^+ dependency, pH sensitivity, and substrate specificity. This increase in activity is referred to as adaptive regulation, or starvation-induced enhancement, and is distinct from *trans* effects (28). The addition of even a single amino acid that is primarily transported by System A to the medium prevented the increase (28). Adaptive regulation has been seen in many cell types (31). Kinetic analysis has generally shown the increase in System A activity in the cells tested to be attributable to an increase in the V_{max} of uptake with little change in the K_m. Using inhibitors of macromolecular synthesis, researchers demonstrated that both RNA and protein synthesis were necessary to observe the starvation-induced enhancement (28, 51). Tunicamycin also blocked starvation-induced enhancement in rat hepatocytes, which suggests that a

System A–associated protein synthesized upon starvation is a glycoprotein (2). Enhancement of System A activity in fibroblasts could be reversed by adding a System A amino acid to the medium. The reversal was also shown to require protein and RNA synthesis (27).

Initially, it was thought that only substrates of the transport system would prevent starvation-induced enhancement (27). It was proposed that the extracellular concentrations of System A amino acids regulated the expression of transport protein genes, through a signal generated upon the binding of System A amino acids to the transport protein (27). However, more recent studies have shown that in some cells, such as the Madin-Darby canine kidney epithelial cell line (5) and Chinese hamster ovary (CHO) cells (44), there is not necessarily a correlation between competition of transport activity and prevention of adaptive regulation. For example, in CHO cells, β-alanine did not inhibit System A activity, but it did prevent starvation-induced enhancement. Alternatively, diaminobutyric acid inhibited transport through System A, but did not prevent starvation-induced enhancement (44). This evidence suggests that the signal for repression is not generated by the substrate binding to the transport protein, but may be generated by changes in intracellular amino acid concentrations through a molecule having similar, but not identical, specificity to the transporter (34, 44, 45, 69). In hepatocytes, no amino acids or analogs have been found to repress System A that did not show inhibition of substrate activity (7). There may be cellular differences in substrate regulation of transport activity.

To observe the enhancement of System A in fibroblasts, serum must be present in the medium (26). The addition of serum to starved fibroblasts caused an immediate increase in the V_{max} of System A activity, which was not inhibited by cycloheximide. These results suggest that the increase is caused by a post-translational modification of the carriers. Insulin stimulation of glucose transport is thought to occur by the translocation of intracellular vesicles containing glucose transporters to the plasma membrane (16). A similar mechanism may be responsible in part for the regulation of amino acid uptake (26).

Englesberg and coworkers (e.g. 44) have been using genetic approaches to study the regulation of amino acid transport System A in CHO cells. They have isolated a mutant of System A that has elevated and unregulated System A transport activity. These cells were fused to normal CHO cells that show adaptive regulation of System A. The hybrid cell lines were able to regulate System A transport activity. These results suggest that the transport contributions from each of the starting cell lines could be regulated by a *trans*-acting element donated by one parental line (44).

Higher organisms need to control the metabolism of amino acids in different tissues and the flow of amino acids to and from these tissues. Hormonal

regulation of these processes includes effects on transport activity. Most of the hormonal effects on transport appear to be on the activity of System A. The effects of a few hormones are described here; additional information is described in recent reviews (31, 59).

Insulin is an anabolic hormone, enhancing protein synthesis in target cells as well as increasing glucose transport activity. Insulin increases System A activity in many cell types (31). The increase in activity is usually accompanied by an increase in the V_{max} of uptake, with little change in the K_m. Most of the insulin effect was inhibited by cycloheximide in chick embryo heart cells (32) and rat hepatocytes (38), an indication that regulation is dependent on protein synthesis. Insulin also caused a cycloheximide-insensitive enhancement in System A activity in chick heart embryo heart cells (32) and kidney epithelial cells (4), which suggests that insulin serves to protect the transport proteins from degradation. Insulin stimulation of System A in rat hepatocytes was shown to be dependent on RNA synthesis as well as on protein synthesis (38).

Glucagon acts to increase blood glucose by breaking down glycogen and accelerating gluconeogenesis. Most System A substrates are gluconeogenic precursors. Glucagon increases the System A transport activity in hepatocytes (37). Most of the glucagon-stimulated System A activity in hepatocytes appeared to require RNA and protein synthesis (24). That stimulation was also blocked by tunicamycin (2) suggests that the synthesis of an asparagine-linked glycoprotein is necessary. Tunicamycin added after glucagon induction had started prevented further induction, which suggests that the glycoprotein must be continually synthesized (2). In some cases the effect can be mimicked by dibutyryl cAMP (37), so glucagon stimulation is probably mediated by cAMP. The increase in System A activity was maintained in plasma membrane vesicles prepared from glucagon-stimulated hepatocytes (56) and from dibutyryl cAMP-stimulated hepatocytes (55); this supports the hypothesis that the increase in activity is the result of an increase in a membrane-associated protein.

Regardless of whether the increase in System A activity is caused by amino acid starvation or hormonal induction, the repression of activity upon addition of amino acids to the medium appears to follow the same course (33). When isolated hepatocytes from glucagon-injected rats were placed in amino acid–containing medium, most of the repression of System A activity was dependent on the synthesis of RNA and protein (33). A model based on these results proposed that hormones or amino acid starvation stimulate RNA and protein synthesis, which leads to an increase in System A. When the hormone is removed, or amino acids are supplied, the synthesis of mRNA and protein, induced by an unknown signal, is necessary to inactivate transport activity (33).

The effects of many of the other hormones on transport activity are also attributable to alterations of System A activity. Other effectors include growth factors (such as epidermal growth factor, nerve growth factor, and platelet-derived growth factor), catecholamines, steroids, and glucocorticoids (31, 59).

Other factors also alter the activity of System A. An increase in cell density results in a decrease in both Systems A and ASC in several cell types (3, 6). These alterations may be the result of a change in the Na^+ gradient or the membrane potential. Transformed cells showed an increase in Systems A and ASC (6). Transformed Madin-Darby canine kidney epithelial cells lost the ability to regulate System A after transformation, showing continuously elevated transport activity (5).

System L

Starvation of cells for all of the amino acids did not lead to an increase in System L activity (29). We have, however, been able to observe regulation of System L in the CHO mutant *ts*H1 (60), which has a temperature-sensitive leucyl-tRNA synthetase (63). At elevated temperatures, CHO-*ts*H1 is starved for leucine because of its inability to charge leucyl-tRNA. When CHO-*ts*H1 cells are incubated for six hours at 39°C, an increase in the V_{max} of leucine uptake by System L was seen, with little change in the K_m (60). When the cells were returned to 34°C System L transport activity returned to normal levels. The increase could be prevented by cycloheximide, but not by actinomycin D at early stages, which suggests that System L is regulated at the level of translation (60). Earlier studies in our laboratory showed that *Escherichia coli* leucine transport activity is regulated by the intracellular level of leucyl-tRNA (47). A similar mechanism may exist in mammalian cells. Regulation of System L can also be seen in CHO cells with normal leucyl-tRNA synthetase activity, such as the CHO-K1 cell line (46). However, the leucine concentration must be 10 μM or lower to observe the increase. Starvation for other System L amino acids, such as valine, isoleucine, and phenylalanine, also caused an increase in System L transport activity, but the increase was only about half of that seen upon leucine starvation. The reason for this special role of leucine in System L regulation is not yet known (46).

Genetic approaches have been applied to the study of regulation of System L. Shotwell et al (57) isolated mutants of CHO-*ts*H1 that are defective in the regulation of System L. Mutagen-treated CHO-*ts*H1 cells were selected for their ability to survive at 37–39°C on reduced concentrations of leucine. These temperature-resistant cell lines showed elevated and unregulated leucine transport activity. The increase was attributible to an increase in the V_{max} of System L activity. The temperature-resistant cell lines still had a temperature-sensitive leucyl-tRNA synthetase, as determined by in vitro

assays. It was concluded that these cells had a defective regulatory element controlling System L transport activity, which led to constitutively elevated transport activity. The increased activity led to increased intracellular levels of leucine and other System L amino acids that complemented the synthetase defect (57). The fusion of one of these regulatory mutants to a temperature-sensitive leucyl-tRNA synthetase mutant that can regulate System L activity resulted in hybrids that were able to regulate System L activity. This evidence suggests that regulation of System L may involve a *trans*-acting factor (E. J. Collarini and D. L. Oxender, unpublished results).

System N

As described earlier, System N has only been fully characterized in hepatocytes and related cells. Upon amino acid starvation, cultured hepatocytes or hepatoma cells showed a doubling in glutamine uptake (36). The increase in System N depends on protein synthesis. Unlike System A, System N generally does not increase in response to insulin or glucagon in vitro (36).

BIOCHEMICAL APPROACHES TO TRANSPORT

The complete understanding of the mechanism of transport will require the isolation, purification, and reconstitution of the transport components into phospholipid vesicles. In the past, several laboratories have achieved solubilization and reconstitution of amino acid transport from Ehrlich cells, but these efforts have not yielded significant purification. In our laboratory, membrane proteins from Ehrlich cells were solubilized with detergents, and the protein fractions reconstituted into liposomes. Functional transport could be measured in the liposomes and thereby provided an assay for following transport activity (8). McCormick et al (42) found that membrane vesicles formed in the presence of K^+, then exposed to a freeze-thaw step, had high levels of Na^+-dependent amino acid transport. The vesicles are enriched in a 125-kDa protein. It is not yet known if this protein is a component of a transport system (42).

Wright & Peerce (71) have made significant progress in labeling transport components. They have used group-specific labeling reagents to identify the Na^+-dependent proline transporter from rabbit intestine (71). Proline is transported by the brush border tissues of the intestine, kidney, and choroid plexus by a Na^+-dependent system, distinct from Systems A and ASC. Proline uptake is inhibited by phenyl isothiocyanate (PITC) and fluorescein isothiocyanate (FITC). The proline transport system can be protected from irreversible inactivation by PITC or FITC if proline and Na^+ are present. The proline transporter was selectively labeled by first protecting membrane vesicles with proline and Na^+ and labeling nonspecific sites with PITC, then

removing the substrates and labeling with FITC. Vesicles labeled in the presence or absence of substrates were solubilized and run on SDS gels. A 100-kDa band was specifically labeled and identified as the proline transporter (71). Similar studies have been carried out to identify and characterize the Na^+-dependent glucose transporter from rabbit intestine (50). Labeled group-specific reagents should prove useful in the isolation and the identification of membrane transport components for other systems. This technique bypasses one of the major obstacles of identifying amino acid transport proteins in that a specific, high-affinity substrate for the system is not necessary. All that is needed is a chemical modifying agent whose action can be inhibited by a substrate of the transport system.

GENETIC APPROACHES TO TRANSPORT

The isolation and characterization of mutants in amino acid transport systems in microorganisms has been extremely useful in identifying the components and mechanisms of transport processes. Several laboratories have recently applied genetic approaches to the study of amino acid transport in mammalian cells (22). The availability of mutants in individual transport systems greatly facilitates the study of the physiological role of transport in the metabolism of amino acids in cells. In addition, mutants are important for cloning the genes encoding transport components. Mutants have been obtained for the major neutral amino acid systems and are reviewed here.

Moffett & Englesberg (44) have isolated mutants in CHO-K1 cells for System A by taking advantage of the fact that CHO-K1 is a proline auxotroph. The cells are defective in one of the enzymes for proline biosynthesis and, therefore, require proline for growth. Mutagen-treated CHO-K1 cells were selected for growth on high concentrations of alanine, which inhibits proline uptake and is therefore toxic. Alanine-resistant mutants showed increased levels of proline uptake (44). One class of mutants was described earlier as being defective in regulation of System A. Another mutant showed an increase in proline transport, but the transport activity was still subject to regulation. Both the K_m and V_{max} of proline uptake were changed in this mutant; therefore the cells may be mutated in one of the transport components rather than in a regulatory element. All of these mutants showed changes in other transport systems as well as in System A (pleiotropic effects). System ASC and a newly characterized System P, a relatively minor Na^+-dependent component in normal CHO cells, showed increased activity (44). A change in more than one transport system, when selecting for mutants in only one system, suggests that the affected systems may share components. Based on the mutational studies, Englesberg proposed that the three systems share a common component. He also proposed that a regulatory repressor molecule,

which requires a corepressor (not necessarily a System A substrate), regulates the synthesis of mRNA for Systems A, ASC, and P proteins (44, 45).

An enrichment of transport proteins as the result of gene amplification can greatly aid the isolation of transport components. Englesberg and coworkers have attempted to obtain amplification of System A activity in the mutants that showed increased System A activity. Using increasing concentrations of alanine in a stepwise selection, they have obtained alanine-resistant lines with an approximately 30-fold increase in System A activity. Two membrane protein components showed increased expression, possibly being related to System A transport (E. Englesberg, personal communication).

Ertsey & Englesberg (23) isolated a MeAIB-resistant mutant from the CHO-K1 cell line that shows an increase in System ASC only. It has been characterized as a recessive regulatory mutant.

There are advantages in also obtaining mutants with a reduction or loss of transport activity. Such mutants can be used to examine the role of transport on the physiology of the intact cell. In addition, if cell growth can be limited by a reduction or loss of transport activity, it may be possible to complement the defect by introducing genes from another species. This would provide a means of isolating the genes coding for transport activity from another species.

Mutants with reduced activity of System A have been isolated by Adelberg and coworkers (25). Mutagenized mouse lymphocytic cells were exposed to ^{3}H-2-aminoisobutyric acid (AIB), a System A substrate, then frozen and stored in liquid nitrogen. Under these conditions, the cells with normal levels of transport activity will take up ^{3}H-AIB and be killed by exposure to internal radiation. Those cells with reduced transport activity will take up less of the radioactive substrate and survive upon thawing. This procedure is known as a tritium-suicide selection. One of the surviving cell lines showed a reduction in the V_{max} of System A activity (25). In another surviving cell line, the V_{max} of System A activity and the V_{max} of glutamate uptake were both reduced (18). Using a similar selection with ^{3}H-proline, Adelberg and coworkers obtained a spontaneous mutant from CHO cells with a decrease in the V_{max} of System A activity (20).

Adelberg and coworkers have also isolated mutants with reduced System L activity (19). Mutagenized CHO cells were grown on limiting leucine so that the cells with reduced System L transport activity would grow slowly. The cells were then exposed to 5-bromodeoxyuridine, which is incorporated into DNA in actively growing cells. Upon irradiation, the normal cells were preferentially killed, enriching the population in cells with defective leucine uptake (19).

We have used a tritium-suicide selection to obtain mutants in System L (49). As mentioned in an earlier section, we had available mutants with

constitutively elevated System L transport activity (57). These cells were used as the starting cell line for a ^{3}H-suicide selection using ^{3}H-leucine as the substrate. Mutagen-treated cells were grown in medium containing five times the normal concentrations of eight of the essential amino acids that are transported mainly by System L to ensure survival of transport-defective cells. The cells were then exposed to ^{3}H-leucine in sodium-free buffer for three minutes. The cells were frozen and radiation damage was allowed to accumulate for three to five days. After thawing, survivors were assayed for leucine transport activity. Several cell lines showed a specific reduction in System L transport activity. These transport-defective mutants were still defective in the regulation of System L activity, and their growth was limited by low leucine concentrations because of the temperature-sensitive leucyl-tRNA synthetase from the starting cell line (49). These transport mutants are being used to attempt to clone the transport genes from human cells.

Cloning Human Amino Acid Transport Genes

DNA containing potential genes can be introduced into transport mutants in several ways. In our laboratory, CHO-*ts*025C1 cells were fused to freshly isolated, peripheral human blood leucocytes. CHO-*ts*025C1 cells have a temperature-sensitive leucyl-tRNA synthetase and cannot grow at 39°C (30). Since the unstimulated human leucocytes are primary cells, they cannot grow in culture. The resultant hybrid cells were grown at 39°C to obtain hybrids that were temperature resistant. Some of these cells showed increased System L transport activity, which suggests that they had acquired human genes for transport System L (39). The hybrids that had increased leucine transport also had retained human chromosome 20; hence this chromosome is the location for transport System L. A hybrid that had acquired two copies of human chromosome 20 showed the expected further increase in System L activity that would result from a gene dosage effect (39).

Human sequences were also introduced by direct transformation of CHO-*ts*025C1 cells with a human DNA cosmid library cloned into a vector carrying resistance to the antibiotic G418 (21). Transformants were first selected in the presence of G418, and then at low leucine concentrations at the nonpermissive temperature of 39°C. Transformants were isolated with increased leucine transport activity, which may mean they acquired the human gene for System L transport. Attempts are now being made to rescue the human DNA fragments that code for the human System L transport components (21).

CONCLUDING REMARKS

Over two decades of research have demonstrated that essentially all types of cells contain multiple transport systems for amino acids. The discrimination of distinct systems and the characterization of these systems has been carried

out using kinetic and competitive inhibitor analysis. Some of these systems are subject to regulation by either nutrient requirements or by hormones. It has been shown that the regulation of amino acid transport can play an important role in regulating the metabolism of specific amino acids, such as the metabolism of alanine in hepatocytes.

Recent success in specific fluorescent labeling of transport components by Wright and coworkers has helped identify the intestinal proline transporter. This technique should also be useful in the identification of other amino acid transport components. Finally, several laboratories are now successfully using genetic approaches to aid in the identification of amino acid transport components in Chinese hamster ovary cells. One outcome of this approach suggests that transport Systems A and L are regulated by *trans*-acting components. The technique of gene amplification is also proving useful for identifying transport components. The application of these techniques will greatly aid in the identification and isolation of transport components, which will in turn greatly increase our knowledge of the molecular aspects of transport in the near future.

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