Regulation and Genetics of Amino Acid Transport

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The major transport systems for the uptake of neutral amino acids in mammalian cells have been designated A, ASC, and L.[1,2] The transport systems and their regulation have been characterized in Chinese hamster ovary (CHO) cells.[3,4] System A is sodium-dependent, subject to *trans*-inhibition, and serves for the uptake of amino acids with short, polar, or linear side chains. System ASC is also sodium dependent and has a strong preference for alanine, serine, and cysteine. In the CHO cell, the ASC system shows a somewhat broader specificity than that found in the Ehrlich cell.[2] Unlike System A, System ASC does not tolerate *N*-methylated substrates such as 2-methylaminoisobutyric acid (MeAIB). System L is sodium-independent and serves for the uptake of branched-chain and aromatic amino acids. We operationally define the systems as follows: System A can be represented by the sodium-dependent uptake of 0.2 m*M* 2-aminoisobutyric acid (AIB) that is inhibited by 25 m*M* MeAIB; System ASC, the sodium-dependent uptake of 0.2 m*M* L-alanine that is not inhibited by 25 m*M* MeAIB; and System L, the sodium-independent uptake of 0.2 m*M* L-leucine that is inhibited by 10 m*M* 2-aminobicyclo-[2,2,1]-heptane-2-carboxylic acid (BCH).[3]

Although the systems have a preferred set of substrates, they do have overlapping specificities. FIGURE 1 shows the contributions of these systems to the uptake of individual amino acids in CHO-K1 cells. This overlap makes the study of transport systems in isolation difficult. The availability of mutations in one or more of the transport systems would greatly facilitate the study of the function and regulation of the transport systems. We are currently combining genetic approaches with kinetic studies using CHO cells because of the relative ease with which mutants can be obtained from these cells. Furthermore, CHO cells can be used to form interspecies hybrids with human cells.[5] The hamster-human hybrid cells preferentially segregate the human chromosomes, permitting the assignment of a phenotype to a particular chromosome. In the present study, we have isolated and characterized CHO mutants defective in the regulation of System L[6] and mutants with reduced System L transport activity. We have also used hamster-human hybrids to map System L transport activity to human chromosome 20.[7]

MATERIALS AND METHODS

Cell Lines and Culture Methods

The CHO-K1 and CHO-tsH1 cell lines were obtained from Dr. L. H. Thompson of the Lawrence Livermore Laboratory, Livermore, California. These cell lines were maintained as described previously.[3,6] The temperature-resistant cell lines C11, C11B6, D10, and F10 were isolated in our lab and maintained as described previously.[6] The transport mutant cell lines C5, C5F6, C9, and D3 were maintained in Eagle's minimal essential medium (MEM) containing Earle's salts and nonessential amino acids, supplemented with 5% (vol/vol) fetal calf serum (KC Biological, Lenexa, KS),

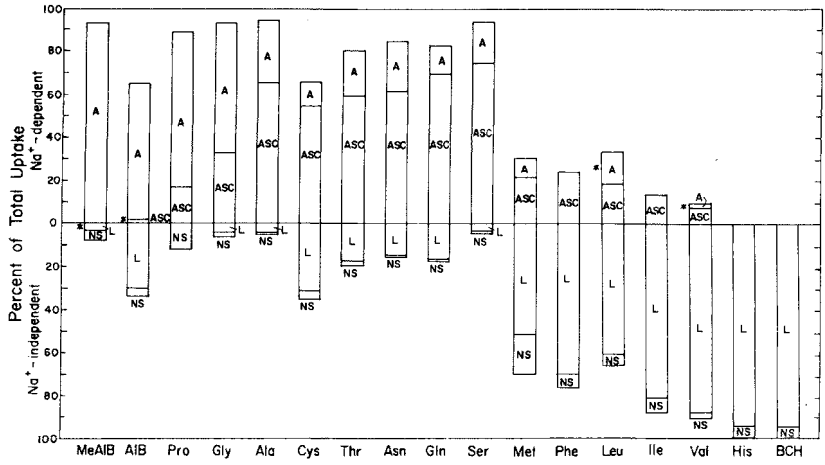

FIGURE 1. Total uptake of neutral amino acids in CHO-K1 cells divided into contributions by the three transport systems. Cells were plated and grown in MEM at 37°C as described in Materials and Methods. The cells were then assayed for the uptake of 0.2 m*M* of the indicated amino acid for one minute at 37°C. The total uptake was divided into the individual transport components as described previously.[3] The components are expressed as a percentage of the total uptake in Na^+-containing buffer. Asterisks indicate statistically insignificant values. NS, nonsaturable component. The values are the averages of three determinations. (Reprinted from Shotwell *et al.*[3])

2.2 g/l $NaHCO_3$, and with the following eight amino acids to the indicated final concentrations: 1 m*M* L-histidine, 2 m*M* L-isoleucine, 2 m*M* L-leucine, 0.5 m*M* L-methionine, 1 m*M* L-phenylalanine, 0.25 m*M* L-tryptophan, 1 m*M* L-tyrosine, and 2 m*M* L-valine. This medium is referred to as 5xMEM. The cell line CHO-ts025C1 and the hamster-human hybrid cell line 158CnpT-1 were the generous gifts of Dr. R. E. Giles of the Advanced Genetics Research Institute, Oakland, California. These cell lines and the segregants of the hamster-human hybrid line were maintained as described previously.[7] All cultures were maintained at pH 7.4 in a humidified atmosphere of 95% air and 5% CO_2. Every three months, cultures were discarded and fresh cultures were started from stock frozen in liquid nitrogen.

The sodium-containing buffer was Dulbecco's phosphate-buffered saline (PBS) at

pH 7.4, consisting of 137 m*M* NaCl, 2.7 m*M* KCl, 10.6 m*M* Na_2HPO_4, and 1.5 m*M* KH_2PO_4. The sodium-free buffer had equimolar amounts of choline chloride and choline phosphate in place of NaCl and Na_2HPO_4, respectively. This buffer is referred to as PBC and was prepared as described previously.[3] For starvation experiments and for depletion and washing steps in transport assays, PBS and PBC were supplemented with 5.6 m*M* D-glucose, 0.49 m*M* $MgCl_2$, and 0.68 m*M* $CaCl_2$. These buffers are referred to as PBS-GMC and PBC-GMC, respectively. All buffers were at pH 7.4.

Transport Assays

Cells were plated in the appropriate medium at the indicated temperatures in Costar 24-well culture dishes with 1.3–1.5 $\times$ 10^5 cells per well. Cells were allowed to recover for 16–24 hours. We used the cluster tray assay as described previously,[3,8] except that in FIGURES 3 and 6 the depletions were for 40 minutes with a change to fresh PBC-GMC at 20 minutes. For FIGURES 1, 2, and 3, the depletions were done at 37°C, and for FIGURES 4, 5, 6, and 8, depletions were done at 34°C.

Cytogenetic Procedures

Giemsa alkaline differential staining (G-11) and the trypsin-Giemsa (G-banding) method of staining were carried out using described procedures.[9,10]

Isozyme Analysis

Procedures for the enzyme assays are as described previously.[7]

Materials

Radioactive amino acids were obtained from Amersham Corp., ICN, and New England Nuclear. Unlabeled amino acids (L-isomers) were obtained from Sigma. 2-Aminobicyclo-[2,2,1]-heptane-2-carboxylic acid was obtained from Calbiochem-Behring Corp. *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, ethyl methanesulfonate and cycloheximide were obtained from Sigma.

RESULTS

Regulation

Mammalian cells adapt to changes in the availability of amino acids by regulating the activity of their transport systems.[11,12] The regulation of System A transport has been well characterized in many cell types, including CHO cells.[11,12] Upon starvation in amino acid–free medium, CHO-K1 cells showed an almost twofold increase in System A activity within six hours (FIG. 2). The presence of a single System A amino acid, even the nonmetabolizable substrate 2-methylaminoisobutyric acid, prevented the starvation-induced derepression. The inhibition of the derepression by cycloheximide suggests that *de novo* protein synthesis may be necessary.

Earlier studies on the regulation of System A transport had also shown that System L transport activity is not regulated.[13,14] Upon starvation in amino acid–free medium, there was generally no change in System L transport activity. Severe starvation of CHO-K1 cells for a single System L amino acid (for example, less than 10 μM leucine), however, resulted in a three- to fourfold increase of leucine transport activity (FIG. 3).[15] Additional dialysis of the commercially available dialyzed fetal calf serum was required to sufficiently starve the cells of leucine. The increase in leucine transport activity following starvation was lost within one hour after refeeding.

Regulation of System L is also seen in the CHO mutant cell line CHO-tsH1, which has a temperature-sensitive leucyl-tRNA synthetase.[16,17] At high temperatures (37°–

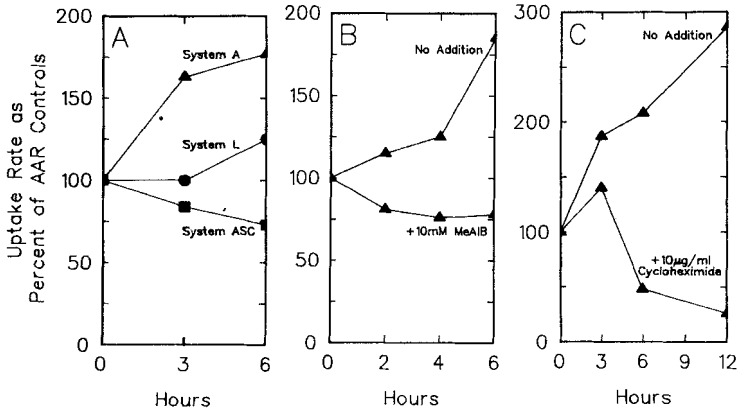

FIGURE 2. Effect of starvation for amino acids on transport activity in CHO-K1 cells. Cells were plated and grown in MEM at 37°C as described in MATERIALS and METHODS. The medium was then replaced with either fresh MEM or PBS-GMC supplemented with 5% dialyzed fetal calf serum. Uptake assays were carried out at the indicated times. The values are expressed as a percentage of the activity of cells kept in MEM, referred to here as amino acid–rich medium (AAR). A, activities of the three systems after starvation. The values are the averages of six determinations. B, Na^+-dependent, 5-min uptake of 0.2 mM MeAIB in PBS by cells incubated in PBS-GMC supplemented with 5% dialyzed fetal calf serum with or without 10 mM MeAIB. The values are the averages of three determinations. C, Na^+-dependent, 5-min uptake of 0.2 mM MeAIB in PBS by cells incubated in PBS-GMC supplemented with 5% dialyzed fetal calf serum with or without 10 μg/ml cycloheximide. The values are the averages of three determinations.

39°C) the cells are starved for leucine because of their inability to charge leucyl-tRNA. They will not grow at these temperatures unless supplied with relatively high concentrations of leucine in the medium.[18] When leucine uptake was measured in CHO-tsH1 cells after incubation at 38°C for several hours, there was a two- to threefold increase in activity over that seen after incubation at 34°C.[19,20] The maximum level of derepression was reached within twelve hours. The derepression was specific for System L activity, as shown in FIGURE 4. When the cells were returned to 34°C, the leucine uptake rate decreased to the original level within six hours. Severe starvation for leucine, either by lowering the leucine concentration in the medium for wild-type cells, or by growing CHO-tsH1 cells at 37°–39°C, results in a derepression of System L transport activity.

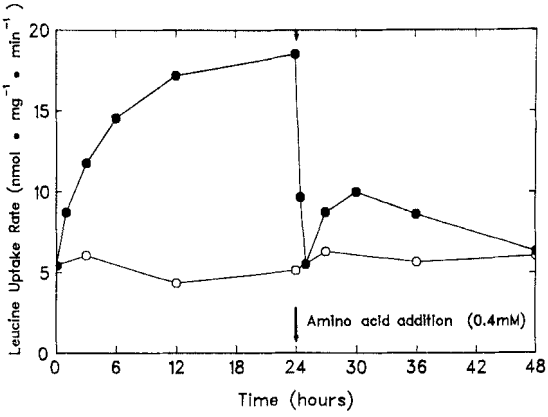

FIGURE 3. Effect of starvation for leucine on leucine uptake in CHO-K1 cells. Cells were plated and grown in MEM at 37°C. The cells were then washed twice with 2 ml of PBS and the medium was either replaced with fresh MEM or with leucine-free MEM supplemented with 5% extensively dialyzed fetal calf serum. After 24 hours leucine was added to a concentration of 0.4 m*M*. At various times the 1-min uptake of 0.2 m*M* leucine in PBC was determined. The values are the averages of four determinations. (○), MEM; (●), leucine-free MEM.

Regulatory Mutants

We have isolated temperature-resistant cells from the CHO-tsH1 cell line that are defective in the regulation of System L transport.[6] After mutagenesis of a population of CHO-tsH1 cells with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, the cells were grown at 38°C in MEM (0.4 m*M* leucine). After several passages, the temperature was raised to 39°C. After several additional passages, clones were isolated and grown in leucine-free MEM supplemented with 0.2 m*M* leucine at 39°C. Survivors were screened for leucine transport activity. The ten clones with the highest leucine transport activity were subjected to further selection by growing them in leucine-free MEM supplemented

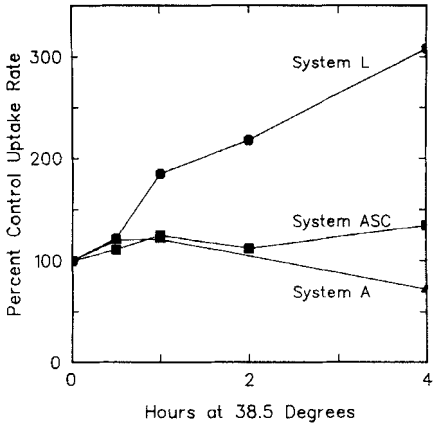

FIGURE 4. Specificity of the temperature-dependent derepression in the cell line CHO-tsH1. Cells were plated and grown in MEM at 34°C. Half the dishes were then shifted to 38.5°C and the other half left at 34°C. The transport systems activities were determined at various times. The data are expressed as the percentage of control values at time zero. The values are the averages of four determinations. (Reprinted from Shotwell *et al.*[20])

with 0.1 m*M* leucine at 39°C. The three cloned cell lines with the highest leucine transport activity (C11, D10, F10) were subcloned to obtain a homogeneous population. The temperature-resistant cell lines are routinely maintained under selective conditions (at 39°C in leucine-free MEM supplemented with 0.1 m*M* leucine).

FIGURE 5 compares the uptake of leucine by the temperature-resistant cell lines, C11 and F10, to that of the parental cell line, CHO-tsH1, after a six-hour incubation at either 34°C or 38.5°C. CHO-tsH1 cells showed a two- to threefold increase in leucine uptake when incubated at 38.5°C as compared to cells incubated at 34°C. The C11 and F10 cell lines had much higher leucine uptake activities than CHO-tsH1 cells at both temperatures, with no significant increase in the activities when incubated at 38.5°C as compared to cells incubated at 34°C. The increase in leucine transport activity in the C11, D10, and F10 cell lines was specific for System L at both temperatures. The activities of System A and ASC were not changed significantly in the C11, D10, and F10 cell lines in comparison to CHO-tsH1 cells.

Measurement of the kinetic parameters of leucine uptake showed that the increased transport activity in the temperature-resistant cell lines was accompanied by

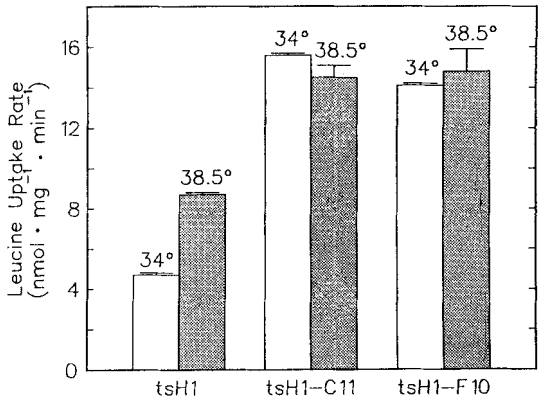

FIGURE 5. Leucine uptake by the cell line CHO-tsH1 and two temperature-resistant cell lines. Cells were plated and grown in MEM at 34°C. Half the dishes were shifted to 38.5°C and the other half left at 34°C. After 6 hours, the 30-sec uptake of 0.2 m*M* leucine in PBC was determined. Values are the averages of four determinations.

a two- to threefold increase in the V_{max} of leucine uptake with no significant change in the K_m when compared to CHO-tsH1 cells at 34°C. At 38.5°C the V_{max} of leucine uptake in CHO-tsH1 cells increased threefold over that seen at 34°C, while in the temperature-resistant cell lines there was only a small increase.

We measured the 20-minute steady-state accumulation of several amino acids in the CHO-tsH1 and the temperature-resistant cell lines. The accumulation of proline, mainly transported by System A, and the accumulation of alanine and serine, mainly transported by System ASC, was not significantly different in the temperature-resistant cell lines when compared to the CHO-tsH1 cell line. However, the steady-state accumulation of leucine, isoleucine, and valine, mainly transported by System L, was increased 1.5–3-fold in the temperature-resistant cell lines when compared to the CHO-tsH1 cell line. These results suggest that the increase in System L transport activity in the temperature-resistant lines leads to increased intracellular steady-state levels of System L amino acids.

We examined the possibility that the temperature-resistant cells resulted from a reversion of the leucyl-tRNA synthetase. An examination of the thermolability of the

leucyl-tRNA synthetase in the temperature-resistant lines suggested that the temperature resistance was not related to a reversion of the synthetase.

Several criteria must be met in order to consider a change in transport the result of a mutation. One of these criteria is that the alteration would be expected to persist after the removal of selection pressure.[21] When the temperature-resistant lines were grown under nonselective conditions (at 34°C in MEM, which has 0.4 m*M* leucine) for as long as 62 days, the System L transport activity remained the same as in those cells kept under selective conditions.

We have concluded that the increase in the activity of System L transport in the temperature-resistant lines is the result of a stable mutation in the regulation of System L transport. The kinetic data are consistent with, but do not prove, an increase in the number of active transport carriers in these cell lines, with no change in the affinity of the carrier. The increase in System L transport activity in these cell lines also leads to an elevated intracellular steady-state accumulation of System L amino acids, complementing the leucyl-tRNA synthetase defect and resulting in temperature resistance. These results are consistent with the conclusion that the temperature-resistant cell lines have a defect in a negative regulatory element leading to constitutively derepressed System L transport activity.

A similar type of mutant defective in the regulation of System A transport has been reported by Englesberg.[22] To isolate this mutant, CHO pro$^-$ cells were grown in the presence of high concentrations of alanine, which inhibits their growth by limiting proline uptake.[23] Alanine-resistant cells were obtained that had constitutively derepressed System A transport activity. This higher System A transport activity apparently allowed the cells to overcome proline starvation. These types of mutants contribute to our understanding of transport regulation and can be used in the selection of other types of mutants, as described in the following section.

Transport Mutants

We are attempting to isolate and characterize mutants defective in amino acid transport in order to identify the components of the transport systems. To obtain transport mutants in System L, we have designed a tritium-suicide selection strategy. Tritium-suicide selections using radioactive amino acids have been used successfully to obtain protein synthesis mutants similar to the CHO-tsH1 cell line.[24,25] We have modified the procedure in the following way to select for transport mutants. A tritiated amino acid is used as a toxic substrate. Cells that take up the radioactive amino acid will be subjected to internal radiation and will be killed. However, if in a population there are mutants with a defective transport system, these cells will take up much less of the radioactive amino acid, experience less internal radiation, and survive the procedure. To make sure we are selecting for transport mutants and not for protein synthesis mutants, the exposure to the tritiated amino acid is brief (three minutes) and the cells are frozen as quickly as possible.

Initial attempts to obtain transport mutants from normal CHO-K1 cells using this type of selection did not yield mutants with significant transport defects. It is possible that the ability of these cells to regulate System L transport activity did not permit the selection of transport-defective mutants. We then decided to take advantage of the regulatory mutants isolated in our lab and described in the previous section. Since the regulatory mutants have a much higher System L transport activity, they are more sensitive to toxic substrates. Furthermore, since they have lost the ability to regulate System L activity, a low transport mutant derived from these cells would also no longer be able to regulate System L activity. Finally, mutants from these parental cells would

still have the temperature-sensitive leucyl-tRNA synthetase, a condition which will be to our advantage in future selections.

There were two points to consider in designing the selection. First, since System L serves for the transport of most of the essential amino acids, a mutant with low System L transport activity may not survive unless supplemented with these amino acids. Therefore, after mutagenesis the cells were grown in MEM supplemented with three to five times the normal concentrations of essential System L amino acids. Second, the parental cells are able to survive at 39°C, presumably because their elevated System L transport activity compensates for the temperature-sensitive leucyl-tRNA synthetase. Transport-defective mutants would lose the ability to compensate for the defect, so after mutagenesis the cells were grown at 34°C, the permissive temperature. Approxi-

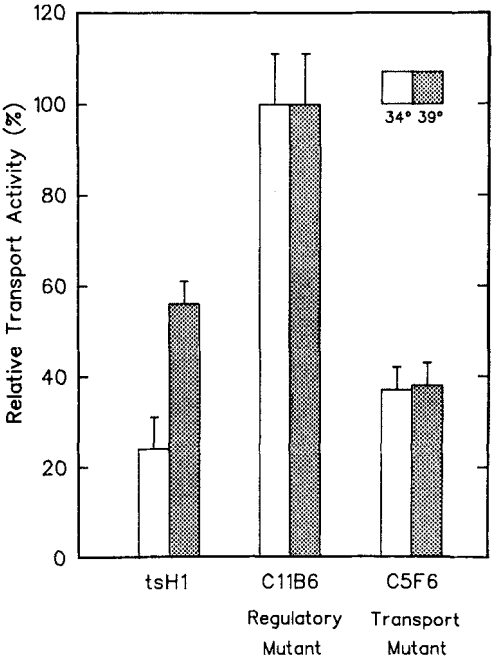

FIGURE 6. Leucine uptake by the cell line CHO-tsH1, the regulatory mutant cell line C11B6, and the low transport cell line C5F6. Cells were plated and grown in 5xMEM at 34°C. Cells were then washed with 2 ml of PBS and the medium was replaced with MEM. Half the dishes were placed at 39°C and the other half left at 34°C. After 6 hours the 30-sec uptake of 50 μM leucine in PBC was determined. The values are the averages of three determinations.

mately 10^7 cells from the C11B6 line, a subclone of the regulatory mutant C11, were mutagenized with 200 μg/ml ethyl methanesulfonate for 16 hours at 39°C and then incubated for six to seven days to allow phenotypic expression. After six to seven days, the cells were harvested by trypsinization, centrifuged, washed with PBC-GMC and exposed to [^{3}H]leucine (80 μCi/ml, 2×10^5 cells/ml) in PBC for three minutes at 37°C. Sodium was removed in order to limit the uptake of the [^{3}H]leucine to System L. The cells were washed with cold PBC-GMC and frozen in 5×MEM supplemented with 10% fetal calf serum and 10% glycerol as quickly as possible. Several days later, the cells were thawed and plated in 5×MEM supplemented with 10% fetal calf serum in 100-mm culture dishes at 34°C. The medium was changed every day for several days to remove dead cells and [^{3}H]leucine. The surviving colonies were either picked and transferred or the cells were harvested, diluted, and dispensed one cell per well in a

96-well dish. The surviving clones were grown up and assayed for leucine uptake. Those with the lowest values were subjected to further study. In one case the population surviving after thawing was mutagenized a second time and subjected to the same procedure. The cell lines C5, C9, and D3 are from this second round of selection. The C5 cell line was subcloned and the subclone with the lowest leucine transport activity (C5F6) was retained. These cells are routinely maintained in 5×MEM at 34°C.

FIGURE 6 shows the uptake of leucine in the cell line CHO-tsH1, the parental cell line C11B6, and the low transport cell line C5F6 after a six-hour incubation at either 34°C or 39°C. As shown previously, leucine uptake activity was increased two- to threefold in CHO-tsH1 cells when incubated at 39°C. The regulatory mutant cell line C11B6 showed increased and unregulated leucine uptake. The low transport cell line C5F6 had 37% and 38% of the leucine transport activity of C11B6 cells when incubated at 34°C and 39°C, respectively. The cell line C5F6 also showed unregulated leucine transport activity.

We measured the ability of the low transport cell lines to grow on low levels of leucine. FIGURE 7 shows the growth curves for low transport cell lines C9, D3, and the parental cell line C11B6 grown in leucine-free MEM supplemented with 75 μM leucine at 39°C. The low transport cell lines showed limited growth in comparison to the parental cell line. These results support our earlier conclusions about the regulatory mutants. The fact that the low transport mutants are temperature-sensitive shows that the leucyl-tRNA synthetase had not reverted. The low transport mutants are now being characterized further. They may also be used as the starting cells for a selection aimed at amplifying the CHO System L transport genes.

Mapping of Human Transport Genes

Somatic cell hybridization studies were initiated based on our early studies on the properties of the CHO-tsH1 cell line. Hybrid cells of a temperature-sensitive leucyl-tRNA synthetase mutant (CHO-ts025C1) and human leukocytes would be expected to become temperature-resistant if they expressed the human leucyl-tRNA synthetase gene or if they expressed human System L transport activity, leading to an increased ability to accumulate leucine. We examined several temperature-resistant hamster-

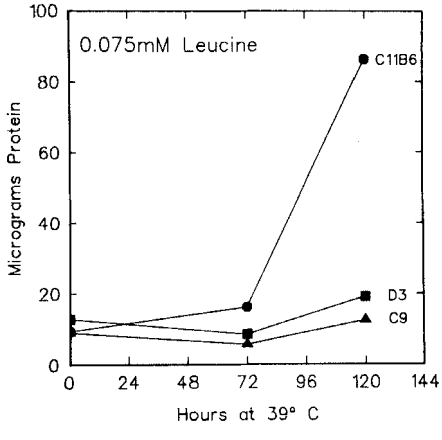

FIGURE 7. Growth of the low transport cell lines C5 and D3 at 39°C. Cells were plated at 2.2×10^4 cells per well in 24-well culture dishes in 5xMEM at 34°C. After recovery (time zero), cells were washed two times with 2 ml of PBS. The medium was replaced with leucine-free MEM supplemented with 75 μM leucine and 5% dialyzed fetal calf serum. The cells were incubated at 39°C for the indicated times. The cells were then washed twice with 2 ml of PBS and precipitated with 220 μl of 10% trichloroacetic acid. The precipitate was dissolved in 100 μl 1 N NaOH and the protein determined.[30] The values are the averages of four determinations.

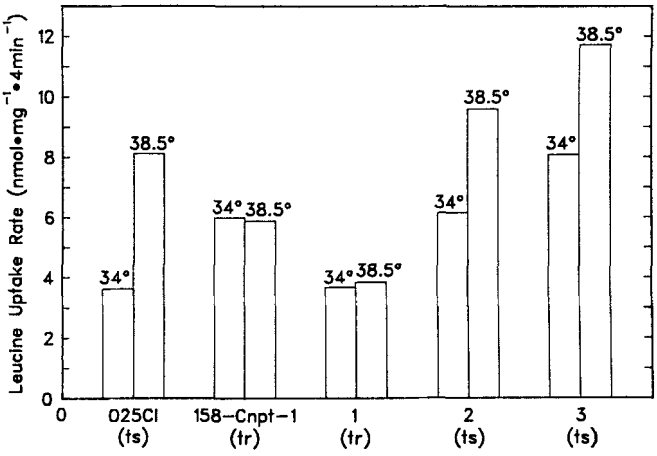

FIGURE 8. Leucine uptake by several segregants of the hamster-human hybrid cell line 158CnpT-1. Cells were plated and grown for 6 hours in MEM at 34°C. Half the dishes were shifted to 38.5°C and the other half kept at 34°C. After 12 hours, the 4-min uptake of 0.1 m*M* leucine in PBC was determined. The values are the averages of four determinations. tr, temperature-resistant; ts, temperature-sensitive. (Reprinted with permission from Shotwell *et al.* 1984. Fed. Proc. **43:** 2269–2272.)

human hybrids obtained from Giles *et al.* that were used to assign the chromosomal location of the human leucyl-tRNA synthetase to chromosome 5.[26,27] We measured the leucine uptake of several of these hybrids and found that the hybrid line 158CnpT-1 had a two- to threefold increase in leucine transport activity over that seen in CHO-ts025C1 cells.[7] This increase was specific for System L activity. The activities of systems A and ASC were not significantly changed. The hybrid cell line accumulated higher steady-state levels of intracellular leucine and other System L amino acids than did CHO-ts025C1 cells, but the accumulation of alanine in the hybrid cells was not significantly different than that in CHO-ts025C1 cells.

Cytogenetic analysis was carried out on the hamster-human hybrid cell line using the alkaline Giemsa (G-11) method to distinguish four human chromosomes and three human translocations in the hamster chromosomes. The trypsin-Giemsa (G-banding) method was used to identify these four chromosomes as numbers 4, 5, 20, and 21.[9,10]

To determine if the high leucine transport activity and the temperature resistance were from independent human chromosomal loci, the hybrid cell line 158CnpT-1 was subjected to conditions that allowed further segregation of the human chromosomes. The cells were grown under permissive conditions (at 34°C in MEM supplemented with 2 m*M* leucine) to allow survival of segregants that had lost human chromosome 5. The cells were treated with a sublethal dose of colcemid (0.02 μg/ml) for 30 hours at 34°C to facilitate the segregation. After recovery from the colcemid (42 hours) the cultures were subcloned. The clones were assayed for leucine uptake and temperature-resistant growth. The segregants fell into four classes: (1) low leucine transport activity and temperature resistance; (2) high leucine transport activity and temperature sensitivity; (3) extra-high leucine transport activity and temperature sensitivity; and (4) low leucine transport activity and temperature sensitivity. FIGURE 8 compares the leucine uptake activity of segregants from the first three classes to that of CHO-ts025C1 cells and the hamster-human hybrid cell line 158CnpT-1. Growth curves

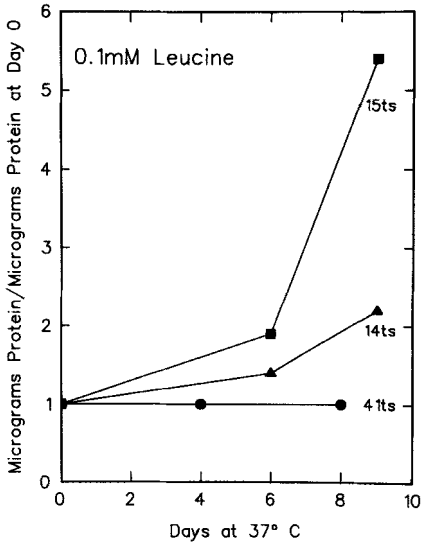

FIGURE 9. Growth of segregants of the hamster-human hybrid cell line 158CnpT-1 at 37°C. Cells were plated at 7.5×10^4 cells per well in 24-well culture dishes in MEM at 34°C. After recovery (time zero), cells were washed with 1 ml PBS. The medium was replaced with leucine-free MEM supplemented with 0.1 m*M* leucine and 5% dialyzed fetal calf serum. The cells were incubated at 37°C for the indicated times. The samples were then prepared as described in FIGURE 7. The values are the averages of two experiments with six determinations each.

showed a slight, but significantly higher temperature resistance in classes 2 and 3 (FIG. 9). The segregant cell line 14ts is from class 2, the segregant cell line 15ts is from class 3, and the segregant cell line 41ts is from class 4.

Karyotypes of the segregants were carried out using G-11 staining and G-banding techniques. The cytogenetic analysis showed that the primary temperature resistance was correlated with the presence of human chromosome 5, confirming the earlier reports.[26,27] The loss of the high leucine transport activity was associated with the loss of one of the two small human chromosomes, 20 or 21.

To distinguish between chromosomes 20 and 21, we assayed for the presence of the marker enzymes for chromosomes 20 and 21, adenosine deaminase and the cytoplasmic form of superoxide dismutase, respectively, in the segregants.[28] This analysis showed a positive correlation between the high leucine transport phenotype and the presence of adenosine deaminase activity, or human chromosome 20 (TABLE 1).

TABLE 1. Analysis of Human Chromosomal Marker Enzymes in the Hamster-Human Hybrid Cell Line 158CnpT-1 and Segregants[a]

Cell Line	Leucine Transport Activity	Presence of Enzyme	
		Adenosine Deaminase	Superoxide Dismutase 1
158CnpT-1	high	+	+
Segregants			
11 (class 1)	low	−	+
23 (class 1)	low	−	+
14 (class 2)	high	+	+
15 (class 3)	extra-high	+	+
41 (class 4)	low	−	+

[a]Adenosine deaminase (chromosome 20) and superoxide dismutase 1 (chromosome 21) were assayed as described in MATERIALS and METHODS.

Segregants in class 3, which had extra-high leucine transport activity, had two copies of chromosome 20, as seen in the karyotype analysis, and also showed greater adenosine deaminase activity. These results allowed us to assign the expression of System L transport activity to human chromosome 20.[7]

CONCLUSIONS

We have used genetic approaches to study amino acid transport and its regulation with the goal of cloning the transport genes. To this end we have isolated several classes of transport mutants that are described in this report. The increased temperature resistance with higher transport activity, seen in the regulatory mutant cell lines and in the hamster-human hybrid cell lines, supports our assumption that mutant selections could be designed in which growth is limited by leucine transport activity. The low transport mutants that we isolated are being used as the recipient cell lines for DNA transformation in transport gene cloning studies. Transformants containing the transport genes can be selected by raising the temperature, by lowering the leucine concentration, or by a combination of the two. The CHO-ts025C1 cell line has been transformed with a human cosmid library and clones have been obtained with increased transport. We are currently attempting to rescue the plasmid containing the human transport genes by a lambda *in vitro* packaging system.[29]

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