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Chinese Hamster Ovary mRNA-Dependent, Na^+-Independent L-Leucine Transport in *Xenopus laevis* Oocytes

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In freshly prepared uninjected folliculated oocytes, Na^+-independent leucine uptake is mediated predominantly by a system L-like transport system. Removal of follicular cells, however, results in an irreversible loss of this transport activity. When total poly(A)$^+$ mRNA derived from Chinese hamster ovary (CHO) cells was injected into prophase-arrested stage V or VI *Xenopus laevis* oocytes, enhanced expression of Na^+-independent leucine transport was observed. The injected mRNAs associated with increased levels of leucine uptake were between 2 and 3 kb in length. The newly expressed leucine transport activity exhibited important differences from the known characteristics of system L, which is the dominant Na^+-independent leucine transporter in CHO cells as well as in freshly isolated folliculated oocytes. The CHO mRNA-dependent leucine uptake in oocytes was highly sensitive to the cationic amino acids lysine, arginine, and ornithine (>95% inhibition). As with the leucine uptake, an enhanced lysine uptake was also observed in size-fractionated CHO mRNA-injected oocytes. The uptakes of leucine and lysine were mutually inhibitable, suggesting that the newly expressed transporter was responsible for uptakes of both leucine and lysine. The inhibition of uptake of lysine by leucine was Na^+ independent, thus clearly distinguishing it from the previously reported endogenous system y^+ activity. Furthermore, the high sensitivity to tryptophan of the CHO mRNA-dependent leucine transport was in sharp contrast to the properties of the recently cloned leucine transport-associated gene from rat kidney tissue, although leucine transport from both sources was sensitive to cationic amino acids. Our results suggest that there may be a family of leucine transporters operative in different tissues and possibly under different conditions.

Mammalian cells transport amino acids across cell membranes by several well-defined overlapping transport systems (9). The criteria used to distinguish one transport route from another have been based largely on kinetic analysis and substrate specificity studies. Accumulation of leucine in mammalian cells is mediated primarily by zwitterionic amino acid-preferring systems, such as system L (12). On the basis of the specificity of amino acids that are transported, the transport systems can be divided into three major categories that are primarily responsible for uptakes of zwitterionic, cationic, and anionic amino acids (10). The transport systems, such as systems ASC and asc, reacting mostly with zwitterionic amino acids can also accept cationic amino acids under certain conditions (34). Similarly, system y^+, serving mainly for cationic amino acid transport, may under certain conditions transport zwitterionic amino acids (35, 42). Additionally, systems with the same reactivity to both zwitterionic and cationic amino acids have been characterized by using mouse blastocysts (36, 39). The interaction between systems for zwitterionic and cationic amino acids is further supported by functional switching from one system to another in a growth- or development-dependent manner (6, 37). These results taken together suggest that a number of the previously described Na^+-independent transport systems, such as systems LI and LII (40), asc_1 and asc_2 (44), y^+ (42), and $b^{0,+}$ (36), may be closely related members of a family (8, 38) that are encoded by a family of closely related genes.

The molecular identities of most of these transporters are only beginning to emerge (4, 20, 32, 41). Progress in the isolation and cloning of amino acid transporters has been hindered by their very low abundance and the fact that transport of a given amino acid is often mediated by several transporters (12). Since Gurdon et al. (16) first described the use of *Xenopus laevis* oocytes as an efficient in vivo translation system, *X. laevis* oocytes have become a particularly valuable system for cloning genes for which biological function is the most suitable method of identification. Membrane proteins, including various transporters, have been expressed in oocytes after microinjection of the corresponding mRNA (28). The growing list of transporters cloned in oocytes includes those for glucose (17), noradrenaline (22), γ-aminobutyric acid (15), dopamine (19), serotonin (5), betaine (43), and glycine (14). Recently, two genes responsible for enhanced zwitterionic and cationic amino acid transport have been cloned independently from kidneys in three laboratories (4, 32, 41). They reported a system $b^{0,+}$-like amino acid transport activity in oocytes injected with cloned cDNAs. In this report, we have characterized the enhanced Na^+-independent leucine transport in oocytes following microinjection with Chinese hamster ovary (CHO) cell poly(A)$^+$ mRNA.

MATERIALS AND METHODS

Oocyte isolation. Adult female South African clawed frogs, *X. laevis* (Xenopus 1, Ann Arbor, Mich.), were anesthetized in a 0.3% solution of ethyl-*m*-aminobenzoate for 15 to 20 min and then placed on ice. One lobe of the ovary was aseptically removed as described by Colman (13) and washed thoroughly in modified Barth's saline (MBS) [88 mM NaCl, 1.0 mM KCl, 2.4 mM $NaHCO_3$, 0.3 mM $Ca(NO_3)_2$, 0.41 mM $CaCl_2$, 0.82 mM $MgSO_4$, 15.0 mM 4-(2-hydroxyethyl)-1-

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piperazine ethanesulfonic acid (HEPES)–NaOH (pH 7.6), 10 U of penicillin per ml, 10 μg of streptomycin sulfate per ml] supplemented with 0.5 μg of gentamicin per ml and 2.5 mM sodium pyruvate. This MBS medium gives an osmolarity of 210 mosM. By using watchmakers' forceps, the ovary lobe was separated into clumps, each containing approximately 10 stage V or VI oocytes. Defolliculation was carried out either manually following preincubation in Ca^{2+}- and Mg^{2+}-free MBS in the presence of 1 mM EDTA for 30 min or by collagenase treatment (2 mg/ml) (Boehringer Mannheim Biochemicals; type B) in Ca^{2+}-free MBS medium at 22°C for 1.5 h. After the oocytes were washed with MBS three times, fully developed, healthy oocytes were selected for microinjection. The oocytes were incubated in MBS at 19°C for 3 to 4 days with changes of medium every 12 to 24 h. During the incubation period, oocytes displaying abnormal pigmentation were discarded.

mRNA isolation and size fractionation. Total cellular RNA was isolated according to an improved single-step guanidinium-thiocyanate-phenol-chloroform extraction procedure (24). CHO-C11B6, a CHO cell line constitutively expressing system L activity (27), was used in this study. Poly(A)$^+$ mRNA was isolated by oligo(dT)-cellulose column chromatography (2). Size fractionation of total mRNA was carried out by using a linear 5 to 30% (wt/vol) sucrose gradient containing 10 mM Tris-HCl (pH 7.4), 1 mM EDTA, and 0.2% sodium dodecyl sulfate (SDS). mRNA samples (100 μg) were heated to 65°C for 5 min and then added to the sucrose gradient and centrifuged at 35,000 rpm in a Beckman SW40 Ti rotor for 12 h. Twenty aliquots (0.5 ml each) were collected from the sucrose gradient. Size-fractionated poly(A)$^+$ mRNA was precipitated from each aliquot by addition of sodium acetate (final concentration, 0.3 M) and 2 volumes of ethanol. Samples were stored frozen at −70°C. The precipitated poly(A)$^+$ mRNA was resuspended in diethyl pyrocarbonate-treated water to a final concentration of 1 μg/μl for microinjection.

Microinjection of poly(A)$^+$ mRNA. Preliminary tests showed that mRNA levels between 34 and 68 ng per oocyte exhibited an optimum enhancement of leucine uptake; thus, the amount of mRNA used for injection in all subsequent studies was 50 ng per oocyte. The oocyte injection was carried out as described by Colman (13) with 50 nl of either diethyl pyrocarbonate-treated water (control) or the poly (A)$^+$ mRNA-containing solution. After injection, oocytes were stored at 19°C in MBS, with daily changes of medium.

Electrophoretic analysis. The mean size of the fractionated mRNA was determined by using a 1.0% formaldehyde denaturing agarose gel (25). The total combined mRNA from two neighboring fractions (1 ml) was precipitated in ethanol and resuspended in diethyl pyrocarbonate-treated water. One-half of each sample was heated at 65°C for 15 min and then loaded on the gel for electrophoresis.

Transport assay. Injected oocytes that had been stored for 3 or 4 days at 19°C were prepared for transport assay by incubation at 22°C for 30 min in either MBS or Na^+-free MBS medium (with Na^+ ion being replaced by equimolar choline). Before transport assays, groups of five or six oocytes were transferred into plastic tubes with truncated ends sealed by nylon mesh (30). The uptake mixture was made up in MBS or Na^+-free MBS medium and contained L-[^{3}H]leucine at a concentration of either 0.2 mM (for most of the transport assays) or 0.05 mM (for substrate specificity assays). When necessary, osmolarity was adjusted by sodium or choline chloride. Transport activity was measured at 22°C for 20 min in a 24-well tray, with each well containing 250 μl of the uptake mixture. The time course of Na^+-independent leucine uptake was linear for at least 1 h; thus, a 20-min uptake for the standard assay approximates the initial rate of transport. Uptake was stopped by an immediate dilution and wash with ice-cold MBS. Individual oocytes were then transferred from the uptake tubes to scintillation vials. The oocytes were lysed by incubation in 0.5 ml of 2% SDS solution overnight. Transport activity was expressed as picomoles oocyte^{-1} hour^{-1}. Unless otherwise specified, the uptake rate refers to saturable uptake, which was calculated by subtracting the nonsaturable rate measured in the presence of excess unlabeled L-leucine (10 mM). Most of the data are expressed as means ± standard deviations for five or six oocytes. Values reported represent experiments with at least two frogs. An analogous procedure was used for assaying L-lysine transport activity.

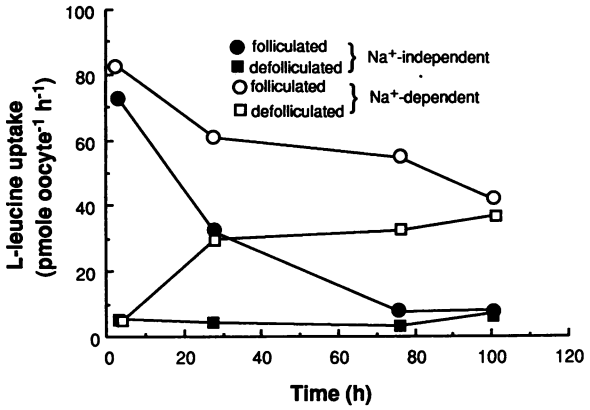

FIG. 1. Temporal changes in L-leucine transport of *X. laevis* oocytes during culture in MBS. Defolliculated oocytes were obtained by collagenase treatment (2 mg/ml). Both defolliculated and folliculated oocytes were incubated at 19°C in MBS, and transport of 0.2 mM L-[^{3}H]leucine into oocytes was determined at the times indicated. The transport assay was carried out as described in Materials and Methods. The rates of Na^+-dependent transport were calculated by subtracting the rates observed in the absence of sodium (Na^+-independent). Measurements represent saturable uptake, which was determined by subtracting the uptake rates measured in the presence of 10 mM L-leucine. Data are expressed as picomoles oocyte^{-1} hour^{-1} and are averages for five or six oocytes. The standard deviations (less than 15%) are omitted for clarity.

RESULTS

Characterization of endogenous Na^+-independent leucine uptake in oocytes. To distinguish the newly expressed leucine transport resulting from microinjected mRNA and the endogenous transport activity, the Na^+-independent leucine uptakes in both folliculated and defolliculated oocytes were characterized. More than 90% of transport of 50 μM L-[^{3}H]leucine into folliculated oocytes was inhibited in the presence of 10 mM unlabeled leucine, indicating that most of the leucine entry was mediated by saturable processes. In freshly isolated folliculated oocytes, this saturable leucine transport (60 to 80 pmol oocyte^{-1} h^{-1}) was equally distributed between Na^+-independent and Na^+-dependent components (Fig. 1). The Na^+-independent component resembled the previously described system L (7, 29). The high levels of activity, however, were observed only in freshly prepared folliculated oocytes. The system L-like activity disappeared

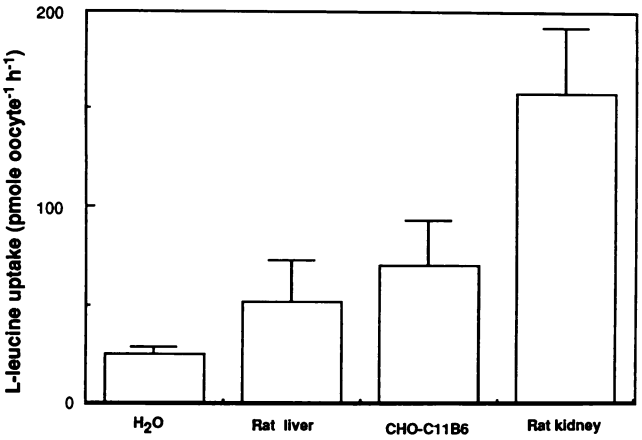

FIG. 2. Expression of the mammalian Na^+-independent L-leucine transporter in *X. laevis* oocytes. Oocytes were injected with water or poly(A)$^+$ mRNA samples (50 ng per oocyte) derived from three different sources as indicated. Oocytes were incubated at 19°C in MBS for 3 days. Total uptake of L-[^{3}H]leucine (0.2 mM) into oocytes was determined as described in Materials and Methods. The data are averages of the sums of saturable and nonsaturable uptake rates for five or six oocytes, expressed as picomoles oocyte^{-1} hour^{-1}. Bars, standard deviations.

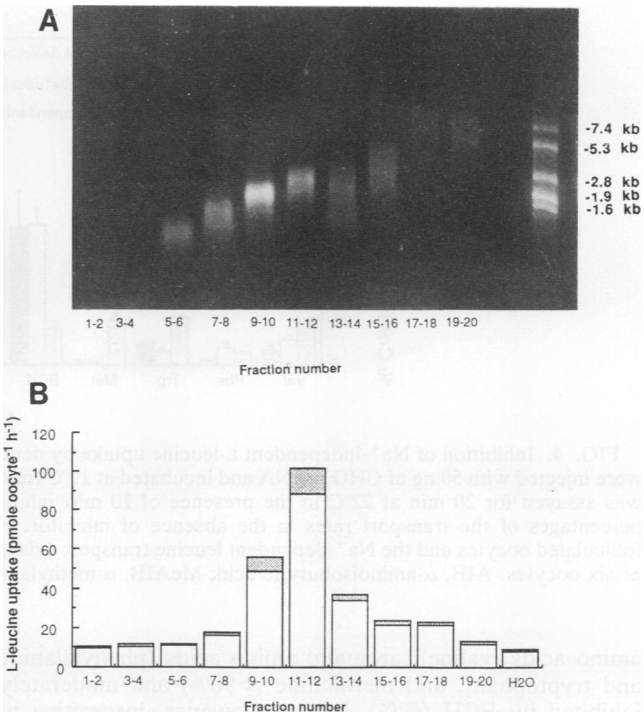

FIG. 3. Sucrose density gradient fractionation and agarose gel electrophoresis of CHO mRNA. Samples of mRNA (100 mg) were fractionated on a 5 to 30% sucrose gradient, and 0.5-ml fractions were collected. Samples of mRNA (50 ng each) obtained from two combined neighboring fractions were injected into oocytes and incubated at 19°C in MBS for 4 days. Total uptake of 0.2 mM L-[^{3}H]leucine was measured in the absence of sodium as described in Materials and Methods. (B) The Na^+-independent transport activity is depicted as a function of fraction number. The data are averages of the sums of saturable and nonsaturable uptake rates for five or six oocytes. (A) Parallel samples from the sucrose gradient were subjected to electrophoresis on a formaldehyde denaturing agarose gel (1%). mRNA size standards (Boehringer Mannheim Biochemicals) are shown on the right. Shaded bars indicate standard deviations.

rapidly during in vitro incubation of the oocytes at 19°C even in the presence of follicular cells (Fig. 1). Figure 1 also indicates that low levels of saturable leucine transport activity (3 to 10 pmol oocyte^{-1} h^{-1}) were retained in defolliculated oocytes. This activity clearly differed from the system L-like transport activity, since it was totally insensitive to β-2-aminobicyclo-(2,2,1)-heptane-2-carboxylic acid (BCH), a model substrate for system L. In contrast to the Na^+-independent process, the Na^+-dependent uptake activity of leucine was restored within 1 to 2 days of incubation after defolliculation (30 to 40 pmol oocyte^{-1} h^{-1}) (Fig. 1). Manual defolliculation produced the same effect as collagenase treatment, although the elimination of residual follicular-cell amino acid transport activities was not as efficient with the former method (70 to 80% elimination). This lower efficiency is assumed to be due to incomplete defolliculation. Water-injected as well as manually defolliculated oocytes showed essentially the same features as collagenase-treated oocytes, as illustrated in Fig. 1. The absence of system L activity and the low levels of endogenous Na^+-independent leucine transport have made the oocytes a useful system for expressing exogenous Na^+-independent leucine transporter.

Functional expression of leucine transport. The maximal enhanced transport of leucine appeared 3 to 4 days after mRNA injection. As shown in Fig. 2, injection of mRNA from CHO-C11B6 cells produced approximately a threefold increase in Na^+-independent leucine uptake compared with that observed in water-injected oocytes (69.6 versus 25.2 pmol oocyte^{-1} h^{-1}). The transport rate observed with mRNA from CHO cells was slightly higher than that with rat liver mRNA (51.6 pmol oocyte^{-1} h^{-1}) (33) and much lower than that with rat kidney mRNA (158.4 pmol oocyte^{-1} h^{-1}) (31). Injection with size-fractionated CHO mRNA demonstrated that most of the transport activity was present between fractions 9 and 14, with the highest responses in fractions 11 and 12 (Fig. 3B). Separation of the size-fractionated mRNA samples on a denaturing agarose gel revealed that the size of the active mRNA corresponded to 2 to 3 kb (Fig. 3A). This size is similar to the size of mRNA which was shown in other reports to be responsible for enhanced amino acid transport in oocytes (31, 33) and for Na^+-dependent glucose transport from the intestine (26).

Substrate specificity. The competitive inhibition of 50 μM L-[^{3}H]leucine uptake by selected inhibitors (10 mM) in oocytes injected with water and oocytes injected with mRNA from CHO-C11B6 cells was measured. In agreement with a previous report (7), the specificity of the endogenous Na^+-independent leucine transport activity in freshly prepared folliculated oocytes injected with water resembled that of system L (Fig. 4). This endogenous Na^+-independent leucine transport activity was strongly inhibited by the system L-preferring substrates (valine, phenylalanine, tryptophan, and BCH). The specificity of the endogenous Na^+-dependent leucine transport activity, however, was similar to that of a transport system referred to as system $B^{0,+}$, reported earlier (39). This Na^+-dependent system has been characterized by its high sensitivity to both bulky zwitterionic and cationic amino acids (e.g., valine, phenylalanine, tryptophan, arginine, and lysine).

As shown in Fig. 4, after injection of oocytes with fractionated CHO mRNA, the enhanced Na^+-independent uptake of leucine was strongly inhibited by branched-chain

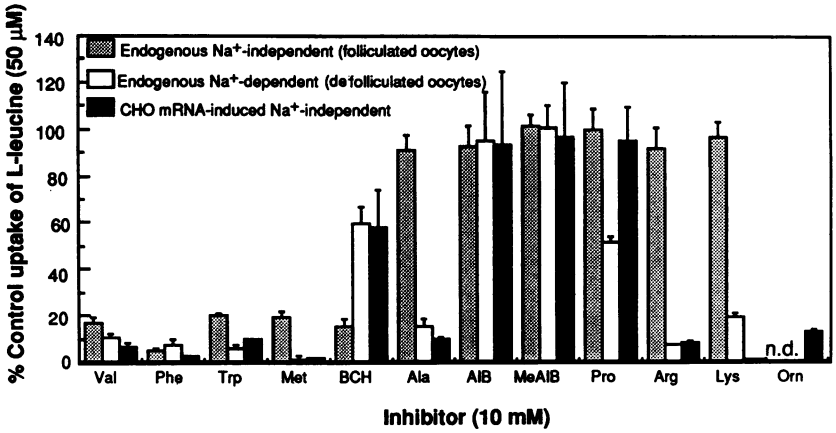

FIG. 4. Inhibition of Na^+-independent L-leucine uptake by neutral and basic amino acids in oocytes injected with CHO mRNA. Oocytes were injected with 50 ng of CHO mRNA and incubated at 19°C for 4 days. The Na^+-independent transport of 50 μM L-[^{3}H]leucine in oocytes was assayed for 20 min at 22°C in the presence of 10 mM inhibitor as described in Materials and Methods. The data are expressed as percentages of the transport rates in the absence of inhibitor. For comparison, the endogenous Na^+-independent leucine transport in folliculated oocytes and the Na^+-dependent leucine transport 4 days after defolliculation were also determined. The data are averages for five or six oocytes. AIB, α-aminoisobutyric acid; MeAIB, α-methylaminoisobutyric acid; n.d., not determined.

amino acids (valine), aromatic amino acids (phenylalanine and tryptophan), and methionine (>90%) and moderately inhibited by BCH (40%). It was, however, insensitive to proline, α-aminoisobutyric acid, and α-methylaminoisobutyric acid, typical substrates for system A (<20%). Furthermore, when the oocytes were preloaded with 10 mM unlabeled leucine for 4 h, *trans* stimulation of Na^+-independent labeled leucine uptake and *trans* inhibition of Na^+-dependent leucine uptake were observed (Table 1). This pattern of substrate specificity is similar to that described for system L in CHO cells. It should be noted, however, that the newly expressed transporter was very sensitive to cationic amino acids, such as arginine, lysine, and ornithine (Fig. 4). The latter results are not consistent with the properties of system L. The characteristics of this transport activity resemble those of system $b^{0,+}$, which previously has been observed only in the mouse blastocyst (36).

To test whether a mutual inhibition between leucine and cationic amino acids exists, transport of lysine in water- and CHO mRNA-injected oocytes was measured. As with leucine uptake, an enhancement of Na^+-independent lysine uptake was observed after CHO mRNA injection (Table 2). Although high levels of endogenous Na^+-independent lysine transport activity were found in defolliculated oocytes (58 pmol oocyte^{-1} h^{-1}), this Na^+-independent uptake of lysine increased approximately threefold 4 days after the CHO mRNA injection (157 pmol oocyte^{-1} h^{-1}). The data in Table 2 also show that transport of 50 μM L-[^{3}H]lysine was strongly inhibited by 10 mM leucine, indicating that uptakes of leucine and lysine are mutually inhibitable. As reported previously for arginine uptake (7), the endogenous lysine transport activity of oocytes is analogous to that ascribed to the Na^+-independent system y^+. As expected for system y^+ activity (42), unlabeled leucine inhibited the endogenous lysine transport only weakly in the absence but strongly in the presence of sodium (Table 2). In contrast to the Na^+-dependent inhibition of the endogenous uptake of lysine by leucine, the CHO mRNA-directed lysine transport activity could be completely inhibited by leucine in the absence of sodium; therefore, the CHO mRNA-directed lysine entry was mediated by a route clearly distinguishable from the activity of the endogenous system y^+ (Table 2).

TABLE 1. *trans* effect of preloaded L-leucine on CHO mRNA-directed L-leucine transport activity

Oocyte[a]	Na^+-independent		Na^+-dependent	
	Uptake rate[b]	Ratio	Uptake rate	Ratio
Unloaded	61.7 ± 2.2	1	64.3 ± 7.0	1
Preloaded	113.9 ± 14.3	1.85	21.9 ± 5.4	0.34

[a] Oocytes were preincubated in MBS in the presence (preloaded) or absence (unloaded) of 10 mM L-leucine at 22°C for 4 h and then washed thoroughly with MBS or Na^+-free MBS. The transport of 50 μM L-[^{3}H]leucine was assayed as described in Materials and Methods.

[b] Average ± standard deviation (in picomoles oocyte^{-1} hour^{-1}) for five or six oocytes.

DISCUSSION

In this study, we have demonstrated that microinjection of poly(A)$^+$ mRNA prepared from CHO cells into *X. laevis* oocytes is associated with enhanced Na^+-independent and Na^+-dependent leucine transport activities (Table 1). The percentages of the enhanced Na^+-independent and Na^+-dependent leucine transport activities could vary from 30 to 60% and from 10 to 20%, respectively. In other reported studies, microinjections of total mRNAs from the kidney (11, 31), the liver (18, 30, 33), and the small intestine (3) as well as several tissue cell lines (30, 31) into oocytes were associated with increased amino acid transport activity. The reported functional expression of mammalian system L-like transport in oocytes following the injection of rat kidney mRNA was essentially based on the transport activity for leucine or phenylalanine, which was significantly higher than that for the water-injected oocytes (11, 31). Without sufficient characterization of the endogenous and the stimulated leucine transport activities, the possibility that the enhancement resulted from increased expression of endogenous transport activity cannot be ruled out (3, 28, 33). Consequently, it is important to be able to distinguish between newly expressed and endogenous transport activities in oocytes. Although Na^+-independent leucine transport activity was high in freshly prepared folliculated oocytes (Fig. 1)

TABLE 2. Inhibition of L-lysine uptake by L-leucine in oocytes injected with water or CHO mRNA

Inhibitor	Oocytes injected with:				CHO mRNA enhancement	
	Water		CHO mRNA			
	Uptake rate[a]	%	Uptake rate[a]	%	Uptake rate[b]	%
Without Na^+						
None	58.0 ± 1.5	100.0	156.8 ± 7.8	100.0	98.8	100.0
L-Lysine	3.2 ± 1.4	5.5	4.7 ± 1.0	3.0	1.5	1.5
L-Leucine	39.3 ± 1.9	67.8	35.4 ± 4.7	22.6	−3.9	−4.0
With Na^+						
None	88.9 ± 7.0	100.0	210.2 ± 34.5	100.0	121.3	100.0
L-Lysine	4.3 ± 4.8	4.8	4.3 ± 1.2	2.0	0.0	0.0
L-Leucine	9.1 ± 1.7	10.2	12.0 ± 2.2	5.7	2.9	2.4

[a] Average ± standard deviation (in picomoles $oocyte^{-1}$ $hour^{-1}$) for five or six oocytes. The transport of 50 μM L-[^{3}H]lysine was measured for 20 min at 22°C in the presence or absence of 10 mM inhibitor in MBS or Na^+-free MBS as described in Materials and Methods.

[b] Calculated by subtracting the lysine uptake rate for water-injected oocytes from that for CHO mRNA-injected oocytes.

(7), this activity was completely lost during in vitro culture and could not be recovered. Furthermore, defolliculation by either enzymatic or manual means also resulted in an irreversible loss of leucine transport activity, suggesting either that the Na^+-independent transporter is located in the follicular cells or that the follicular cells are required for its expression. The appearance of Na^+-independent leucine transport activity after the injection of mRNA into defolliculated oocytes, therefore, likely does not result from enhancement of an endogenous leucine transporter.

System L is the predominant Na^+-independent leucine transport system in CHO cells (12) and in freshly isolated folliculated oocytes (Fig. 4). Following CHO mRNA injection, however, the enhanced leucine transport exhibited a high level of sensitivity to cationic amino acids (>95% inhibition), a characteristic not associated with the typical system L transport activity (10, 12). Functional changes have been reported for expression of other mammalian membrane proteins, such as the *p*-aminohippurate transporter and Na^+/K^+ ATPase, in oocytes (21, 26). While overlapping inhibition of transport by zwitterionic and cationic amino acids is quite common and a switch from system L properties to system $b^{0,+}$ properties during the development of preimplantation mouse conceptuses has been reported (37), system L has not generally been shown to be reactive with cationic amino acids. Therefore, the cationic amino acid-sensitive leucine transport activity is unlikely to result from expression of the same system L transporter as that found in CHO cells. Our results demonstrate that microinjection of CHO mRNA is associated with enhanced uptake of both leucine and lysine and that these two amino acids are mutually inhibitory with respect to each other's transport, suggesting that they are mediated by a common, newly expressed transporter. This type of transport activity, in fact, resembles the previously described system $b^{0,+}$ (36) rather than system L, although neither oocytes nor CHO cells exhibit detectable levels of a system $b^{0,+}$-like transport activity.

Recently, the cDNAs which are responsible for enhanced cystine uptake into oocytes have been cloned from rat and rabbit kidneys (4, 32, 41). These two structurally homologous cDNAs show stimulated uptakes of both zwitterionic and cationic amino acids. In contrast to the 10 to 12 membrane-spanning regions postulated to occur for many transporters, the deduced amino acid sequence from the two kidney cDNAs suggests that only one membrane-spanning domain is present (4, 41). This finding has led the authors to conclude that the enhanced amino acid transport activity may be the result of cloning a regulatory factor or an activator of amino acid transport rather than the transporter gene itself. It is also possible that this cloned factor functions as one subunit of a membrane transport complex. Interestingly, they have shown that expression of human 4F2 surface antigen in oocytes results in stimulation of system y^+-like amino acid transport (3). There is significant similarity between the 4F2 protein and the deduced amino acid sequence of the cloned cDNA which is responsible for enhanced uptake of cystine, implying that transport of neutral and dibasic amino acids in oocytes may be induced by a family of type II membrane glycoproteins (4).

It should be noted that the high sensitivity to tryptophan of the CHO mRNA-dependent leucine transport activity contrasts with the transport effects associated with injection of the cloned rat kidney cDNA in oocytes (32). In that study, expression of the cDNA did not result in enhanced tryptophan uptake nor was the enhanced leucine uptake inhibited by tryptophan. Although the enhanced transport activity in oocytes produced by the kidney-derived cDNAs has been assigned to system $b^{0,+}$ (4, 41), this tryptophan insensitivity contrasts with the previously described properties of system $b^{0,+}$ (36). Tryptophan sensitivity has been used in the past as one of the main criteria to distinguish system LI from system LII (40) and system b^+1 from system b^+2 (20). Our data demonstrate that the inhibition by tryptophan of the CHO mRNA-dependent leucine transport was about 80%, suggesting that the newly expressed leucine transporter differed somewhat from that expressed from the cloned kidney cDNAs. These results suggest that there may be a family of activators or subunits from different sources involved in mRNA-dependent leucine transport activity. The cloning and characterization of individual transporters or their activators should help clarify the molecular bases of the different transport properties observed following the injection of tissue mRNA into oocytes.

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REFERENCES

1. **Aoshima, H., K. Tomita, and S. Sugio.** 1988. Expression of amino acid transport systems in *Xenopus* oocytes injected with mRNA of rat small intestine and kidney. Arch. Biochem. Biophys. **265:**73–81.
2. **Aviv, H., and P. Leder.** 1972. Purification of biologically active globin messenger RNA by chromatography on oligothymidylic acid-cellulose. Proc. Natl. Acad. Sci. USA **69:**1408–1412.
3. **Bertran, J., S. Magagnin, A. Werner, D. Markovich, J. Biber, X. Testar, A. Zorzano, L. C. Kuhn, M. Palacin, and H. Murer.** 1992. Stimulation of system y^+-like amino acid transport by the heavy chain of human 4F2 surface antigen in *Xenopus laevis* oocytes. Proc. Natl. Acad. Sci. USA **89:**5606–5610.
4. **Bertran, J., A. Werner, M. L. Moore, G. Stange, D. Markovich, J. Biber, X. Testar, A. Zorzano, M. Palacin, and H. Murer.** 1992. Expression cloning of a cDNA from rabbit kidney cortex that induces a single transport system for cystine and dibasic and neutral amino acids. Proc. Natl. Acad. Sci. USA **89:**5601–5605.
5. **Blakely, R. D., H. E. Berson, R. T. Fremeau, Jr., M. G. Caron, M. M. Peek, H. K. Prince, and C. C. Bradley.** 1991. Cloning and expression of a functional serotonin transporter from rat brain. Nature (London) **354:**66–70.
6. **Boerner, P., M. Evans-Layng, H. S. Ü, and M. H. Saier, Jr.** 1986. Polarity of neutral amino acid transport and characterization of a broad specificity transport activity in a kidney epithelial cell line, MDCK. J. Biol. Chem. **261:**13957–13962.
7. **Campa, M. J., and M. S. Kilberg.** 1989. Characterization of neutral and cationic amino acid transport in *Xenopus* oocytes. J. Cell. Physiol. **141:**545–652.
8. **Christensen, H. N.** 1984. Organic ion transport during seven decades: the amino acids. Biochim. Biophys. Acta **779:**255–269.
9. **Christensen, H. N.** 1985. On the strategy of kinetic discrimination of amino acid transport systems. J. Membr. Biol. **84:**97–103.
10. **Christensen, H. N.** 1990. Role of amino acid transport and countertransport in nutrition and metabolism. Physiol. Rev. **70:**43–77.
11. **Coady, M. J., A. M. Pajor, E. M. Toloza, and E. M. Wright.** 1990. Expression of mammalian renal transporters in *Xenopus laevis* oocytes. Arch. Biochem. Biophys. **283:**130–134.
12. **Collarini, E. J., and D. L. Oxender.** 1987. Mechanisms of transport of amino acids across membranes. Annu. Rev. Nutr. **7:**75–90.
13. **Colman, A.** 1984. Translation of eukaryotic messenger RNA in *Xenopus* oocytes, p. 271–302. *In* B. D. Hames and S. J. Higgins (ed.), Transcription and translation: a practical approach. IRL Press, Washington, D.C.
14. **Guastella, J., N. Brecha, C. Weigmann, H. A. Lester, and N. Davidson.** 1992. Cloning, expression, and localization of a rat brain high-affinity glycine transporter. Proc. Natl. Acad. Sci. USA **89:**7189–7193.
15. **Guastella, J., N. Nelson, H. Nelson, L. Czyzyk, S. Keynan, M. C. Miedel, N. Davidson, H. A. Lester, and B. I. Kanner.** 1990. Cloning and expression of a rat brain GABA transporter. Science **249:**1303–1306.
16. **Gurdon, J. B., C. D. Lane, H. R. Woodland, and G. Marbaix.** 1971. Use of frog eggs and oocytes for the study of messenger RNA and its translation in living cells. Nature (London) **233:**177–182.
17. **Hediger, M. A., M. J. Coady, T. S. Ikeda, and E. M. Wright.** 1987. Expression cloning and cDNA sequencing of the Na^+/glucose co-transporter. Nature (London) **330:**379–381.
18. **Hediger, M. A., T. Ikeda, M. Coady, C. B. Gundersen, and E. M. Wright.** 1987. Expression of size-selected mRNA encoding the intestinal Na/glucose cotransporter in *Xenopus laevis* oocytes. Proc. Natl. Acad. Sci. USA **84:**2634–2637.
19. **Kilty, J. E., D. Lorang, and S. G. Amara.** 1991. Cloning and expression of a cocaine-sensitive rat dopamine transporter. Science **254:**578–579.
20. **Kim, J. W., E. I. Closs, L. M. Albritton, and J. M. Cunningham.** 1991. Transport of cationic amino acids by the mouse ecotropic retrovirus receptor. Nature (London) **352:**725–728.
21. **Kwon, O., H. M. Kwon, S. K. Hong, and J. M. Goldinger.** 1989. Size selected mRNA induces expression of *p*-aminohippurate transport in *Xenopus* oocytes. Proc. Soc. Exp. Biol. Med. **192:**205–208.
22. **Pacholczyk, T., R. D. Blakely, and S. G. Amara.** 1991. Expression cloning of a cocaine- and antidepressant-sensitive human noradrenaline transporter. Nature (London) **350:**350–353.
23. **Palacin, M., A. Werner, J. Dittmer, H. Murer, and J. Biber.** 1990. Expression of rat liver Na^+/L-alanine co-transport in *Xenopus laevis* oocytes. Biochem. J. **270:**189–195.
24. **Puissant, C., and L. M. Houdebine.** 1990. An improvement of the single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. BioTechniques **8:**148–149.
25. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed., p. 7.26–7.45. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
26. **Schwarz, W., and Q. B. Gu.** 1988. Characteristics of the Na^+/K^+-ATPase from *Torpedo californica* expressed in *Xenopus* oocytes: a combination of tracer flux measurements with electrophysiological measurements. Biochim. Biophys. Acta **945:**167–174.
27. **Shotwell, M. A., E. J. Collarini, A. Mansukhani, A. E. Hampel, and D. L. Oxender.** 1983. Isolation of Chinese hamster ovary cell mutants defective in the regulation of leucine transport. J. Biol. Chem. **258:**8183–8187.
28. **Sigel, E.** 1990. Use of *Xenopus* oocytes for the functional expression of plasma membrane proteins. J. Membr. Biol. **117:**201–221.
29. **Su, T. Z., and D. L. Oxender.** 1991. Characterization and expression of L-leucine transport in *Xenopus laevis* oocytes. FASEB J. **5:**A1188.
30. **Tarnuzzer, R. W., M. J. Campa, N. X. Qian, E. Englesberg, and M. S. Kilberg.** 1990. Expression of the mammalian system A neutral amino acid transporter in *Xenopus* oocytes. J. Biol. Chem. **265:**13914–13917.
31. **Tate, S. S., R. Urade, T. V. Getchell, and S. Udenfriend.** 1989. Expression of the mammalian Na^+-independent L system amino acid transporter in *Xenopus laevis* oocytes. Arch. Biochem. Biophys. **275:**591–596.
32. **Tate, S. S., N. Yan, and S. Udenfriend.** 1992. Expression cloning of a Na^+-independent neutral amino acid transporter from rat kidney. Proc. Natl. Acad. Sci. USA **89:**1–5.
33. **Taylor, P. M., B. Mackenzie, S. Y. Low, and M. J. Rennie.** 1992. Expression of rat liver glutamine transporters in Xenopus laevis oocytes. J. Biol. Chem. **267:**3873–3877.
34. **Thomas, E. L., and H. N. Christensen.** 1970. Indications of spatial relations among structures recognizing amino acids and Na^+ at a transport receptor site. Biochem. Biophys. Res. Commun. **40:**277–283.
35. **Thomas, E. L., T. C. Shao, and H. N. Christensen.** 1971. Structural selectivity in interaction of neutral amino acids and alkali metal ions with a cationic amino acid transport system. J. Biol. Chem. **246:**1677–1681.
36. **Van Winkle, L. J., A. L. Campione, and J. M. Gorman.** 1988. Na^+-independent transport of basic and zwitterionic amino acids in mouse blastocysts by a shared system and by processes which distinguish between these substrates. J. Biol. Chem. **263:**3150–3163.
37. **Van Winkle, L. J., A. L. Campione, and J. M. Gorman.** 1990. Changes in the activities of amino acid transport system $b^{0,+}$ and L during development of preimplantation mouse conceptuses. Biochim. Biophys. Acta **1021:**77–84.
38. **Van Winkle, L. J., A. L. Campione, J. M. Gorman, and B. D. Weimer.** 1990. Inhibition of transport system $b^{0,+}$ in blastocysts by inorganic and organic cations yields insight into the structure of its amino acid receptor site. Biochim. Biophys. Acta **1025:**215–224.
39. **Van Winkle, L. J., H. N. Christensen, and A. L. Campione.** 1985. Na^+-dependent transport of basic, zwitterionic, and bicyclic amino acids by a broad-scope system in mouse blastocysts. J. Biol. Chem. **260:**12118–12123.
40. **Weissbach, L., M. E. Handlogten, H. N. Christensen, and M. S. Kilberg.** 1982. Evidence for two Na^+-independent neutral amino

acid transport systems in primary cultures of rat hepatocytes. J. Biol. Chem. **257**:12006–12011.

41. **Wells, R. G., and M. A. Hediger.** 1992. Cloning of a rat kidney cDNA that stimulates dibasic and neutral amino acid transport and has sequence similarity to glucosidases. Proc. Natl. Acad. Sci. USA **89**:5596–5600.
42. **White, M. F., and H. N. Christensen.** 1982. Cationic amino acid transport into cultured animal cells. J. Biol. Chem. **257**:4450–4457.
43. **Yamauchi, A., S. Uchida, H. M. Kwon, A. S. Preston, R. B. Robey, A. Garcia-Perez, M. B. Burg, and J. S. Handler.** 1992. Cloning of a Na(+)- and Cl(−)-dependent betaine transporter that is regulated by hypertonicity. J. Biol. Chem. **267**:649–652.
44. **Young, J. D., D. K. Mason, and D. A. Fincham.** 1988. Topographical similarities between harmaline inhibition sites on Na^+-dependent amino acid transport system ASC in human erythrocytes and Na^+-independent system asc in horse erythrocytes. J. Biol. Chem. **263**:140–143.