The Amino Acid Transport System y^+L Induced in *Xenopus laevis* Oocytes by Human Choriocarcinoma Cell (JAR) mRNA Is Functionally Related to the Heavy Chain of the 4F2 Cell Surface Antigen†

You-Jun Fei, Puttur D. Prasad, Frederick H. Leibach, and Vadivel Ganapathy*

Department of Biochemistry and Molecular Biology, Medical College of Georgia, Augusta, Georgia 30912-2100

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ABSTRACT: Injection of mRNA isolated from human placental choriocarcinoma cells (JAR) into *Xenopus laevis* oocytes induced the transport of the neutral amino acid leucine as well as the transport of the cationic amino acid arginine. The induced transport of leucine was predominantly Na^+-dependent, whereas that of arginine was Na^+-independent. The ratio of transport activity for these amino acids in mRNA-injected oocytes versus water-injected oocytes was much greater if the transport activity was measured at pH 5.5 instead of at pH 7.5. Leucine transport in mRNA-injected oocytes was inhibited to a marked extent by arginine and lysine. The bicyclic amino acid BCH and the *N*-methyl amino acid MeAIB had no effect. Arginine transport in mRNA-injected oocytes was insensitive to *N*-ethylmaleimide and inhibited markedly by micromolar concentrations of leucine in the presence of Na^+. The inhibitory potency of leucine was reduced severalfold in the absence of Na^+. These results indicated that the arginine transport activity induced in *Xenopus* oocytes by JAR cell mRNA was due to an amino acid transport system which is identical with or highly similar to y^+L and that this system was responsible for a major portion of leucine transport activity measured in these oocytes. Northern blot analysis showed that normal placenta and JAR cells do not possess detectable levels of mRNA transcripts for D2, a protein closely related to the function of another transport system, namely $b^{o,+}$, which is also involved in the cellular uptake of neutral and cationic amino acids. On the other hand, the mRNA transcripts for the heavy chain of the 4F2 cell surface antigen are expressed at high levels in placenta and JAR cells. This protein is also known to induce the transport of neutral as well as cationic amino acids in *Xenopus* oocytes. Hybrid depletion of JAR cell mRNA using an antisense oligomer specific for the mRNA of this protein completely abolished the induction of the y^+L-like activity in oocytes. It is concluded that the 4F2 cell surface antigen is responsible for or is a component/inducer of an amino acid transport system which is most likely identical with system y^+L.

There is evidence for the presence of at least five different transport systems in animal cells which are involved in the cellular uptake of cationic amino acids (Christensen, 1989; Van Winkle, 1993; Kilberg *et al.*, 1993; Ganapathy *et al.*, 1994; McGivan & Pastor-Anglada, 1994). They are CAT-1 (y^+), CAT-2, $b^{o,+}$, $B^{o,+}$, and y^+L. Recent studies have provided important information on the molecular nature of three of these transport systems, CAT-1, CAT-2, and $b^{o,+}$. The murine receptor for the ecotropic murine leukemia virus has been shown to be functionally related to the activity of the CAT-1 system (Kim *et al.*, 1991; Wang *et al.*, 1991). The human homolog of this protein has also been identified and characterized (Yoshimoto *et al.*, 1991). At least two subtypes of the CAT-2 system are known which differ in their affinities for cationic amino acids and exhibit differential tissue expression (MacLeod *et al.*, 1990; Closs *et al.*, 1993a,b). The predicted membrane topology of CAT-1 and the subtypes of CAT-2 depict these proteins as having multiple transmembrane domains, a distinct characteristic of most known transporters.

Expression cloning in *Xenopus laevis* oocytes has recently led to the isolation of cDNAs encoding a novel class of membrane proteins which are closely related to the function of the $b^{o,+}$ system (Bertran *et al.*, 1992a; Wells & Hediger, 1992; Tate *et al.*, 1992; Lee *et al.*, 1993; Yan *et al.*, 1994). Though differently labeled (BAT for the rabbit clone and D2 or NBAT for the rat and human clones), injection of cRNAs from these cDNAs into *Xenopus* oocytes leads to induction of an amino acid transport system with characteristics similar to those described for the $b^{o,+}$ system. Unlike the proteins related to the function of the CAT system, the $b^{o,+}$-related proteins consist of fewer membrane-spanning domains, though the exact number of these domains remains controvertial (Bertran *et al.*, 1992a; Wells & Hediger, 1992; Mosckovitz *et al.*, 1994). The gene for the human homolog has been mapped to chromosome 2 (Lee *et al.*, 1993; Yan *et al.*, 1994). It has been recently reported that mutations in this gene are involved in the inherited amino acid transport defect cystinuria (Calonge *et al.*, 1994). Consistent with this are the findings that the transport system induced in *Xenopus* oocytes by the $b^{o,+}$-related proteins accepts cystine as a substrate in addition to the cationic amino acids.

Interestingly, the $b^{o,+}$ system-related proteins have significant homology to another membrane glycoprotein, namely the heavy chain of the 4F2 cell surface antigen (4F2HC) (Bertran *et al.*, 1992a; Wells & Hediger, 1992). The cDNA

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* Address correspondence to this author at Department of Biochemistry and Molecular Biology, Medical College of Georgia, Augusta, GA 30912-2100. Tel: 706-721-7652. Fax: 706-721-6608.

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encoding this protein has been isolated (Lumadue *et al.*, 1987; Quackenbush *et al.*, 1987; Teixeira *et al.*, 1987). Injection of human 4F2HC cRNA into *Xenopus* oocytes induces an amino acid transport activity with characteristics significantly different from those of D2- or BAT-induced transport activity (Bertran *et al.*, 1992b; Wells *et al.*, 1992). Thus, the 4F2HC does not appear to be related to the $b^{o,+}$ system. Although the 4F2HC-induced activity exhibits some characteristics of CATs, $b^{o,+}$, and $B^{o,+}$, the activity is not the result of activation of all these systems together. It is most likely that the activity represents a single distinct amino acid transport system. In a recent review of amino acid transport systems expressed in *Xenopus* oocytes, Van Winkle (1993) suggested that the system induced by 4F2HC is most likely y^+L.

System y^+L is the most recent addition to the group of transport systems involved in the cellular uptake of cationic amino acids in mammalian cells. Its presence has so far been described only in human erythrocytes (Deves *et al.*, 1992, 1993) and human placenta (Eleno *et al.*, 1994). This transporter shares some of the properties of the transport activity induced in *Xenopus* oocytes by D2 (or BAT) and 4F2HC. The purpose of the present investigation was to determine the functional relationship between system y^+L and D2 or 4F2HC in human placenta. The experimental approach was to functionally express placental cationic amino acid transport activity in *Xenopus* oocytes by injection of mRNA from human choriocarcinoma cells, characterize the transport activity, and assess the involvement of D2 or 4F2HC in the expression of the activity. We have chosen JAR cells for this purpose because we have previously shown that this cell line expresses several amino acid transport systems known to be present in normal placenta which include the taurine transporter (Miyamoto *et al.*, 1987; Kulanthaivel *et al.*, 1991; Ramamoorthy *et al.*, 1994), the amino acid transport system L (Ganapathy *et al.*, 1986; Ramamoorthy *et al.*, 1994), and the glycine transporter GLYT 1 (Dicke *et al.*, 1993; Liu *et al.*, 1994). The results of the investigation demonstrate that the amino acid transport system that is induced in *Xenopus* oocytes by JAR cell mRNA and exhibits characteristics similar to those of y^+L is functionally related to 4F2HC and not to D2.

MATERIALS AND METHODS

Materials. [2,3,4,5-^{3}H]Arginine (specific radioactivity, 60 Ci/mmol) and [α-^{32}P]dCTP (specific radioactivity, 3000 Ci/mmol) were obtained from Amersham (Arlington Heights, IL), and [4,5-^{3}H]leucine (specific radioactivity, 60 Ci/mmol) was obtained from Du Pont-New England Nuclear (Boston, MA). Oligo(dT)-cellulose was from Collaborative Research, Inc. (Bedford, MA). Amino acids and other chemicals were from Sigma (St. Louis, MO). *X. laevis* were purchased from Xenopus I (Ann Arbor, MI). The choriocarcinoma cell line JAR was from the American Type Culture Collection (Rockville, MD).

Oocytes and Injections. A small piece of ovarian lobe was dissected from *X. laevis* under anesthesia and incubated in a Ca^{2+}-free medium (82.5 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 5 mM Hepes/Tris, pH 7.5)[1] containing 1.6 mg/mL collagenase type 1A for 30 min at room temperature. Healthy oocytes in stages V−VI were manually defolliculated. After allowing 1 day for recovery, oocytes were injected with 50 nL of solution containing JAR cell mRNA (0.5 μg/μL) or water. Oocytes were maintained at 19 °C for the desired number of days in sterile modified Barth's saline [88 mM NaCl, 1 mM KCl, 0.82 mM $MgSO_4$, 2.4 mM $NaHCO_3$, 0.41 mM $CaCl_2$, 0.33 mM $Ca(NO_3)_2$, 10 mM Hepes/Tris, pH 7.4] supplemented with 50 μg/mL gentamycin prior to use in uptake measurements.

Uptake Measurements. Uptake of [^{3}H]leucine and [^{3}H]-arginine into oocytes was measured in a 24-well microtiter plate. Briefly, three to five oocytes were incubated at room temperature for 60 min in the desired uptake buffer (100 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, buffered with 3 mM Hepes/3 mM Mes/Tris, pH 5.5 or 7.5). Uptake was found to be approximately linear up to 60 min of incubation ($r = 0.98$). Concentration of labeled amino acids was 0.1 μM. Uptake was terminated by washing the oocytes with ice-cold uptake medium (pH 7.5) four times. Each oocyte was then dissolved in 0.2 mL of 10% SDS, and radioactivity associated with the oocyte was determined by liquid scintillation spectrometry. Each experiment was repeated at least twice.

Poly(A)$^+$ mRNA Isolation. JAR cells were cultured in RPMI-1640 medium with 10% fetal bovine serum as described (Kulanthaivel *et al.*, 1991; Ramamoorthy *et al.*, 1992). The cells were scraped and collected by centrifugation at 2500*g* for 15 min at 4 °C. The cells were washed three times with phosphate-buffered saline. Total RNA was extracted from these cells by the guanidinium isothiocyanate method and purified by centrifugation on a cesium trifluoroacetate gradient. Poly(A)$^+$ RNA was isolated from total RNA by two rounds of affinity chromatography on an oligo-(dT)-cellulose column.

Hybrid Depletion. Sense and antisense oligomer nucleotides were designed on the basis of the sequence of the 4F2HC cDNA (Lumadue *et al.*, 1987; Quakenbush *et al.*, 1987; Teixeira *et al.*, 1987) in such a way that the position of the initiation codon was in the middle of the oligomers. The sequence of the sense oligomer was 5′-CTGCAG-GCACCATGAGCCAGGAC-3′, and that of the antisense oligomer was 5′-GTCCTGGCTCATGGTGCCTGCAG-3′. JAR cell mRNA was denatured by heating at 65 °C for 5 min and incubated with the sense or antisense oligomer for 20 min at 42 °C in the presence of 50 mM NaCl prior to injection into oocytes. Concentrations of the JAR cell mRNA and the oligomers during the incubation were 0.5 and 0.025 μg/μL, respectively.

Northern Blot Analysis. The D2H (human D2) cDNA probe (a 2.3 kb *Eco*RI fragment) and the 4F2HC cDNA probe (a 1.8 kb *Eco*RI fragment) were kindly provided by Dr. Matthias A. Hediger, Department of Medicine, Harvard University, Boston, MA. The probes were radiolabeled with [α-^{32}P]dCTP by random priming using an oligolabeling kit (Pharmacia). Total RNA from normal term human placenta (obtained immediately after cesarian section) and from the outer cortex of the human kidney (obtained at an autopsy done within 12 h of death) was prepared by using RNAzol

[1] Abbreviations: NMDG, *N*-methyl-D-glucamine; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; Mes, 4-morpholineethanesulfonic acid; SDS, sodium dodecyl sulfate; SSC, sodium chloride (150 mM)−sodium citrate (15 mM); MeAIB, α-(methylamino)isobutyric acid; BCH, 2-aminobicyclo[2.2.1]heptane-2-carboxylic acid; NEM, *N*-ethylmaleimide.

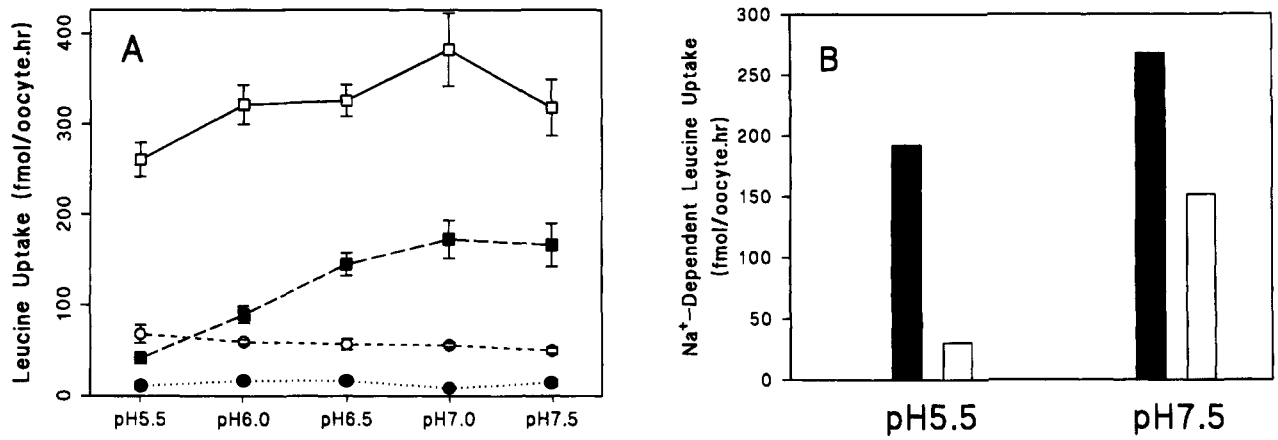

FIGURE 1: Influence of Na^+ and pH on leucine uptake in water- and mRNA-injected oocytes. Oocytes were injected with water or mRNA (25 ng), and uptake of leucine (0.1 μM) was measured with a 1 h incubation on day 6 postinjection. Uptake buffers of varying pH were prepared by changing the relative concentrations of Hepes, Mes, and Tris. All buffers contained 2 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, and either 100 mM NaCl (Na^+-containing buffer) or 100 mM NMDG-Cl (Na^+-free buffer). A. Uptake of leucine in water-injected (●, ■) and mRNA-injected (○, □) oocytes at different pH in the absence (○, ●) and presence (□, ■) of Na^+. B. Na^+-dependent leucine uptake (*i.e.*, uptake in the presence of Na^+ minus uptake in the absence of Na^+) at pH 5.5 and 7.5 in water-injected (open bars) and mRNA-injected (closed bars) oocytes.

(Biotecx) according to the manufacturer's protocol. Poly-$(A)^+$ RNA was then isolated by affinity chromatography on an oligo(dT)-cellulose column. Poly$(A)^+$ RNA was size-fractionated on a denaturing formaldehyde-agarose gel and transferred onto an activated nylon membrane (Hybond N^+, Amersham). The blots were hybridized to ^{32}P-labeled cDNA probes under high stringency conditions. Posthybridization washes included one wash with 3 × SSC−0.5% SDS at room temperature for 30 min, two washes with 3 × SSC−0.5% SDS at 55 °C for 30 min, one wash with 1 × SSC−0.1% SDS at 55 °C for 30 min, and one wash with 0.1 × SSC−0.1% SDS at 65 °C for 30 min. The blots were exposed to autoradiographic film with an intensifying screen for 2 days. Sizes of hybridizing RNAs were determined on the basis of parallel electrophoresis of RNA standards.

RESULTS AND DISCUSSION

Distinguishing Characteristics of the Cationic Amino Acid Transport Systems. The present investigation required specific experiments which would lead to unambiguous identification of each of the five known cationic amino acid transport systems, namely CAT-1 (y^+), CAT-2, $b^{o,+}$, $B^{o,+}$, and y^+L. These transport systems exhibit considerable similarity in their characteristics (Christensen, 1989; Van Winkle, 1993; Kilberg *et al.*, 1993; Ganapathy *et al.*, 1994; McGivan & Pastor-Anglada, 1994), and hence there is a significant potential for erroneous identification. A careful analysis of available information in the literature on these five transport systems revealed certain clearly distinguishing features of these systems. CATs are specific for cationic amino acids, do not interact with cystine, and are Na^+-independent. CAT-1 is known to interact weakly with certain neutral amino acids such as homoserine in the presence of Na^+. $b^{o,+}$ accepts cationic amino acids, cystine, and several neutral amino acids as substrates, but the transport of all these substrates via this system is Na^+-independent. Even though $B^{o,+}$ also interacts with cationic amino acids and neutral amino acids, there are important differences between $B^{o,+}$ and b^{o+} in substrate selectivity and Na^+ dependence. Transport of cationic amino acids as well as neutral amino acids via $B^{o,+}$ is Na^+-dependent. y^+L, like $b^{o,+}$ and $B^{o,+}$, also interacts with cationic and neutral amino acids. The distinguishing feature of y^+L however is that while the transport of cationic amino acids by this system is Na^+-independent, the transport of neutral amino acids is Na^+-dependent. The specificity of system y^+L toward neutral amino acids is similar but not identical to that of system L. Even though system y^+L as well as system y^+ interact with neutral amino acids in the presence of Na^+, the affinity of system y^+L for these amino acids is severalfold greater than that of system y^+. Especially the neutral amino acids leucine, glutamine, and methionine interact with system y^+L very strongly. The experimental approach employed in the present investigation to identify system y^+L was to study the transports of the neutral amino acid leucine and the cationic amino acid arginine, determine the Na^+ dependence of these two transport processes, and examine the mutual interaction between the two amino acids during transport.

Characteristics of Leucine Uptake in Xenopus Oocytes That Is Induced by JAR Cell mRNA. Oocytes were injected with either water or mRNA isolated from JAR choriocarcinoma cells, and uptake of leucine was studied in these oocytes on day 6 following injection. Figure 1A describes the influence of Na^+ and pH on the uptake of leucine (0.1 μM). At pH 7.5, injection of mRNA enhanced the uptake of leucine 3.4-fold in the absence of Na^+ and 1.9-fold in the presence of Na^+. The Na^+-dependent stimulation was much higher for the endogenous activity (11.4-fold; 166 ± 24 versus 15 ± 3 fmol/oocyte/h in the presence and absence of Na^+, respectively) than for the activity in mRNA-injected oocytes (6.4-fold; 318 ± 31 versus 50 ± 3 fmol/oocyte/h in the presence and absence of Na^+, respectively). Reducing the pH of the uptake medium did not have any significant effect on the Na^+-independent leucine uptake in water-injected or mRNA-injected oocytes. Interestingly, the Na^+-dependent leucine uptake was differentially affected by pH in water-injected and mRNA-injected oocytes. Reducing the pH of the uptake medium markedly decreased leucine uptake in the presence of Na^+ in water-injected oocytes. The decrease was 75% when the pH was changed from 7.5 to 5.5. Under similar conditions, the uptake in mRNA-injected oocytes was affected only minimally by the pH change. The

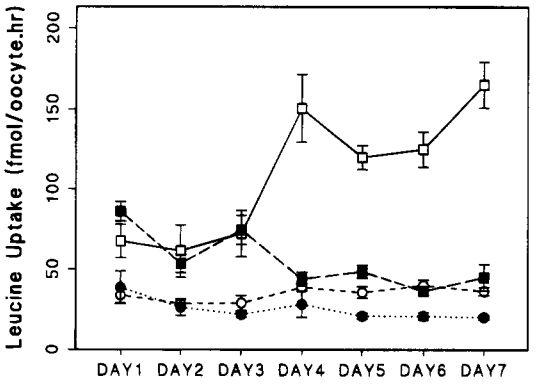

FIGURE 2: Time course of expression of leucine uptake in oocytes. Oocytes were injected with water (●, ■) or 25 ng of mRNA (○, □); 1−7 days after injection, uptake of leucine (0.1 μM) was measured with a 1 h incubation in the absence (○, ●) or presence (□, ■) of Na^+.

decrease in the uptake when the pH was changed from 7.5 to 5.5 was only 20% in oocytes injected with mRNA. Because of this differential effect of pH on the endogenous versus mRNA-induced leucine uptake, the enhancement of Na^+-dependent uptake (*i.e.*, uptake in the presence of Na^+ minus uptake in the absence of Na^+) induced by JAR cell mRNA was 1.8-fold at pH 7.5, whereas the corresponding value was 6.3 at pH 5.5 (Figure 1B). The uptake of leucine measured at pH 5.5 in mRNA-injected oocytes was predominantly Na^+-dependent, the uptake in the presence of Na^+ being 4-fold greater than the uptake in the absence of Na^+. Subsequent experiments on leucine uptake were routinely done at pH 5.5 in the presence of Na^+.

Figure 2 describes the time course of induction of the Na^+-dependent leucine uptake in *Xenopus* oocytes by JAR cell mRNA. The endogenous leucine uptake activity, whether measured in the absence or presence of Na^+, decreased with time. On the other hand, uptake in mRNA-injected oocytes increased with time, and maximal uptake was observed between days 4 and 7. Subsequent experiments were therefore done after culturing the oocytes for 6 days.

The substrate specificity of the transport process responsible for the uptake of leucine in the presence of Na^+ in water- and mRNA-injected oocytes was studied by examining the effect of various amino acids on the uptake of [^{3}H]-leucine (Table 1). The endogenous activity measured in water-injected oocytes was partially inhibitable by glutamine, alanine, and arginine but was insensitive to the bicyclic amino acid BCH. The uptake activity measured in mRNA-injected oocytes was also insensitive to BCH and MeAIB and partly inhibitable by glutamine, alanine, arginine, and lysine. An important difference however was that the uptake of leucine in mRNA-injected oocytes was more potently inhibited by glutamine and arginine than in water-injected oocytes. When the uptake of leucine that was induced by JAR cell mRNA (*i.e.*, uptake in mRNA-injected oocytes minus uptake in water-injected oocytes) was analyzed, it was found that glutamine at 1 mM inhibited the uptake almost completely (95% inhibition). The inhibition caused by 1 mM arginine was 76%. Increasing the arginine concentration to 10 mM did not increase this inhibition any further. These results show that the leucine uptake process induced in oocytes by JAR cell mRNA is Na^+-dependent and interacts with neutral as well as cationic amino acids. The process is not related to system A because of the lack of interaction with MeAIB.

Table 1: Effects of Various Amino Acids on the Uptake of [^{3}H]Leucine in *Xenopus* Oocytes Injected with Water or JAR Cell mRNA[a]

	[^{3}H]leucine uptake (fmol/oocyte/h)	
unlabeled amino acid	water	mRNA
control	60.3 ± 5.2 (100)	226.2 ± 24.8 (100)
leucine	ND	15.6 ± 1.5 (7)
glutamine	19.2 ± 0.7 (32)	27.1 ± 4.8 (12)
arginine	21.2 ± 0.8 (35)	60.8 ± 7.7 (27)
lysine	ND	64.0 ± 6.2 (28)
alanine	35.5 ± 1.5 (59)	136.2 ± 27.2 (60)
MeAIB	ND	232.1 ± 35.5 (103)
BCH	57.6 ± 2.5 (96)	249.6 ± 38.2 (110)

[a] Oocytes were injected with water or JAR cell mRNA, and uptake of [^{3}H]leucine (0.1 μM) was measured in the presence of Na^+ and at pH 5.5 on postinjection day 6. When present, concentration of unlabeled amino acids was 1 mM. Results are given as means ±SE for 6−10 oocytes. Values in parantheses are percent of the respective control uptake. ND, not determined.

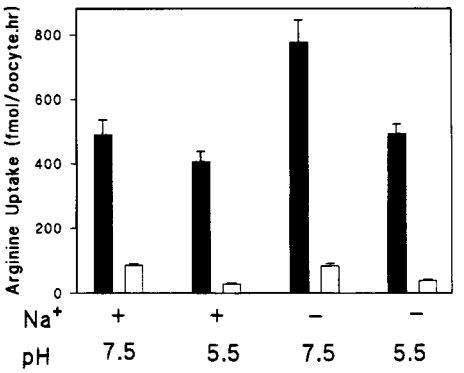

FIGURE 3: Influence of pH and Na^+ on arginine uptake in water- and mRNA-injected oocytes. Oocytes were injected with water (open bars) or 25 ng of mRNA (closed bars). Uptake of arginine (0.1 μM) was measured with a 1 h incubation on day 6 postinjection at pH 7.5 or 5.5. Uptake buffer contained either NaCl (Na^+-containing buffer) or an equimolar concentration of NMDG-Cl (Na^+-free buffer).

The Na^+-dependent characteristic and the lack of interaction with BCH exclude the involvement of system L. The marked inhibition of the uptake process by glutamine and arginine indicated that system $B^{o,+}$ and/or system y^+L is/are at least partly responsible for the process.

Characteristics of Arginine Uptake in Xenopus Oocytes That Is Induced by JAR Cell mRNA. To evaluate the participation of system $B^{o,+}$ and/or system y^+L in the Na^+-dependent uptake of leucine measured in mRNA-injected oocytes, we studied the characteristics of the uptake of the cationic amino acid arginine. First, we examined the effect of Na^+ and pH on arginine uptake in water- and mRNA-injected oocytes (Figure 3). The uptake was found to be Na^+-independent, whether the uptake was measured at pH 7.5 or 5.5. Injection of JAR cell mRNA enhanced the uptake at both pH values, but the magnitude of enhancement was significantly greater at pH 5.5 than at pH 7.5. The mRNA-induced stimulation of arginine uptake was 6−9-fold at pH 7.5 and 13−15-fold at pH 5.5. The greater increase seen at pH 5.5 was primarily due to a preferential decrease in the endogenous arginine uptake activity.

The substrate specificity of the transport process responsible for the uptake of arginine in water- and mRNA-injected oocytes was studied by examining the effect of various amino

Table 2: Effects of Various Amino Acids on the Uptake of [^{3}H]Arginine in *Xenopus* Oocytes Injected with Water or JAR Cell mRNA[a]

unlabeled amino acid	[^{3}H]arginine uptake (fmol/oocyte/h)	
	water	mRNA
control	74.8 ± 5.4 (100)	245.4 ± 21.4 (100)
leucine	6.4 ± 0.3 (9)	12.3 ± 1.2 (5)
glutamine	9.6 ± 1.4 (13)	11.5 ± 0.9 (5)
lysine	ND	8.3 ± 1.0 (3)
alanine	36.1 ± 2.8 (48)	65.7 ± 6.9 (27)
BCH	63.8 ± 4.0 (85)	232.6 ± 18.0 (95)

[a] Oocytes were injected with water or JAR cell mRNA, and uptake of [^{3}H]arginine (0.1 μM) was measured in the presence of Na^+ and at pH 5.5 on postinjection day 6. When present, concentration of unlabeled amino acids was 1 mM. Results are given as means ±SE for 6−10 oocytes. Values in parantheses are percent of the respective control uptake. ND, not determined.

acids on the uptake of [^{3}H]arginine (Table 2). The uptake in water-injected oocytes was almost completely inhibitable by glutamine and leucine. Alanine caused moderate inhibition, and BCH showed very little inhibitory effect. The uptake in mRNA-injected oocytes was also completely inhibited by leucine and glutamine, moderately inhibited by alanine, and insensitive to BCH. The Na^+-independent nature of the uptake process in water- and mRNA-injected oocytes indicated the noninvolvement of system $B^{o,+}$ in the process. The Na^+-independent nature of the arginine uptake and the strong interaction of the neutral amino acids leucine and glutamine with the uptake process are compatible with involvement of system y^+L in the process.

The experiments with regard to the interaction of amino acids with the arginine uptake process (Table 2) were carried out in the presence of Na^+. A distinct characteristic of system y^+L is that the neutral amino acids such as leucine, glutamine, and methionine interact with the system with high affinity but only in the presence of Na^+. Therefore, we studied the influence of Na^+ on the inhibition of arginine uptake by leucine in oocytes injected with JAR cell mRNA (Figure 4). In the presence of Na^+, leucine at a concentration of 10 μM inhibited the uptake of arginine by 60%. At 1 mM leucine, the inhibition was 92%. Thus, the affinity of the arginine uptake process in mRNA-injected oocytes is very high for leucine in the presence of Na^+. When Na^+ was absent, the affinity decreased dramatically. Leucine at 10 μM had no effect on the uptake of arginine in the absence of Na^+. Much higher concentrations of leucine were required to cause significant inhibition of arginine uptake. Even at a concentration of 1 mM, the inhibition caused by leucine was only 65−70%. The high affinity of the arginine uptake process for leucine in the presence of Na^+ strongly suggests that system y^+L may be responsible for the uptake process. Even though system y^+ is known to interact with neutral amino acids in the presence of Na^+, the interaction is very weak. Therefore, it is unlikely that system y^+ contributes to arginine uptake in mRNA-injected oocytes to any significant extent. Another distinguishing characteristic between system y^+L and system y^+ is that while the former is insensitive to the thiol reagent *N*-ethylmaleimide (NEM), the latter is inhibitable by this reagent (Deves *et al.*, 1993). Therefore, we studied the effect of NEM on the uptake of arginine in water- and mRNA-injected oocytes. The oocytes were treated with or without 0.25 mM NEM for 15 min at room temperature, following which uptake of arginine (0.1 μM) was measured. In water-injected oocytes, the uptake was 57.6 ± 3.4 fmol/oocyte/h in the absence of NEM treatment which remained almost the same (48.3 ± 3.8 fmol/oocyte/h) in the presence of NEM treatment. Similarly, the uptake was insensitive to NEM in mRNA-injected oocytes (absence of NEM treatment, 183.1 ± 15.2 fmol/oocyte/h; presence of NEM treatment, 190.4 ± 15.3 fmol/oocyte/h). Under these experimental conditions, NEM is known to cause complete inhibition of system y^+ in erythrocytes (Deves *et al.*, 1993).

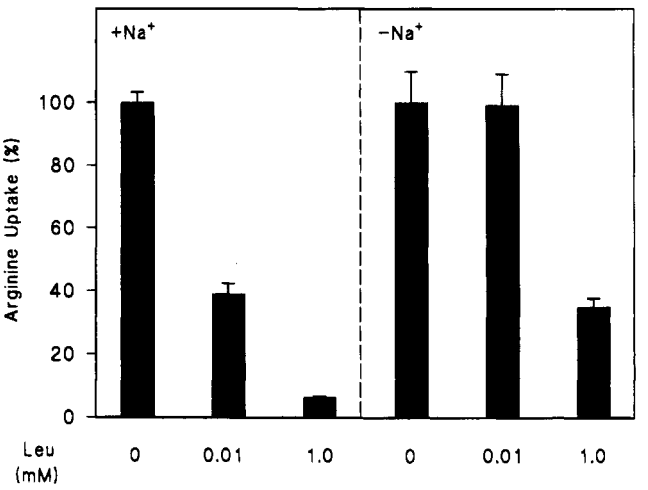

FIGURE 4: Influence of Na^+ on the inhibition of arginine uptake by leucine in mRNA-injected oocytes. Oocytes were injected with 25 ng of mRNA, and uptake of arginine (0.1 μM) was measured with a 1 h incubation on day 6 postinjection at pH 5.5 in the presence or absence of Na^+. Na^+-free buffers contained NMDG-Cl instead of NaCl. The uptake of arginine measured in the absence of leucine was taken as 100%. This value was 270.6 ± 21.3 fmol/oocyte/h in the presence of Na^+ and 332.7 ± 31.2 fmol/oocyte/h in the absence of Na^+.

The characteristics of the transport process studied thus far in this investigation can be summarized as follows. Injection of JAR cell mRNA into *Xenopus* oocytes induces a transport process which accepts neutral as well as cationic amino acids as substrates. The uptake of the neutral amino acid leucine is Na^+-dependent, whereas the uptake of the cationic amino acid arginine is Na^+-independent. These two amino acids are mutually inhibitory. The uptake of leucine is inhibitable by arginine to a marked extent, though not completely. Similarly, the uptake of arginine is almost totally inhibitable by leucine. However, even though the uptake of arginine does not require Na^+, the presence of Na^+ is necessary for maximal inhibition of arginine uptake by leucine. The arginine uptake process interacts with leucine with high affinity in the presence of Na^+. These characteristics suggest that the JAR cell mRNA-induced arginine uptake is mediated by an amino acid transport system that is identical with or highly similar to system y^+L. The uptake of leucine measured in mRNA-injected oocytes is mediated by this system to a large extent. However, it appears that JAR cell mRNA induces in oocytes additional transport systems which also contribute to leucine uptake. This is evident from the inability of arginine to block leucine uptake completely. Even in the presence of 10 mM arginine, an appreciable fraction (~20%) of leucine uptake remained unaffected. Furthermore, the inhibition studies with alanine indicated the presence of heterogeneity in leucine uptake in mRNA-injected oocytes (Tables 1 and 2). The most

distinguishing characteristic of system y^+L is the differential role of Na^+ in the transport of cationic and neutral amino acids. This interesting transport system, so far described only in human erythrocytes (Deves *et al.*, 1992, 1993) and human placenta (Eleno *et al.*, 1994), mediates the uptake of cationic amino acids by a completely Na^+-independent mechanism but requires Na^+ to mediate the uptake of neutral amino acids. The role of Na^+ in y^+L-mediated neutral amino acid transport is to increase the affinity of the system for these amino acids. The potency of neutral amino acids to compete with cationic amino acids for the transport process decreases 60−90-fold when Na^+ is replaced by K^+. Although this system was originally named y^+L because of its interaction with leucine (Deves *et al.*, 1992), it also interacts with methionine and glutamine with comparable affinity. Interestingly, the bicyclic amino acid BCH is conspicuously excluded by system y^+L. The amino acid transport system that is induced in *Xenopus* oocytes by JAR cell mRNA and responsible for the uptake of arginine and, to a large extent, leucine as well exhibits all of the above-described characteristics ascribed to system y^+L. Therefore, we conclude that the JAR cell mRNA-induced arginine uptake system in *Xenopus* oocytes observed in this study is most likely system y^+L.

Evidence for Expression of 4F2HC But Not D2 in Human Placenta and JAR Cells. Recent studies have revealed that 4F2HC and D2 are involved in the transport of cationic amino acids (Bertran *et al.*, 1992a; Wells & Hediger, 1992; Tate *et al.*, 1992; Lee *et al.*, 1994; Yan *et al.*, 1994). The transport systems induced by these proteins in *Xenopus* oocytes accept cationic as well as neutral amino acids as substrates. However, they differ in the involvement of Na^+. The D2-induced system shows no requirement for Na^+ whether the transported substrate is a cationic or neutral amino acid. In contrast, the 4F2HC-induced system catalyzes the transport of cationic amino acids in a Na^+-independent manner and the transport of neutral amino acids in a Na^+-dependent manner. The characteristics of the 4F2HC-induced transport system are thus very similar to those of the transport system studied in this investigation. The expression of D2 appears to be restricted only to the intestine and kidney (Bertran *et al.*, 1992a; Wells & Hediger, 1992), whereas the expression of 4F2HC is evident in several tissues (Parmacek *et al.*, 1989). There is no information available on the expression of these two proteins in human placenta. In order to assess the relationship between these proteins and the transport of cationic amino acids in placenta, we probed the placental and JAR cell mRNA for the presence of 4F2HC and D2 mRNA transcripts (Figure 5). The human 4F2HC cDNA probe was found to hybridize to a 2.1 kb mRNA species in the placenta, JAR cell, and kidney. But, there was no evidence for the presence of D2 mRNA transcripts in the placenta and JAR cell. As shown by other investigators (Bertran *et al.*, 1992a; Lee *et al.*, 1993), the human D2 cDNA probe used here however hybridized to a 2.3 kb mRNA species in human kidney. These data show that 4F2HC but not D2 is expressed in the placenta and JAR choriocarcinoma cells. Since the properties of the amino acid transport system induced in *Xenopus* oocytes by JAR cell mRNA resemble those of y^+L and are similar to the characteristics of the 4F2HC-induced system, the expression of 4F2HC in these cells raises the possibility that y^+L and 4F2HC are functionally related.

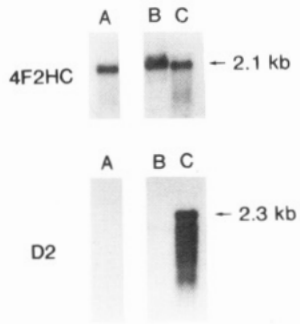

FIGURE 5: Northern blot analysis of mRNA from human kidney, human placenta, and JAR cell with cDNA probes of 4F2HC and D2H. Poly(A)$^+$ RNA (6 μg) from human placenta (lane A), JAR cell (lane B), and human kidney (lane C) was size-fractionated and probed with ^{32}P-labeled 4F2HC cDNA or ^{32}P-labeled D2H cDNA by sequential hybridization. The indicated RNA sizes were based on parallel electrophoresis of RNA standards.

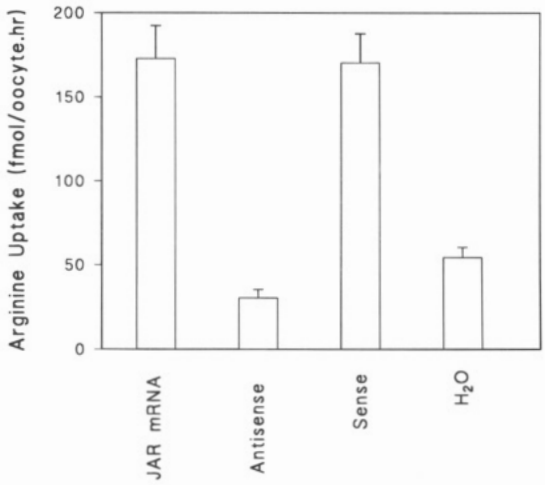

FIGURE 6: Hybrid depletion of JAR cell mRNA-induced arginine uptake with an antisense oligomer specific for 4F2HC. Oocytes were injected with water, 25 ng of mRNA, or 25 ng of mRNA hybridized with 1.25 ng of sense or antisense oligomer specific for 4F2HC. Uptake of arginine (0.1 μM) was measured in oocytes on day 6 following injection in the presence of Na^+. The pH of the uptake buffer was 5.5, and the incubation time for uptake measurement was 1 h.

Influence of Hybrid Depletion of 4F2HC mRNA from JAR Cell mRNA on the Induction of the y^+L-like System in Xenopus Oocytes. If the y^+L-like system activity induced in *Xenopus* oocytes by JAR cell mRNA is due to the 4F2HC mRNA transcripts, hybrid depletion of these transcripts should abolish the mRNA-induced activity. Figure 6 describes the results of the hybrid depletion experiment. JAR cell mRNA was incubated with a sense and an antisense oligomer to a region comprising the initiation codon of the 4F2HC mRNA and then injected into oocytes. Incubation of mRNA with the sense oligomer did not have any effect on the mRNA-induced arginine uptake, whereas incubation of mRNA with the antisense oligomer completely blocked the induction of arginine uptake, strongly suggesting that 4F2HC mRNA is responsible for the induction of the transport system which is most likely identical with y^+L.

The results of the present investigation on the relationship between the cationic amino acid transport system y^+L and 4F2HC are significant for several reasons. Transport of cationic amino acids across the placenta plays a very important role in the growth and development of the fetus. Lysine is an essential amino acid. Although arginine may

not be essential in adults, this amino acid is very much essential during the early stages of development because of the incomplete development of the biosynthetic capacity for this amino acid. Thus, arginine is essential for fetal development. These two amino acids have to be supplied to the fetus by the mother via transfer across the placenta. In addition, arginine is the precursor for the synthesis of nitric oxide, an important regulator of cellular function (Bredt & Snyder, 1994). Therefore, the mechanisms responsible for the transfer of lysine and arginine across the placenta assume great importance. Transport of cationic amino acids has been characterized in placental brush border and basal membrane vesicles (Furesz *et al.*, 1991; Eleno *et al.*, 1994). The transport system y^+L is present in both membranes (Eleno *et al.*, 1994). An interesting observation is that the abundance of y^+L in the basal membrane is manyfold greater than in the brush border membrane. Since the activity of the y^+L system is minimally affected by membrane potential, it has been suggested that this transporter might be primarily involved in the efflux of cationic amino acids from the syncytiotrophoblast into fetal circulation across the basal membrane (Eleno *et al.*, 1994).

The current investigation is the first known instance of the functional expression of the y^+L system in *Xenopus* oocytes with exogenous mRNA. The transport of arginine that is induced in oocytes following injection of JAR cell mRNA is predominantly catalyzed by y^+L. This induction is completely abolished by hybrid depletion of 4F2HC mRNA. Northern blot analysis provides clear evidence for the presence of 4F2HC mRNA transcripts in JAR cells. Therefore, the activity of system y^+L is functionally related to 4F2HC. Two independent studies have shown that injection of 4F2HC cRNA induces a unique amino acid transport system (Bertran *et al.*, 1992b; Wells *et al.*, 1992). The identity of this system was not established in these studies, but a careful analysis of the results of these studies reveals a close similarity between the 4F2HC-induced system and the y^+L system (Van Winkle, 1993). Interestingly, the activity of the system, measured as the Na^+-dependent leucine uptake, in cRNA-injected oocytes was only 3−4-fold greater than in water-injected oocytes. In the present investigation, injection of total mRNA from JAR cells has been shown to enhance the y^+L activity 2-fold at pH 7.5 and 6-fold at pH 5.5. This is a surprisingly high level of induction when compared to the results obtained with cRNA. The 4F2HC exists as a heterodimer with a highly hydrophobic light chain subunit, linked together by disulfide bonds (Hemler & Strominger, 1982). The induction of y^+L system in *Xenopus* oocytes following injection of the human 4F2HC cRNA may be due to the association of the human heavy chain subunit with the endogenous light chain subunit present in *Xenopus* oocytes. There is evidence for endogenous expression of system y^+L activity in *Xenopus* oocytes (Campa & Kilberg, 1989; Van Winkle, 1993). The association between the human 4F2HC and the *Xenopus* light chain may not be very efficient due to species differences in the structure of the light chain. This might explain the relatively poor induction of the transport activity seen with 4F2HC cRNA. In the present investigation, total mRNA from JAR cells was injected into the oocytes. Since this mRNA sample might contain the transcripts for 4F2HC as well as the light chain subunit, injection of total mRNA may lead to the expression of 4F2HC as well as the homologous human light chain. The efficient interaction between these two subunits might underlie the relatively high level of induction of the y^+L activity observed in this study. The molecular nature of the light chain subunit remains unknown.

There is substantial evidence indicating that the 4F2 antigen, comprised of both the heavy chain and the light chain, is intimately involved in the regulation of intracellular calcium levels (Michalak, 1986; Bron *et al.*, 1986; Posillico *et al.*, 1987). Our results show that this antigen is functionally related to the cationic amino acid transport system y^+L. It is not known whether the antigen is identical with the transport protein or alternatively represents an essential component or an activator of the transport system. Whatever may be the mode of action, the antigen plays a critical role in the uptake of cationic amino acids in cells expressing this antigen. Since arginine is the precursor of nitric oxide, the 4F2 antigen might modulate the intracellular production of this second messenger through its role in the cellular uptake of the precursor amino acid. There is evidence for cross-talk between nitric oxide and intracellular calcium levels (Schuman & Madison, 1994). The role of the 4F2 antigen in the cellular uptake of arginine might explain the observed involvement of this antigen in the regulation of calcium levels inside the cell.

The absence of detectable levels of D2 mRNA in human placenta and JAR cells is interesting. Mutations in the D2 gene have been shown to be responsible for the inherited amino acid transport defect cystinuria (Calonge *et al.*, 1994). The lack of D2 gene expression in normal human placenta suggests that the placental transport of cationic amino acids would not be affected in cystinuric mothers. Since these amino acids are essential for the growth and development of the fetus, the absence of functional expression of the cystinuria gene in normal placenta is clearly advantageous to the fetus.

In summary, injection of mRNA from human choriocarcinoma (JAR) cells into *X. laevis* oocytes leads to a robust induction of a cationic amino acid transport activity which is most likely identical with system y^+L on the basis of its functional characteristics. This system is functionally related to the heavy chain of the 4F2 cell surface antigen. Human placenta and JAR cells possess 4F2HC mRNA transcripts. It is concluded that the 4F2 antigen is responsible or is a component/inducer of an amino acid transport system which is most likely identical with system y^+L.

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