Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 5601–5605, June 1992
Physiology

Expression cloning of a cDNA from rabbit kidney cortex that induces a single transport system for cystine and dibasic and neutral amino acids

JOAN BERTRAN*†, ANDREAS WERNER*, MARILYN L. MOORE*, GERTI STANGE*, DANIEL MARKOVICH*, JÜRG BIBER*, XAVIER TESTAR†, ANTONIO ZORZANO†, MANUEL PALACIN*†, AND HEINI MURER*‡

*Institute of Physiology, University of Zürich, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland; and †Department of Biochemistry and Physiology, University of Barcelona, Avda. Diagonal 645, E-08028 Barcelona, Spain

Communicated by Maurice B. Burg, February 21, 1992 (received for review December 16, 1991)

ABSTRACT **We have isolated a cDNA clone by screening a rabbit kidney cortex cDNA library for expression of sodium-independent transport of L-arginine and L-alanine in *Xenopus laevis* oocytes. Expressed uptake relates to a single component of sodium-independent transport for dibasic and neutral amino acids. This transport activity resembles the functionally defined system $b^{0,+}$ and carries cystine and dibasic amino acids with high affinity. The rBAT ($b^{0,+}$ amino acid transporter-related) mRNA is found mainly in kidney and intestinal mucosa. It encodes a predicted 77.8-kDa protein with only one putative transmembrane domain and seven potential N-glycosylation sites. This protein could either be a constitutive element or a specific activator of system $b^{0,+}$.**

Renal proximal tubular and small intestinal epithelial cells are involved in vectorial transcellular fluxes of different groups of amino acids. The basolateral membranes of proximal tubular and small intestinal epithelial cells are believed to contain amino acid carriers that are similar to those described for nonpolarized cells, whereas in the brush border membranes additional transport systems are present (1). Among the latter, a sodium-independent transport activity for neutral and dibasic amino acids has been described (2); it has been suggested that this activity is related to that of system $b^{0,+}$, as first defined in mouse blastocysts (3). In general, our knowledge about the structural identity of the different amino acid transport systems is limited. Recently, however, the system y^+ for dibasic amino acids has been identified as the previously known receptor for the ecotropic murine leukemia virus (4, 5).

Two observations made it possible to clone a cDNA from rabbit kidney that encodes a protein related to amino acid transport (6): (*i*) Injection of poly(A)$^+$ RNA from rabbit kidney cortex into *Xenopus laevis* oocytes resulted in the expression of saturable sodium-independent uptake of L-arginine and L-alanine; these uptake activities showed inhibition characteristics that suggest preferential expression of system $b^{0,+}$. (*ii*) After size selection of poly(A)$^+$ RNA by sucrose density gradient centrifugation, the mRNA coding for the expressed amino acid uptakes was found in the 1.8- to 2.4-kilobase (kb) fractions. Increased amino acid uptake activities have been reported by others in oocytes injected with mRNA from rat and rabbit kidney and intestine (e.g., refs. 7 and 8).

In the present study, we have isolated a rabbit kidney cDNA clone[§] (named rBAT; related to $b^{0,+}$ amino acid transporter) that, upon *in vitro* transcription and injection in *X. laevis* oocytes, induces a single sodium-independent transport system for dibasic and neutral amino acids, including cystine. The characteristics of this amino acid transport system strongly resemble system $b^{0,+}$ activity. The rBAT mRNA is found mainly in kidney and intestinal mucosa. Wells and Hediger (9) have independently isolated a rat kidney cDNA clone that is highly homologous to rBAT (79% identity in the nucleotide sequence) and that induces a transport activity in the oocytes that resembles also system $b^{0,+}$; their results are reported in the preceding paper (9).

METHODS

***X. laevis* Oocytes and Amino Acid Transport Assay.** All techniques and methods concerning the handling of oocytes as well as the assay for transport activity have been described in detail (6). Oocytes were injected with 2.5 ng of rBAT complementary RNA (cRNA), and 3–4 days later amino acid uptake was measured for 10 min (L-arginine and L-alanine) or 5 min (L-cystine and L-leucine), because time course of uptake studies showed linearity during these time periods (data not shown). When L-cystine was used, uptake solutions contained 10 mM diamide. When L-cysteine was used as an inhibitor, 10 mM dithiothreitol was present in the uptake solution. The stop solution described in ref. 6 was modified to increase the concentrations of unlabeled amino acids (L-arginine and L-leucine) from 2 mM to 20 mM to eliminate a binding component in L-[^{35}S]cystine and L-[4,5-^{3}H]leucine uptakes. This modification did not alter the uptake values for L-[2,3-^{3}H]arginine and L-[3-^{3}H]alanine, which did not show a binding component. All radiochemicals were from New England Nuclear and were used at a specific activity of 10–50 μCi/ml (1 Ci = 37 GBq).

cDNA Cloning. We used the rabbit kidney cortex expression library described in ref. 10. This library was constructed with cDNAs ≥2 kb and was unidirectionally ligated into Bluescript SK$^+$; *in vitro* transcription was performed as described (10). cRNA was dissolved in water (0.3–0.5 μg/μl) and injected (50 nl) into oocytes. Uptakes of L-[2,3-^{3}H]arginine and L-[3-^{3}H]alanine were assayed 3 days later. Initially, pools of about 1000 clones were tested. The only positive group was subdivided until a single clone (rBAT) stimulating L-alanine and L-arginine transport was obtained.

The sequencing of rBAT cDNA was performed in both directions directly in Bluescript SK$^+$. We generated a series of subclones by cutting the insert (rBAT) with different restriction endonucleases and subcloning the fragments obtained. To complete the sequence, oligonucleotides were used as primers. Sequencing was carried out by the chain-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: α-AIB, 2-aminoisobutyric acid; MeAIB, 2-(methylamino)isobutyric acid; cRNA, complementary RNA; 4F2hc, 4F2 surface antigen heavy chain.

‡To whom reprint requests should be addressed.

§The sequence reported in this paper has been deposited in the GenBank data base (accession no. M90096).

termination method (11) using a T7 polymerase sequencing kit (Pharmacia).

Northern Blot Analysis. RNA was extracted from different tissues according to the protocol described elsewhere (6), and when specified poly(A)$^+$ RNA was purified by oligo(dT) chromatography (Boehringer Mannheim). Total RNA (25 μg) or poly(A)$^+$ RNA (10 μg) was denatured and loaded onto 1% agarose/formaldehyde gels; the gels were transferred to a GeneScreen nylon membrane (DuPont) by a vacuum procedure. Blots were prehybridized and hybridized in 50% formamide. After hybridization, blots were washed four times in 2× standard saline citrate/0.1% SDS at room temperature and then twice for 10 min each in 0.4× standard saline citrate/0.1% SDS at 55°C. The cDNA probe for rBAT was a 2140-base-pair (bp) *Bam*HI/*Kpn* I fragment, lacking 117 bp in the 5′ end of the complete rBAT cDNA.

RESULTS AND DISCUSSION

A single clone (rBAT) was isolated by screening for expression of sodium-independent L-arginine and L-alanine uptake in *X. laevis* oocytes (data not shown). Injection of cRNA synthesized from this clone increased the uptake of L-arginine and L-alanine 50 and 30 times over background, respectively, and that of L-cystine and L-leucine 250 and 50 times, respectively (Fig. 1). On the contrary, expression of 2-aminoisobutyric acid (α-AIB) and L-glutamic acid uptake activity was only slightly positive, and there was no expression of 2-(methylamino)isobutyric acid (MeAIB) uptake in those oocytes.

To characterize all these induced uptake activities, we measured their inhibition by different amino acids at a 100-fold excess concentration (5 mM). As shown in Fig. 2, dibasic and neutral L amino acids inhibited the uptake of all the assayed amino acids in the same order: dibasic L amino acids and L-leucine > L-phenylalanine > L-histidine and unbulky amino acids including amino acids that branch at the β-carbon >> α-AIB and 2-amino-2-norbornanecarboxylic acid > proline and MeAIB. Interestingly, the pattern of inhibition of the expressed uptake of L-cystine is similar to that of the other tested substrates. L-Cystine at 200 μM inhibited the uptake of all the substrates tested. This pattern of expressed uptakes and their inhibitions resemble $b^{0,+}$ activity (3) and is not compatible with the induction of systems y^+ and L, which are two sodium-independent transport pathways for dibasic and neutral amino acids, respectively, that are present in most mammalian cells (12). System y^+ does not transport L-leucine in the absence of sodium (4, 5), and for system L, 2-amino-2-norbornanecarboxylic acid is a specific analogue with high affinity for the carrier (in the micromolar range), whereas dibasic L amino acids show very low interaction with it (12).

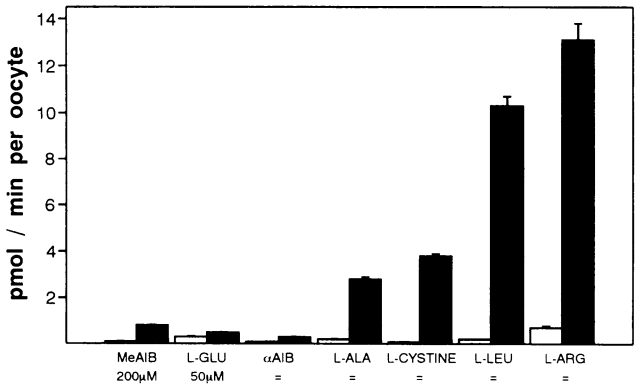

FIG. 1. Amino acid transport by water and rBAT cRNA-injected oocytes. Substrate concentrations were 50 μM for L-glutamic acid, α-AIB, L-alanine, L-cystine, L-leucine, and L-arginine and 200 μM for MeAIB. Each bar is the mean ± SEM of the uptake values in water (open bars) and in cRNA-injected oocytes (solid bars) measured in seven oocytes.

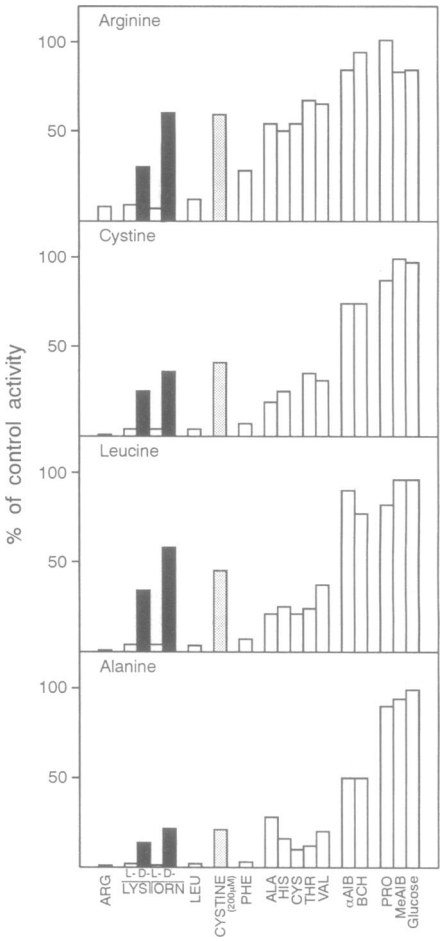

FIG. 2. Inhibition of rBAT-induced transport activity by different amino acids. The rBAT-induced transport activities (for L-arginine, L-cystine, L-leucine, and L-alanine), calculated by subtracting the uptake values obtained in water-injected oocytes from the uptake values in cRNA-injected oocytes, were tested for inhibition by different amino acids and glucose at a concentration of 5 mM and by L-cystine at 200 μM. L Isomers and nonenantiomeric amino acids [α-AIB, MeAIB, and 2-amino-2-norbornanecarboxylic acid (racemic)] are represented by open bars; D isomers, by solid bars; and L-cystine, by shaded bars. Uptake was measured at 50 μM substrate concentration. Each data point is the mean of values obtained in seven oocytes and is expressed as the residual percentage of uptake. Basal values of uptake (mean ± SEM), expressed in pmol/min per oocyte, were 11.0 ± 0.5 (n = 10 independent experiments) for L-arginine, 5.2 ± 0.6 (n = 5 independent experiments) for L-cystine, 7.8 ± 1.4 (n = 5 independent experiments) for L-leucine, and 1.7 ± 0.3 (n = 4 independent experiments) for L-alanine.

The induced uptake of L-arginine (10 μM–2 mM), L-leucine (10 μM–10 mM), and L-cystine (10–200 μM) in the rBAT cRNA-injected oocytes showed complete saturation and was independent of the presence of sodium (100 mM) in the uptake media (data not shown). Apparent kinetic parameters for these substrates were measured (Table 1). V_{max} values for L-arginine and for L-leucine were ≈3 times higher than V_{max} values for L-cystine uptake, whereas all three substrates showed K_m values in the range of 100 μM (Table 1). To further examine whether the amino acid transport activity expressed in the rBAT cRNA-injected oocytes corresponds to a single component of transport, Dixon plot inhibition analysis of the uptake of L-arginine, L-cystine, and L-leucine was performed (Table 1). All the amino acids tested as

Table 1. Kinetic parameters for the rBAT-induced transport activity

Substrate	V_{max}, pmol/min per oocyte	K_m or K_i, mM						
		L-Arginine	L-Cystine	L-Leucine	L-Lysine	L-Ornithine	L-Alanine	D-Ornithine
L-Arginine	29	0.105	0.184	0.199	0.298	0.222	4.9	3.7
L-Cystine	10	0.047	0.060	0.172	0.106	0.197	0.8	7.6
L-Leucine	35	0.056	0.090	0.128	0.075	0.199	1.3	3.8

The transport activity induced in the rBAT cRNA-injected oocytes is a single component. Kinetic parameters for the induced activity of transport and inhibition of transport of L-arginine, L-cystine, and L-leucine are given. Values of K_m are reported when an amino acid is listed as its own inhibitor. Oocytes were prepared and injected; uptakes were measured as described in *Methods*. To obtain the K_i values, Dixon plot analysis was performed by using three different concentrations of substrate against four different concentrations of inhibitor, correcting osmolarities with choline chloride. For each concentration of substrate used, regression lines of the Dixon plots were estimated by minimal squares. Then, for each pair of curves, the value of $[I] = -K_i$ was calculated when $1/v_1 = 1/v_2$, where v_1 and v_2 are substrate influxes at S_1 and S_2 concentrations of substrate, respectively, and [I] is the inhibitor concentration. K_i values are the means of the three values given by each experiment. K_m and V_{max} values correspond to the average of two independent experiments.

inhibitors showed Dixon plots compatible with competitive inhibition (data not shown). The K_i values reached in these studies, as well as the K_m values for L-arginine, L-cystine, and L-leucine, were in the same range (50–200 μM) as the K_i values for the other two dibasic L amino acids (lysine and ornithine) tested. L-Alanine as well as the D isomer of ornithine show competitive inhibition, with K_i values in the millimolar range for the three substrates tested. In conclusion, rBAT cRNA induces in oocytes a homogeneous single transport activity with high affinity for L-cystine, dibasic L amino acids, and L-leucine. These observations are compatible with expression of system $b^{0,+}$.

To eliminate the possibility that rBAT may induce the recently identified system y^+ (4, 5), we searched for inhibition of the expressed uptake of L-arginine in water-injected and rBAT cRNA-injected oocytes by L-homoserine (Fig. 3). Identical results were obtained using L-cystine as a substrate (data not shown); L-homoserine inhibits system y^+ only in the presence of sodium (12). The expressed uptake of 50 μM L-arginine (and 50 μM L-cystine) in the cRNA-injected oocytes was inhibited by 5 mM L-homoserine to the same extent in the absence and in the presence of sodium (100 mM). This suggests that system y^+ is absent in the expressed transport activity of the rBAT cRNA-injected oocytes. On the contrary, the uptake of L-arginine in the water-injected oocytes was unaffected by the inhibitor in the absence of sodium and clearly inhibited in its presence, confirming previous observations on the presence of endogenous system y^+ activity (6, 13). Under our uptake conditions, the $b^{0,+}$-like system observed in the rBAT cRNA-injected oocytes is virtually nonfunctional in water-injected oocytes—i.e., L-arginine uptake was not inhibited by a 10-fold excess of L-leucine (6), and the uptake of L-cystine was very small (0.02 pmol/min per oocyte at 50 μM L-cystine) and was not saturable (data not shown).

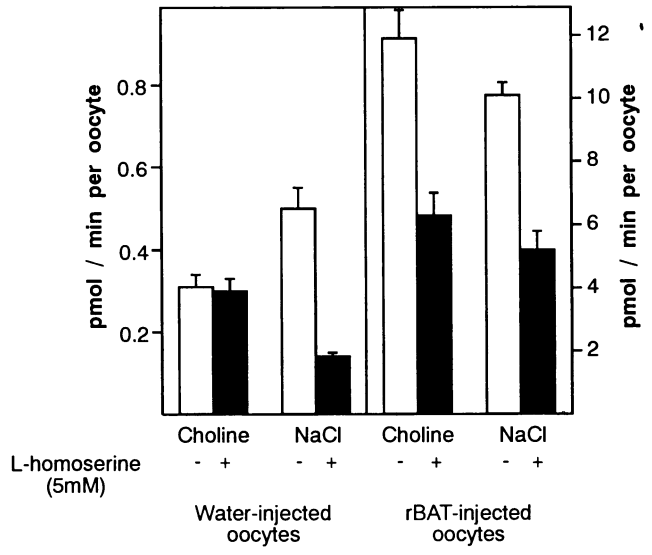

FIG. 3. Inhibition of L-arginine uptake in water and cRNA-injected oocytes by L-homoserine, in the presence and absence of sodium. Each data point is the mean + SEM from seven oocytes. L-Arginine (50 μM) uptake was measured in the presence of sodium chloride (100 mM) or choline chloride (100 mM) and in the presence (solid bars) or absence (open bars) of L-homoserine (5 mM).

Tissue expression of the mRNA corresponding to rBAT was examined by Northern blot analysis (Fig. 4). In the rabbit, mRNA species of 2.2–2.3 kb and 3.7–3.9 kb from kidney tissues and intestinal mucosa hybridized with the rBAT cDNA. Since the relative intensities of both bands do not change with stringency (data not shown), they could be the result of using differential polyadenylylation sites. Brain mRNA showed very weak signals of about 3.8 kb and 5.4 kb,

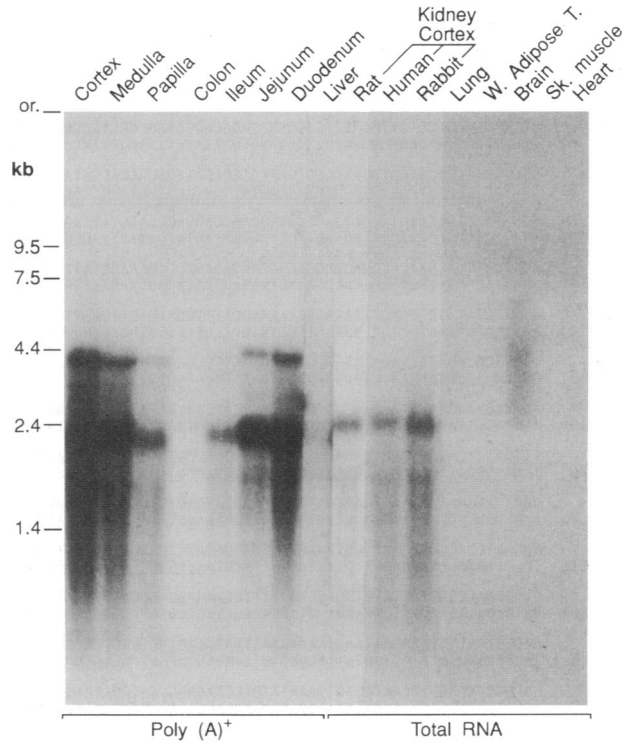

FIG. 4. Northern blot analysis for rBAT mRNA in different tissues. rBAT cDNA hybridizes to transcripts present mainly in kidney tissues and intestinal mucosa at high-stringency conditions. mRNA from rabbit kidney and intestinal mucosa probed with ^{32}P-labeled rBAT cDNA shows two transcripts (2.2 kb and 3.8 kb). Human and rat kidney cortex RNA show only the smaller band (2.3–2.5 kb). There are weak signals in rabbit brain (3.8 kb and 5.4 kb) and in rabbit liver (2.2 kb); these signals are only visible after long exposures. W. Adipose T., white adipose tissue; Sk. muscle, skeletal muscle; or., origin.

and liver mRNA showed a signal (about 2.2 kb) only after long exposures. Other tissues, of nonepithelial origin (skeletal muscle, heart, and adipose tissue), did not show detectable signals. Within the kidney and intestinal mucosa tissues, there was a gradient of abundance of rBAT transcripts that parallels the amino acid transport activities in these organs (1, 14): cortex > medulla >> papilla; and duodenum and jejunum > ileum >>> colon. Human and rat kidney cortex transcripts of 2.3–2.5 kb hybridized with rBAT cDNA. Furthermore, we examined cell lines derived from kidney tissues for transcript hybridization with rBAT cDNA: OK and LLC-PK_1 cells contain a positive signal of 2.3–2.4 kb and 2.4–2.6 kb, respectively (data not shown).

The nucleotide and deduced amino acid sequences of rBAT are presented in Fig. 5. The size of the rBAT clone (2247 bp) corresponds to that of the lower transcript seen on Northern blots. The first ATG codon lies within a good consensus initiation sequence (15). The open reading frame continues to the first stop codon (TGA) at base 2043 and codes for 677 amino acid residues with molecular mass of 77,832 Da. Hydrophobicity analysis (16) shows a single putative membrane-spanning domain (residues 80–102). There are seven potential N-glycosylation sites downstream of the transmembrane domain (i.e., in the putative extracellular domain). *In vitro* reticulocyte translation of rBAT cRNA resulted in several protein bands; the sizes of the major products were approximately 78, 74, and 69 kDa (data not shown). The higher molecular mass band is compatible with the size of the protein deduced from the predicted open reading frame. Addition of microsomes to the *in vitro* translation system resulted in the shift of those major molecular mass translation products to a higher molecular mass (approximately 99 kDa and 85–92 kDa); endoglycosidase H treatment shifted the protein bands back to the original molecular mass of nonglycosylated proteins (data not shown). These observations suggest that rBAT codes for a type II membrane glycoprotein (17), encompassing a cytoplasmic N terminus, a single transmembrane domain, and an N-glycosylated extracellular C terminus.

A search through protein sequence data bases (October, 1991) found amino acid sequence homology in the proposed extracellular domain of rBAT to a protein family of carbohydrate-metabolizing enzymes and related proteins that lack membrane-spanning domains—e.g., a maltase-like gene of the mosquito *Aedes aegypti* (ref. 18; 34% identity, 55% similarity) and *Bacillus cereus* oligo-1,6-glucosidase (ref. 19; 30% identity, 49% similarity). In addition, rBAT protein shows amino acid sequence homology to the N-terminus, transmembrane, and C-terminus domains of the 4F2 heavy chain (4F2hc) human and mouse surface antigens (refs. 20 and 21; i.e., 30% identity, 52% similarity to the human 4F2hc). The rBAT protein shows no homology with the ecotropic murine leukemia virus receptor, the recently identified y^+ carrier for dibasic amino acids (4, 5).

In contrast to the predicted structure of rBAT, known plasma membrane carriers for organic and inorganic substrates in mammals (e.g., refs. 9, 22, and 23) share a common structural feature, a variable number (i.e., 6–12) of putative

```
                                                -11 CCCGGGGAGAC

   1    ATGGCTGAGGAAGGAAGCAAGAGAGACTCCATCAAGATGAATATGAAAGGATGCCAGACA
     1  MetAlaGluGluGlySerLysArgAspSerIleLysMetAsnMetLysGlyCysGlnThr

  61    AACAATGGCTTTGTGCAAAATGAAGACATCCCAGAGCTGGACCTGGATCCAGGCAGCTCG
    21  AsnAsnGlyPheValGlnAsnGluAspIleProGluLeuAspLeuAspProGlySerSer

 121    GAGCACATCCTTGGGCCAGAGGAGCCCAACGCCAAGAACATCCAGCCCTACGCAGGGATG
    41  GluHisIleLeuGlyProGluGluProAsnAlaLysAsnIleGlnProTyrAlaGlyMet

 181    CCCAAGGAGGTGCTGTTCCAGTTCTCGGGCCAGGCCCGCTACCGCGTGCCCCGGGAGATC
    61  ProLysGluValLeuPheGlnPheSerGlyGlnAlaArgTyrArgValProArgGluIle

 241    CTCTTCTGGCTCGTCGTGGTTTCTGTGCTCGTGCTCATTGGGGCCACCATAGCCATCATC
    81  LeuPheTrpLeuValValValSerValLeuValLeuIleGlyAlaThrIleAlaIleIle

 301    GCCATCTCTCCAAAGTGCCTTGATTGGTGGCAGGCAGGGCCCATGTATCAGATCTACCCG
   101  AlaIleSerProLysCysLeuAspTrpTrpGlnAlaGlyProMetTyrGlnIleTyrPro

 361    AGGTCATTCAAGGACAGCGACAAGGACGGGAATGGAGACCTGAAAGGTATTCAAGATAAA
   121  ArgSerPheLysAspSerAspLysAspGlyAsnGlyAspLeuLysGlyIleGlnAspLys

 421    CTGGACTATATCACAACTTTAAATATAAAAACCATTTGGATTACTTCATTTTATAAATCA
   141  LeuAspTyrIleThrThrLeuAsnIleLysThrIleTrpIleThrSerPheTyrLysSer

 481    TCCCTCAGAGATTTTAGATATGGTGTTGAAGATTTCCGAGAAATTGATCCTATTTTTGGA
   161  SerLeuArgAspPheArgTyrGlyValGluAspPheArgGluIleAspProIlePheGly

 541    ACAATGGAAGATTTTGAGAACCTGCTGGCAGCCATCCATGATAAAGGTTTAAAACTAATC
   181  ThrMetGluAspPheGluAsnLeuLeuAlaAlaIleHisAspLysGlyLeuLysLeuIle

 601    ATTGATTTCATACCAAATCACACCAGTGACAAACACCCTTGGTTTCAATTGAGTCGGACA
   201  IleAspPheIleProAsnHisThrSerAspLysHisProTrpPheGlnLeuSerArgThr

 661    CGGACAGGGAAGTATACTGATTATTATATCTGGCATGACTGTGCCCATGAAAATGGCATA
   221  ArgThrGlyLysTyrThrAspTyrTyrIleTrpHisAspCysAlaHisGluAsnGlyIle

 721    ACCACTCCACCCAACAACTGGTTAAGTGTTTATGGAAACTCCAGTTGGCACTTCGATGAA
   241  ThrThrProProAsnAsnTrpLeuSerValTyrGlyAsnSerSerTrpHisPheAspGlu

 781    GTGCGAAACCAGTGCTATTTTCACCAGTTTTTGAAAGAGCAACCAGATTTAAATTTCCGC
   261  ValArgAsnGlnCysTyrPheHisGlnPheLeuLysGluGlnProAspLeuAsnPheArg

 841    AACCCTGATGTTCAAGAAGAAATAAAAGAAATAATGCAGTTCTGGCTCACCAAAGGTGTT
   281  AsnProAspValGlnGluGluIleLysGluIleMetGlnPheTrpLeuThrLysGlyVal

 901    GATGGCTTTAGTTTCAATGCTGTTAAATTTCTCCTAGAGGCAATGCATCTGAGAAATGAG
   301  AspGlyPheSerPheAsnAlaValLysPheLeuGluGluAlaMetHisLeuArgAsnGlu

 961    ATTCAAGTAAACGCATCACAAATCCCGGACACGGTCACACGCTACTCAGAGCTGTACCAT
   321  IleGlnValAsnAlaSerGlnIleProAspThrValThrArgTyrSerGluLeuTyrHis

1021    GACTTCACCACTACACAGGAGGGGATGCATGACCTTGTCCGCAGCTTCCGGCAGACCATG
   341  AspPheThrThrThrGlnGluGlyMetHisAspLeuValArgSerPheArgGlnThrMet

1081    GACAAGTACAGCAGGGAGCCCGGCAGATACAGGTTCCTGGGCACAGAAGCCTATGCGGAG
   361  AspLysTyrSerArgGluProGlyArgTyrArgPheLeuGlyThrGluAlaTyrAlaGlu

1141    AGCATTGACAGGACCATGAGGTACTACGGGTTGTCATTTATCCAAGAAGCCGATTTTCCT
   381  SerIleAspArgThrMetArgTyrTyrGlyLeuSerPheIleGlnGluAlaAspPhePro

1201    TTCAACAATTACTTCACCACTCTAGACACGCTTTCTGGGAACACTGTGTATGAGGTTATC
   401  PheAsnAsnTyrPheThrThrLeuAspThrLeuSerGlyAsnThrValTyrGluValIle

1261    ACAGCCTGGATGGAAAACATGCCCGAAGGAAAATGGCCCAATTGGATGACTGGTGGACCA
   421  ThrAlaTrpMetGluAsnMetProGluGlyLysTrpProAsnTrpMetThrGlyGlyPro

1321    GACATCACACGGCTGACTTCTCGTCTGGGGAACCAGTATGTCAACATCATGAACATGCTG
   441  AspIleThrArgLeuThrSerArgLeuGlyAsnGlnTyrValAsnIleMetAsnMetLeu

1381    CTTTTTACACTTCCTGGAACTCCCATAACTTACTATGGAGAAGAAATAGGGATGGGAAAT
   461  LeuPheThrLeuProGlyThrProIleThrTyrTyrGlyGluGluIleGlyMetGlyAsn

1441    ATTTTAGCCACAAATCTCAATGAAAGCTATGATGTGAACACCCTTCTCTCCAAGTCACCA
   481  IleLeuAlaThrAsnLeuAsnGluSerTyrAspValAsnThrLeuLeuSerLysSerPro

1501    ATGCAGTGGGACAACAGCTCCAATGCTGGTTTCTCTGAAGGCAATCACACCTGGTTACCC
   501  MetGlnTrpAspAsnSerSerAsnAlaGlyPheSerGluGlyAsnHisThrTrpLeuPro

1561    ACCAGTTCAGATTACCACACTGTGAATGTTGACGTCCAAAAGACCCAGCCCACATCAGCT
   521  ThrSerSerAspTyrHisThrValAsnValAspValGlnLysThrGlnProThrSerAla

1621    TTGAAGTTGTACCAAGCTTTAAGTCTACTTCATGCCAATGAACTGCTGCTCAGCAGGGGC
   541  LeuLysLeuTyrGlnAlaLeuSerLeuLeuHisAlaAsnGluLeuLeuLeuSerArgGly
                *                   *                   *

1681    TGGTTTTGTCTTCTGAGGAATGACAGCCGCGTTTTGGTGTACACAAGAGAGCTGGATGGC
   561  TrpPheCysLeuLeuArgAsnAspSerArgValLeuValTyrThrArgGluLeuAspGly
                *

1741    ATAGACAGAGTCTTTATTGTGGTTCTGAATTTTGGAGAATCAACACTGCTGAATCTACAG
   581  IleAspArgValPheIleValLeuAsnPheGlyGluSerThrLeuLeuAsnLeuGln

1801    GAAATGATTTCAGGACTCCCCGTAAGACTGAGCATAAAATTAAGTACCAATTCTGCCTCC
   601  GluMetIleSerGlyLeuProValArgLeuSerIleLysLeuSerThrAsnSerAlaSer

1861    ACAGGCAGCCAGGTTGATACGAGAGGCATTTTTCTTGAGAGGGGAGAGGGAGTCCTCCTT
   621  ThrGlySerGlnValAspThrArgGlyIlePheLeuGluArgGlyGluGlyValLeuLeu

1921    GAACACAAAATGAAGAACCTCCTTCACCGCCAAACAGCTTTCAGAGACAGATGTTTCATC
   641  GluHisLysMetLysAsnLeuLeuHisArgGlnThrAlaPheArgAspArgCysPheIle

1981    TCCAGTCGAGCATGCTACTCCAGCGCATTAGACATACTGTACAGCTCCTGTTAGGCAGCT
   661  SerSerArgAlaCysTyrSerSerAlaLeuAspIleLeuTyrSerSerCys***

2041    GAGAGGACGGAGACGCGGCACCCGCAGCACTGTAAGCATTAGCAGCATGCGGCTAGGGCA
2101    GCGCTGGGAATGGCCATTAGGTGCTTCTGTAGATTCAGTTACACTGTTAGGAAAGCTTCC
2161    CAAATCTTTTCAAAGAATAATAAATATGTTTCAAAAGAAAAAAAAAAAAAAAAAAAAAAA
2221    AAAAAAAAAAAAAAA
```

FIG. 5. Nucleotide and deduced amino acid sequence of clone rBAT. The first line shows the nucleotide sequence of rBAT cDNA. Nucleotides are numbered in the 5′ to 3′ direction, starting with the first nucleotide of the first ATG codon. The deduced amino acid sequence is shown below. The size of the transcript is 2247 bp and contains a poly(A) tail of 39 bp. The first ATG codon in the described open reading frame lies within a consensus initiation sequence (15). The stop codon (TAG) is indicated by three stars. The possible polyadenylylation signal (AATAAA) is underlined. The putative transmembrane domain, as deduced by hydrophobicity analysis (16), is double underlined. The four leucine residues in a motif resembling a leucine zipper are indicated by stars. Seven potential N-glycosylation sites (Asn-Xaa-Ser or Asn-Xaa-Thr) are underlined.

membrane-spanning domains. This suggests that rBAT protein may not be the complete transporter. Three hypotheses could explain the functional relationship of rBAT protein to system $b^{0,+}$. First, the functional $b^{0,+}$ carrier could be a homooligomer of rBAT protein. In analogy, Takumi *et al.* (24) reported the induction in oocytes of a slow voltage-gated potassium current by a protein with a single predicted transmembrane domain. The second hypothesis is that rBAT protein could be a subunit of a heterooligomeric carrier and associates to silent endogenous subunits of the oocyte carrier. The protein rBAT presents clear similarities with the 4F2hc surface antigen (20, 21); this surface antigen has a heterodimeric structure (25). If this also applies to rBAT protein, the structure of the mature $b^{0,+}$ carrier would then be heterodimeric, a structural feature not yet described for carriers of organic substrates. The third hypothesis is that rBAT could be a specific activator (e.g., glycosidase) of inactive carriers already present in the oocyte. This possibility seems unlikely because, although they have amino acid sequence homology to α-amylases and α-glucosidases, neither rBAT nor 4F2hc proteins conserve the catalytic aspartic acid (or glutamic acid) residues proposed for amylases, α-glucosidases, and transglucanosylases (26).

The reabsorption of L-cystine in kidney and intestine has been attributed to at least two transport systems that share transport with dibasic and/or neutral amino acids such as L-leucine and L-phenylalanine (1). rBAT cRNA induces in oocytes transport of cystine through a system having high affinity for these dibasic and neutral amino acids. Human cystinurias are genetic diseases that involve abnormalities of intestinal and renal absorption of cystine and dibasic amino acids, which lead to malabsorption and hyperexcretion of these amino acids with normal plasma levels (27). A defect in brush border membrane $b^{0,+}$ activity could be involved in cystinuria. In this instance an intact system involved in neutral amino acid reabsorption [e.g., neutral brush border (1)] would compensate for the absorption of neutral amino acids but not of dibasic amino acids and cystine. It is worth mentioning that hybrid depletion of kidney and jejunum mRNA with an rBAT antisense cRNA (or the equivalent D2 antisense cRNA from rat cortical tissue) results in almost complete (>90%) inhibition of L-cystine uptake induced in oocytes (ref. 9; S. Magagnin, J. Bertran, A.W., D.M., J. Biber, M.P., and H.M., unpublished results). Certainly this and the preferential location of rBAT mRNA, related to a protein apparently involved in transport of cystine, in kidney cortex and small intestinal mucosa is in agreement with such a hypothesis.

This work was performed at the University of Zürich. We thank Bruno Stieger for the performance of the *in vitro* translation experiments; Christian Gasser and Denise Rossi helped us in preparing the manuscript. We also thank Ned Mantei for the opportunity to use the rabbit kidney cortex cDNA library. This work was supported by the Swiss National Science Foundation (Grants 3.854.088 and 32-30785.91 to H.M.) and the "Stiftung für Wissenschaftliche Forschung an der Universität Zürich." J. Bertran received a fellowship from the Spanish Ministry of Science and Education and from the Juvenile Diabetes Foundation International (to A.Z.) for his stay in Zürich. We thank the Federation of European Biochemical Societies for providing M.P. with a short-term fellowship to work at the University of Zürich.

1. Silbernagl, S. (1988) *Physiol. Rev.* **68,** 911–1007.
2. Mircheff, A. K., Kippen, I., Hirayama, B. & Wright, E. M. (1982) *J. Membr. Biol.* **64,** 113–122.
3. Van Winkle, L. J., Campione, A. L. & Gorman, M. J. (1988) *J. Biol. Chem.* **263,** 3150–3163.
4. Kim, J. W., Closs, E. I., Albritton, L. M. & Cunningham, J. M. (1991) *Nature (London)* **352,** 725–728.
5. Wang, H., Kavanaugh, M. P., North, R. A. & Kabat, D. (1991) *Nature (London)* **352,** 729–731.
6. Bertran, J., Werner, A., Stange, G., Markovich, D., Biber, J., Testar, X., Zorzano, A., Palacín, M. & Murer, H. (1992) *Biochem. J.* **281,** 717–723.
7. Tate, S. S., Urade, R., Getchell, T. V. & Udenfriend, S. (1989) *Arch. Biochem. Biophys.* **275,** 591–596.
8. McNamara, P. D., Rea, C. T. & Segal, S. (1991) *J. Biol. Chem.* **266,** 986–989.
9. Wells, R. G. & Hediger, M. A. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 5596–5600.
10. Werner, A., Moore, M. L., Mantei, N., Biber, J., Semenza, G. & Murer, H. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 9608–9612.
11. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5463–5467.
12. Christensen, H. N. (1990) *Physiol. Rev.* **70,** 43–77.
13. Campa, M. J. & Kilberg, M. S. (1989) *J. Cell. Physiol.* **141,** 645–652.
14. Munck, B. G. (1981) in *Physiology of the Gastrointestinal Tract,* ed. Johnson, L. R. (Raven, New York), Chapter 44.
15. Kozak, M. (1989) *J. Cell Biol.* **108,** 229–241.
16. Kyte, J. & Doolittle, R. F. (1982) *J. Mol. Biol.* **157,** 105–132.
17. Holland, E. C. & Drickamer, K. (1986) *J. Biol. Chem.* **261,** 1286–1292.
18. James, A. A., Blackmer, K. & Racioppi, J. V. (1989) *Gene* **75,** 73–83.
19. Watanabe, K., Kitamura, K., Iha, H. & Suzuki, Y. (1990) *Eur. J. Biochem.* **192,** 609–620.
20. Teixeira, S., Di Grandi, S. & Kühn, L. C. (1987) *J. Biol. Chem.* **262,** 9574–9580.
21. Parmacek, M. S., Karpinski, B. A., Gottesdiener, K. M., Thompson, C. B. & Leiden, J. M. (1989) *Nucleic Acids Res.* **17,** 1915–1931.
22. Mueckler, M., Caruso, C., Baldwin, S. A., Panico, M., Blench, I., Morris, H. R., Allard, W. J., Lienhard, G. E. & Lodish, H. F. (1985) *Science* **229,** 941–945.
23. Hediger, M. A., Coady, M. J., Ikeda, T. S. & Wright, E. M. (1987) *Nature (London)* **330,** 379–381.
24. Takumi, T., Ohkubo, H. & Nakanishi, S. (1988) *Science* **242,** 1042–1045.
25. Hemler, M. E. & Strominger, J. L. (1982) *J. Immunol.* **129,** 623–628.
26. Svensson, B. (1988) *FEBS Lett.* **230,** 72–76.
27. Bergeron, M. & Scriver, C. R. (1985) in *The Kidney, Physiology and Pathophysiology,* eds. Seldin, D. W. & Giebisch, G. (Raven, New York), Vol. 2, pp. 1725–1745.