

A NEW FAMILY OF PROTEINS (rBAT AND 4F2hc) INVOLVED IN CATIONIC AND ZWITTERIONIC AMINO ACID TRANSPORT: A TALE OF TWO PROTEINS IN SEARCH OF A TRANSPORT FUNCTION

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Summary

The currently identified cDNA clones of mammalian amino acid transporters can be grouped into five different families. One family is composed of the proteins rBAT and the heavy chain (hc) of the cell surface antigen 4F2. RNAs encoding these two proteins induce a system b^{0,+}-like (rBAT) and a system y^{+L}-like (4F2hc) activity in *Xenopus* oocytes. Surprisingly, rBAT and 4F2hc do not seem to be pore-forming proteins. This finding supports the hypothesis that rBAT and 4F2hc are subunits or modulators of the corresponding amino acid transport systems. Expression of rBAT in oocytes induces high-affinity transport of cystine, which is shared with transport of cationic and zwitterionic amino acids. The *rBAT* gene is expressed mainly in kidney and small intestine. The rBAT protein is localized to the microvilli of proximal straight tubules of the kidney and mucosa from the small intestine. This finding is consistent with the involvement of rBAT in a high-affinity resorption system for cystine in the proximal straight tubule of the nephron. All of these characteristics suggest that *rBAT* is a good candidate for a cystinuria gene. Cystinuria is an inheritable defect in high-affinity transport of cystine, shared with cationic amino acids, through epithelial cells of the renal tubule and intestinal tract. Very recently, point missense mutations have been found in the *rBAT* gene of cystinuria patients. The most frequent rBAT mutation, M467T (threonine substitution of methionine at residue 467) nearly abolished the amino acid transport activity elicited by rBAT in oocytes. This result offers convincing evidence that *rBAT* is a cystinuria gene. Biochemical, cytological and genetic approaches are now needed to delineate the mechanism of action of rBAT and 4F2hc in the transport of amino acids.

Introduction

Amino acid transport across the plasma membrane of mammalian cells is catalyzed by proteins that recognise, bind and shuttle these metabolites between the extracellular and the intracellular compartments. In the last 4 years, a rapidly growing number of cDNA sequences encoding proteins related to amino acid plasma membrane transport have appeared (reviews include: Bertran *et al.* 1994; Kanai *et al.* 1994; Kanner and Kleinberger-Doron, 1994; Macleod *et al.* 1994). However, cDNAs encoding other

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significant amino acid transport activities have yet to be cloned (e.g. sodium-dependent systems, such as NBB, B^{0,+} and N, and sodium-independent systems, such as the ubiquitous system L and anionic amino acid transporters; see the introduction to this section in this volume by MacLeod *et al.* 1994). Although only a few amino acid transporter structures are known, functionally similar transporters can already be grouped into four different gene families (Table 1): (i) sodium-independent transporters for cationic amino acids; CAT, (ii) amino acid transporters within the sodium- and chloride-dependent neurotransmitter transporter superfamily, (iii) sodium-dependent, and probably potassium-dependent and chloride-independent, anionic and zwitterionic amino acid transporters, and (iv) sodium-dependent transporters for sugars, amino acids, vitamins and nucleosides. In addition to these amino acid transporters, which are predicted to have multiple transmembrane domains, two homologous proteins, rBAT (also named D2, NAA-Tr or NBAA-Tr) and the heavy chain of the cell surface antigen 4F2 (4F2hc), which are believed to contain a single transmembrane domain, induce amino acid transport activity in *Xenopus* oocytes. It has been postulated that these two proteins may not be the actual transporters but may represent activating subunits of multimeric transporters. These two proteins are the subject of the present review.

Cloning and identification of rBAT and 4F2hc, a new family of proteins involved in amino acid transport

Expression-cloning resulting in amino acid transport activity in *Xenopus* oocytes was used in three laboratories to isolate cDNAs encoding putative transporters from rabbit, rat and human kidney (Bertran *et al.* 1992b, 1993; Lee *et al.* 1993; Tate *et al.* 1992; Wells and Hediger, 1992). These cDNA clones encode proteins that share 80–85% sequence identity. The human cDNA cloned by Hediger's group (Lee *et al.* 1993) lacks 66 nucleotides encoding 22 amino acids at the C terminus that are present in our human clone and in the rat and rabbit clones (Bertran *et al.* 1993). For clarity, the term rBAT will refer to human, rat and rabbit clones in this review. rBAT shares 30% amino acid sequence identity (50% similarity) with another protein, the heavy chain (hc) of the mouse and human surface antigen 4F2 (Parmacek *et al.* 1989; Quackenbush *et al.* 1987; Teixeira *et al.* 1987). The cDNA encoding this protein was originally cloned in 1987 for its ability to react with a monoclonal antibody raised against a lymphoblastic cell surface antigen of unknown function (Quackenbush *et al.* 1987; Teixeira *et al.* 1987). Because 4F2hc shared sequence similarity with rBAT, synthetic 4F2hc RNA was tested in *Xenopus* oocytes and found to induce amino acid transport activity in this system (Bertran *et al.* 1992a; Wells *et al.* 1992). Both rBAT and 4F2hc proteins share several structural features: both lack a leader sequence, have almost identical hydrophilicity profiles (Fig. 1) and share four highly conserved (67–80% identity) regions (10–18 amino acids long) in the putative extracellular domain (Fig. 2A). Both proteins contain an extracellular cysteine residue located four amino acids from the transmembrane region and both extracellular domains show significant homology with a family of α -amylases and α -glucosidases. Interestingly, the catalytic consensus site of these glycosidases is not conserved in rabbit rBAT or 4F2hc, which is consistent with the

Table 1. Protein families related to amino acid transport in mammals

Family	Proteins	Probable related system	Number of transmembrane domains
Cationic amino acid transporters (CAT)	CAT1; CAT2; CAT2a	y ⁺ isoforms	12-14
Na ⁺ - and Cl ⁻ -dependent transporters	GAT1; GAT2 GAT3; GAT4; Taut GLYT BGT-1; PRO	GABA isoforms GABA and beta isoforms Gly	12
Na ⁺ - and K ⁺ -dependent transporters	GLAST; GLT-1; EAAC-1 SATT; ASCT1	X _{AG} isoforms ASC isoforms	6, 8, 10
Na ⁺ -dependent transporters for sugars, amino acids, vitamins and nucleosides	SAATI	A	12
Subunits of amino acid transporters	rBAT 4F2hc	b ^{0,+} y ⁺ L	1, 4 1

Cloned amino acid transporters in mammals have been grouped into five families with their probable amino acid transport systems. In most cases this connection is not clearly established. The deduced proteins of the first four families share a multiple membrane-spanning model. In contrast, the family composed by rBAT and 4F2hc (tentatively shown as subunits of amino acid transporters) contain 1-4 transmembrane domains, depending on predictions of protein structure. Cationic amino acid transporters are reviewed by MacLeod *et al.* (1994). The amino acid transporters within the superfamily of Na⁺- and Cl⁻-dependent neurotransmitter transporters are included here as Na⁺- and Cl⁻-dependent transporters (Na⁺ and Cl⁻ cotransporters). Within this family, Kanner and Kleinberger-Doron (1994) subdivided GABA transporters into five subtypes: GAT1, GAT2, GAT3 (including brain GAT3a and heart GAT3b), GAT4 (including brain GAT4a, GAT4b and GAT4c) and GABA/betaine (also named BGT-1). Three high-affinity glutamate transporter cDNAs (GLAST, GLT-1 and EAAC-1) and two highly homologous cDNAs (SATT and ASCT1), which express amino acid transport activity resembling system ASC, share sequence homology and cotransport of Na⁺ and countertransport of K⁺. The cDNA SAATI (sodium-dependent amino acid transporter-1) showed system A amino acid transport activity when expressed in COS cells and it is highly homologous to the sodium/glucose cotransporters, within the family of Na⁺-dependent transporters for sugars, amino acids, vitamins and nucleosides of eukaryotes and prokaryotes (reviewed in Bertran *et al.* 1994).

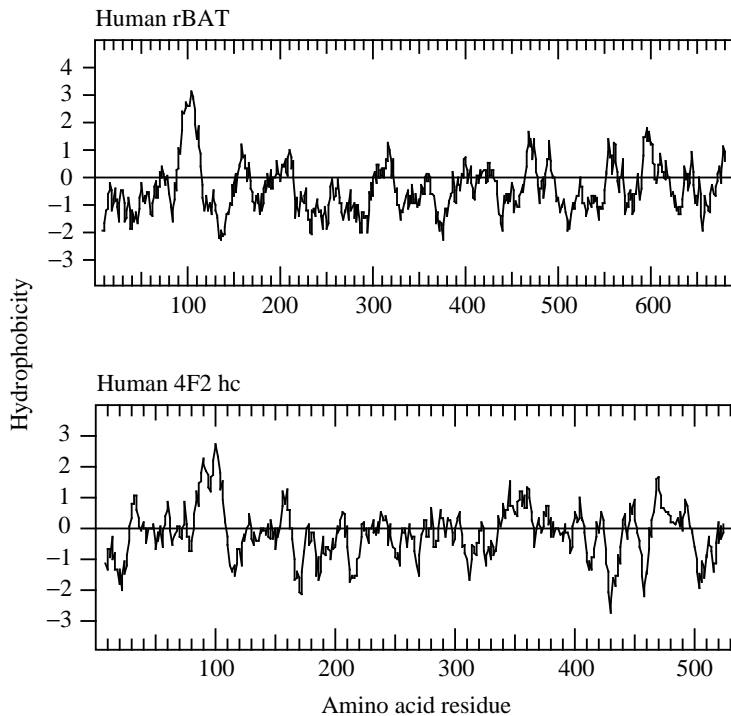


Fig. 1. Hydropathy plots for human rBAT and human 4F2hc deduced proteins. The plots have been drawn using Kyte-Doolittle's algorithm with a window of nine amino acids (Bertran, 1993). The y-axis shows the hydrophobicity scale, and the x-axis shows the amino acid residue. Human rBAT and 4F2hc proteins are 685 and 529 amino acids long, respectively. Hydrophobic regions have positive y values and are considered to be potential membrane-spanning domains if the value is higher than 2.5. Both deduced proteins (rBAT, Bertran *et al.* 1993; 4F2hc, Teixeira *et al.* 1987) have an overall similar hydropathy plot and contain a single region (around amino acid residue 100) with a hydrophobicity value higher than 2.5.

finding that rBAT expression in *Xenopus* oocytes confers no α -amylase or maltase activity (Wells and Hediger, 1992).

The two proteins appear to have different functions. rBAT induces a sodium-independent $b^{0,+}$ -like activity in *Xenopus* oocytes. The $b^{0,+}$ system was first described in mouse blastocysts (Van Winkle *et al.* 1988) and is a high-affinity, sodium-independent transport system for cationic and zwitterionic amino acids, but not for cystine (L. J. Van Winkle, personal communication). Nevertheless, rBAT induces the transport of both cationic/zwitterionic amino acids and cystine, when expressed in the *Xenopus* oocytes, with K_m values in the micromolar range (Table 2). The kinetics of L-cystine transport are shown in Fig. 3. Kinetic analysis and cross-inhibition studies provide evidence that rBAT induces a single transport activity (Bertran *et al.* 1992b). This transport activity is not present in stage VI *Xenopus* oocytes unless synthetic rBAT RNA is injected (Fig. 3; Bertran *et al.* 1992b,c; McNamara *et al.* 1991). Microinjected synthetic 4F2hc RNA

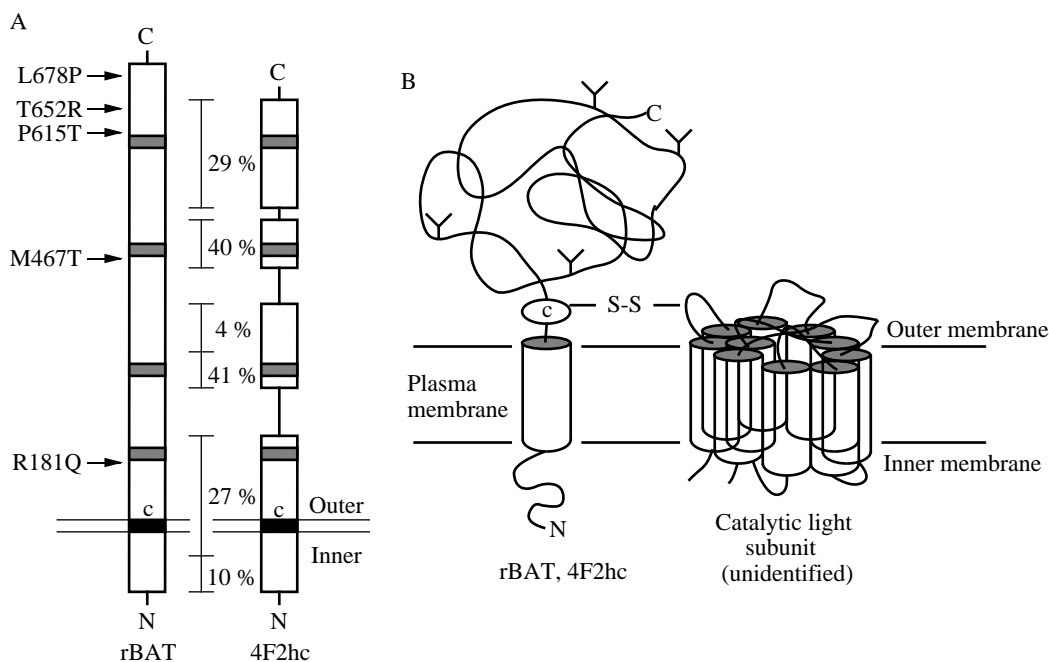


Fig. 2. (A) Schematic comparison of human rBAT and human 4F2hc deduced proteins. Percentages indicate amino acid sequence identity between both proteins. Shaded areas represent small regions of 10–18 amino acid residues of high homology (identity 67–80%) between both proteins. Gaps for the alignment smaller than 10 amino acid residues are not represented or combined. The locations of cystinuria-specific missense mutations in the protein rBAT (R181Q, arginine-188→glutamine; M467T, methionine-467→threonine; P615T, proline-615→threonine; T652R, threonine-652→arginine; L678P, leucine-678→proline) are indicated. Mutations R181Q and M467T (also mutation M467K; methionine-467→lysine; which is not indicated in the figure) are located in well-conserved regions of rBAT and 4F2hc. Interestingly, mutation P615T involves a proline residue which is conserved in human rBAT and 4F2hc. (B) Hypothetical heterodimeric representation of rBAT and 4F2 proteins. This diagram represents the proteins rBAT and 4F2hc (left) as type II membrane glycoproteins, with an intracellular N terminus, a single membrane-spanning domain (not drawn to scale) and a large extracellular domain with potential N-glycosylation sites (four out of six potential N-glycosylation sites in human rBAT are indicated by Y) and the C terminus. In the extracellular domain, next to the transmembrane segment a conserved cysteine residue (c) is present in both proteins. To the right, a putative 'catalytic light subunit' is represented as a multiple membrane-spanning protein (i.e. a pore-forming protein). The 4F2 'light subunit' has been detected as a highly hydrophobic and non-glycosylated approximately 40 kDa protein after immunoprecipitation of the 4F2 complex in non-reducing conditions (see text for details). This light subunit has not been cloned; therefore, its topological representation here is only speculative. The sketch shows a disulfide bridge (-S-S-) between the subunits. The putative 'rBAT light subunit' has not yet been identified.

elicits a transport activity quite distinct from rBAT activity (Bertran *et al.* 1992a; Wells *et al.* 1992). It appears to enhance a pre-existing transport activity with y^+ -like characteristics. Indeed, expression of 4F2hc induces sodium-dependent uptake of both L-

Table 2. Kinetic variables for the rBAT-induced transport activity in oocytes

Amino acid	K_m ($\mu\text{mol l}^{-1}$)	K_i ($\mu\text{mol l}^{-1}$)	V_{max} (pmol min^{-1})	Reference
L-Arginine	105	47–56	29	a
L-Lysine		75–298		a
L-Ornithine		197–222		a
L-Cystine	30–67	90–184	4–16	a,b,d,e
L-Leucine	22(r)–128	172–199	35; 97 % (r)	a,c
L-Phenylalanine	29(r)		100 % (r)	c
L-Histidine	167(r)		126 % (r)	c
L-Methionine	71(r)		151 % (r)	c
L-Alanine		800–4900	56 % (r)	a,c
L-Tryptophan	250(r)		11 % (r)	c
L-Serine*				b
L-Glutamine*				b
Citrulline*				b
L-Cysteine		†		a
L-Threonine		†		a
L-Valine		†		a
D-Ornithine		3700–7600		a
D-Lysine		†		b

Uptake values and kinetic variables refer to human, rat and rabbit rBAT-induced transport in *Xenopus* oocytes. The range of kinetic variables for L-cystine transport corresponds to human, rat and rabbit rBAT expression. No kinetic variables for amino acids other than L-cystine have been determined for human rBAT expression.

The K_i values have been determined only for the expression of rabbit rBAT.

(r) refers to rat rBAT.

V_{max} values are given as $\text{pmol min}^{-1} \text{oocyte}^{-1}$. When given as a percentage, the values are referred to that of Phe.

* indicates amino acids which have been shown to be carried by the expressed transport activity, but no kinetic data are available.

† indicates those amino acids that have been shown to inhibit rBAT-induced uptake activity. rBAT does not induce transport of L-proline, methyl-aminoisobutyric acid, L-glutamate, L-aspartate or taurine (references a and b). ^aBertran *et al.* (1992b); ^bWells and Hediger (1992); ^cTate *et al.* (1992); ^dBertran *et al.* (1993); ^eLee *et al.* (1993).

leucine and L-methionine and sodium-independent uptake of cationic amino acids, but the latter activity is increased by only 2- to 10-fold (K_m for L-arginine near $40 \mu\text{mol l}^{-1}$). This activity is reminiscent of the y^+L system recently described in human erythrocytes, which exhibits sodium-independent high-affinity transport of cationic amino acids and high-affinity transport of L-leucine in the presence of sodium (Devés *et al.* 1992).

The proteins rBAT and 4F2hc are less hydrophobic than common transporter proteins

Biochemical and immunocytochemical studies have demonstrated that the rBAT and 4F2hc proteins are integral plasma membrane glycoproteins expressed in the cell

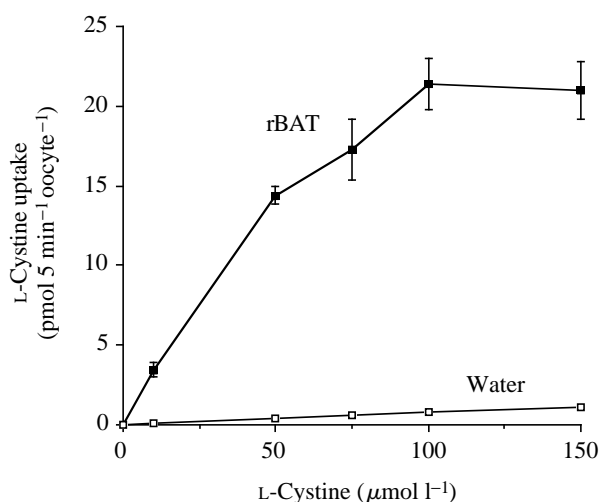


Fig. 3. Transport of L-cystine elicited by human rBAT in *Xenopus* oocytes. Oocytes were injected with 50 nl of water alone (open squares) or water containing 5 ng of synthetic RNA from human rBAT (filled squares). Three days later, the uptake of L-[^{35}S]cystine, at the indicated concentrations, was determined for 5 min incubations. Notice the saturation of the rBAT-induced uptake at concentrations of L-cystine above $50 \mu\text{mol l}^{-1}$; in contrast, uptake of L-cystine in water-injected oocytes shows no detectable saturability at concentrations below 150 mmol l^{-1} . Values are mean \pm S.E.M. of the uptake values from seven oocytes in a representative experiment. This experiment was performed by J. Chillarón in our laboratory.

membrane. The experimental proof for rBAT is as follows. (i) Addition of microsomes to the reticulocyte translation system for synthetic rBAT RNA increases the mass of the major rBAT protein product, which is sensitive to endoglycosidase H treatment (Markovich *et al.* 1993; Wells and Hediger, 1992). (ii) *Xenopus* oocyte translation of synthetic human rBAT RNA results in an rBAT-specific protein band ([^{35}S]methionine labeling) of approximately 94 kDa in crude oocyte membranes, prepared after stripping with sodium carbonate; treatment of the oocytes with tunicamycin shifts the rBAT translation product to a molecular mass of approximately 72 kDa (Bertran *et al.* 1993). The size of this protein product is reasonably similar to the size of the protein deduced from the predicted open reading frame (M_r approximately 79×10^3). A similar size for the glycosylated protein (approximately 90 kDa) has been reported for rBAT in rat renal brush-border membranes with anti-rBAT polyclonal antibodies (Furriols *et al.* 1993; Mosckovitz *et al.* 1993). (iii) Immunoelectron microscopy studies show that rBAT is expressed in the microvilli of the proximal straight tubule of the rat nephron (Furriols *et al.* 1993; Pickel *et al.* 1993).

The main argument against the hypothesis that rBAT and 4F2hc are the actual amino acid transporters, $\text{b}^{\text{o},+}$ -like and $\text{y}^{\text{+L}}$ -like respectively, relies on the predicted structure of these two proteins. The hydrophobicity plot for the human rBAT and 4F2hc proteins is shown in Fig. 1. Only a single membrane-spanning domain is predicted around amino acid residue 100 in the two proteins. At present, the most accepted structural model

indicates that the rBAT and 4F2hc proteins are type II membrane glycoproteins (see Fig. 4). Alternatively, Udenfriend's group (Tate *et al.* 1992) have suggested that rBAT could be arranged in the membrane with at least four transmembrane domains, with three amphipathic α -helices (see Fig. 5). Experimental evidence is needed to elucidate the topology of rBAT insertion into cell membranes.

It is more likely that rBAT and 4F2hc proteins are modulators or components of amino acid transport systems (Bertran *et al.* 1992a; Wells *et al.* 1992): rBAT could 'activate' a silent system $b^{0,+}$ -like endogenous transporter of the oocyte, while 4F2hc may 'activate' a y^+L -like endogenous system partially 'inactive' in the oocyte. rBAT and 4F2hc could act as modulators of these amino acid transporters. In this sense, recent findings suggest that several integral membrane proteins with a single predicted transmembrane domain may specifically modulate the action of particular channels or transporters in oocytes or transfected cells (e.g. IsK and phospholemman, or the putative modulators of the Na^+ /glucose cotransporter and intestinal peptide transport) (Attali *et al.* 1993; Dantzig *et al.* 1994; Veyhl *et al.* 1993). Alternatively, rBAT and 4F2hc proteins could be essential subunits of heterologomeric transporters and could be associated with silent endogenous catalytic subunits of the oocyte transporters (see Fig. 2B). The 4F2hc cell surface antigen is a disulfide-linked heterodimer (approximately 125 kDa) composed of a glycosylated heavy chain of 85 kDa (i.e. 4F2hc) covalently linked by disulfide bridges to a non-glycosylated light chain of 40 kDa (Fig. 2B) (Haynes *et al.* 1981; Hemler and Strominger, 1982). To our knowledge, the light chain has not been cloned or microsequenced. Similarly, rBAT seems to be linked by disulfide bridges to an unidentified putative 'light subunit' within a complex of approximately 125 kDa in rat renal brush-border membranes (C. Mora, J. Chillarón and M. Palacín, in preparation). In addition, expression of rBAT in COS cells results neither in amino acid transport expression nor in the association of rBAT into a higher molecular mass complex under non-reducing conditions (C. Mora, J. Chillarón and M. Palacín, in preparation). These findings suggest that proper functional expression of rBAT might depend on the expression of this putative essential subunit. This hypothetical mechanism is analogous to the role proposed for the Na^+/K^+ -ATPase β subunit (another type II membrane glycoprotein) which, upon injection of its RNA into oocytes, supports the maturation of active pumps containing the endogenous catalytic α subunit (Geering *et al.* 1989). If this hypothesis holds true for rBAT and 4F2hc, the structure of the mature $b^{0,+}$ -like and y^+L -like transporters would then be heterodimeric, a feature not yet described for transporters of organic substrates in mammals. Elucidation of the mechanisms involved in 4F2hc and rBAT expression of amino acid transport will require the isolation and/or cloning of the light chain of the 4F2 cell surface antigen and the putative complementary subunit of rBAT.

Identification of rBAT as a cystinuria gene

Cystinuria is an autosomal recessive disease, with an overall prevalence of one case in 7000 people, which is characterized by urinary hyperexcretion of cystine and cationic amino acids (Levy, 1973; McKusick, 1990; Segal and Thier, 1989). Cystine has a low solubility and its precipitation results in the formation of calculi in the urinary tract, which

leads to obstruction, infections and ultimately to renal insufficiency (Segal and Thier, 1989). Three types of classic cystinuria have been described (Rosenberg *et al.* 1966a): type I, in which heterozygotes show normal aminoaciduria, and types II and III, in which heterozygotes show cystine-lysinuria. In contrast to types I and II, type III homozygotes show an increase in cystine plasma levels after oral cystine administration. These different types are thought to be due to allelism of the same gene (Rosenberg *et al.* 1966b).

Cystinuria has been postulated to result from a defect in the high-affinity transport of cystine, shared with cationic amino acids, through epithelial cells of the renal tubule and intestinal tract (Rosenberg *et al.* 1965). Reabsorption of L-cystine in the kidney is not completely understood. Transport of L-cystine in brush-border membrane vesicles is relatively sodium-independent (Foreman *et al.* 1980; McNamara *et al.* 1981, 1992). The driving force for reabsorption of L-cystine is provided by intracellular reduction to L-cysteine, which then leaves the cell by a basolateral transport system (Silbernagl, 1988). Studies with brush-border membrane vesicles have suggested the existence in renal membranes of a high-affinity system, shared with cationic amino acids, and a low-affinity system for L-cystine, which appears to be unshared (McNamara *et al.* 1981; Segal *et al.* 1977). Transport in jejunal vesicles involves a high-affinity system (Ozegovic *et al.* 1982), which is defective in biopsies of intestinal mucosa from cystinuric patients (Coicadan *et al.* 1980; Thier *et al.* 1964). Functional studies indicate that the high-affinity (micromolar range) transport of L-cystine is located in the proximal straight tubule (Schafer and Watkins, 1984). Moreover, the high-affinity transport of L-cystine appears to be shared with some L-zwitterionic amino acids (Foreman *et al.* 1980; Furlong and Posen, 1990; Schafer and Watkins, 1984).

rBAT messenger RNA is localized within kidney and intestinal mucosa (Bertran *et al.* 1992b, 1993; Lee *et al.* 1993; Wells and Hediger, 1992; Yan *et al.* 1992). In keeping with this distribution, hybrid-depletion experiments of renal and intestinal mRNA with rBAT anti-sense oligonucleotides block expression of system b⁰⁺-like activity in oocytes (Bertran *et al.* 1993; Magagnin *et al.* 1992; Wells and Hediger, 1992). Fig. 4 shows two *rBAT* transcripts in kidney and small intestine, which represent alternative polyadenylation of the same gene (Bertran *et al.* 1992b; Markovich *et al.* 1993). *In situ* hybridization and immunodetection studies demonstrate specific rBAT expression in the microvilli of small intestine and the proximal straight tubule of the rat nephron (Furriols *et al.* 1993; Kanai *et al.* 1992; Pickel *et al.* 1993). Brain tissues show a longer *rBAT* transcript (Fig. 4), which is also present in other human tissues (Bertran *et al.* 1993; Yan *et al.* 1992). RNAase protection assays and immunological studies suggest that this long transcript may correspond to a homologous neural-tissue-specific mRNA transcribed from a different gene (Pickel *et al.* 1993; Yan *et al.* 1992).

The specific expression of rBAT in kidney and small intestine and the characteristics of the high-affinity uptake of L-cystine induced by rBAT in oocytes (see above) suggested that *rBAT* would be a good candidate for the cystinuria gene. To test this hypothesis, the search for cystinuria-specific mutations in *rBAT* was undertaken (Calonge *et al.* 1994), taking advantage of illegitimate transcription in lymphoblastoid cells (Chelly *et al.* 1989). Amplified rBAT cDNAs from lymphoblastoid cell lines from several patients were

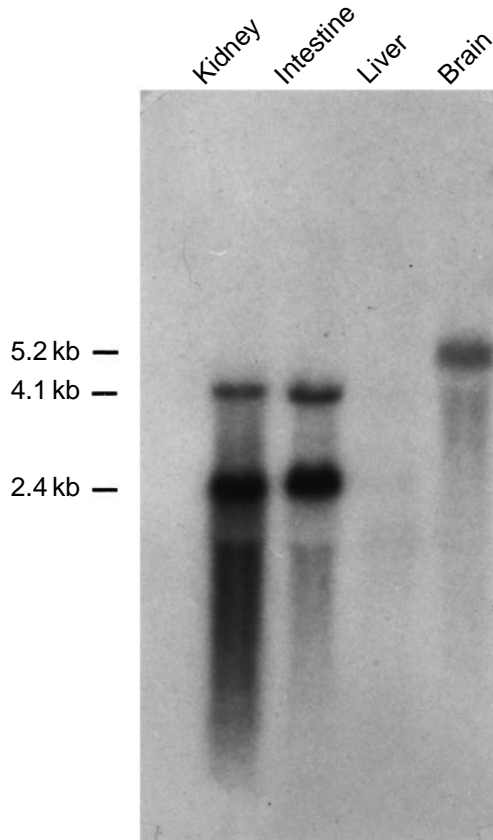


Fig. 4. Tissue distribution of rBAT mRNA. At high stringency conditions, rabbit rBAT cDNA hybridized to transcripts of approximately 2.4 kb and approximately 4.1 kb present in rabbit kidney (i.e. cortex and medulla) and small intestine (i.e. jejunum). rBAT cDNA also hybridized to a rabbit brain transcript of approximately 5.2 kb. rBAT-specific signals are not visible in RNA from rabbit liver. This Northern blot was performed by M. Furriols following the method described in Furriols *et al.* (1993).

analysed by single-strand conformation polymorphism, followed by direct sequencing of electrophoretically altered fragments: six cystinuria-specific point missense mutations in the *rBAT* gene, confirmed in genomic DNA, were found in 30% of the independent cystinuric chromosomes analyzed (Calonge *et al.* 1994). All these mutations affect well-conserved amino acid residues in the human, rat and rabbit rBAT proteins. The localization of these amino acid substitutions in the protein rBAT is indicated in Figs 2A and 5. The main mutation found (present in seven families of cystinuric patients), M467T (i.e. substitution of methionine at residue 467 by threonine), was detected in homozygosis in two cystinuric kindreds (a Spanish family, Calonge *et al.* 1994, and an Italian family, P. Gasparini, personal communication) and seems to be associated with type I cystinuria. Interestingly, mutation M467T greatly impaired (approximately 80%) L-cystine,

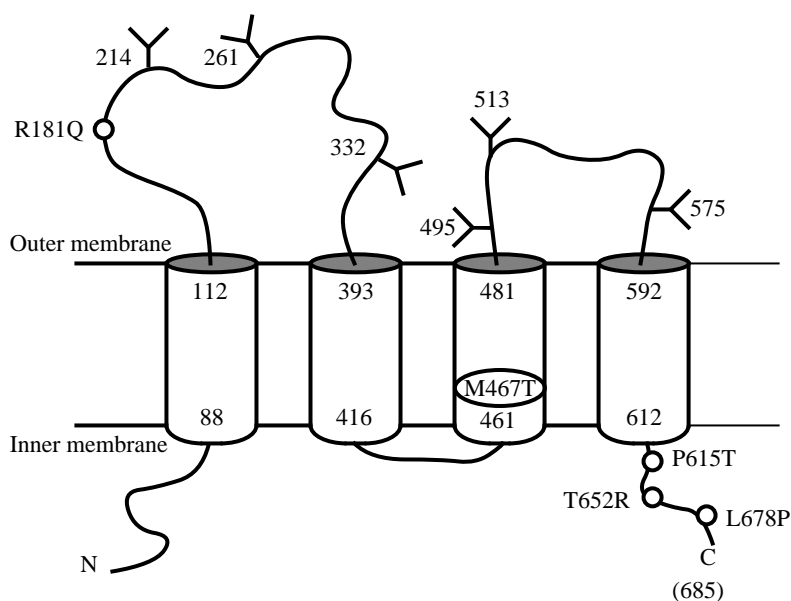


Fig. 5. Schematic location of cystinuria-specific mutations of rBAT in the model of rBAT containing four membrane-spanning domains. The mutations found in the rBAT gene of cystinuric patients are described in the legend to Fig. 2A. Mutation M467T (also M467K) is located in the third transmembrane domain proposed by Tate *et al.* (1992). Three mutations (P615T, T652R and L678P) are grouped towards the C terminus. All six rBAT mutations involve conserved or well-conserved amino acid residues in human, rat and rabbit rBAT. Numbers indicate the first and last amino acid residues in the putative transmembrane domains and the positions of potential N-glycosylation sites (Y).

L-arginine and L-leucine transport activity associated with rBAT in oocytes (Calonge *et al.* 1994). These data provide convincing evidence that *rBAT* is a cystinuria gene.

The *rBAT* gene has been localized to the short arm of human chromosome 2 (i.e. region 2pter–p12) (Calonge *et al.* 1994; Lee *et al.* 1993). The work of Calonge *et al.* (1994) has received independent and simultaneous confirmation by linkage studies with chromosome 2p markers. Pras *et al.* (1994) have found linkage (maximal lod score greater than 9) between cystinuria and microsatellite D2S119 with an approximate location of the cystinuria locus at 7 centimorgans telomeric to this marker.

The involvement of *rBAT* gene in cystinuria has the following consequences. (i) As predicted by functional and immunolocalization studies, rBAT is related to high-affinity reabsorption of cystine in kidney. (ii) System $b^{0,+}$, which transports zwitterionic amino acids in addition to cystine and cationic amino acids, is defective in cystinuria. Why is urinary excretion of zwitterionic amino acids not increased in cystinuria? In keeping with the involvement of zwitterionic amino acids in cystinuria, administration of cycloleucine to humans or rats produces increased urinary excretion of cystine and cationic amino acids in amounts similar to those in cystinuria patients (Brown, 1967). This finding is consistent with the inhibition caused by cycloleucine on the human rBAT-induced

cystine transport in oocytes (J. Chillaron, unpublished results). However, the normal urinary excretion of zwitterionic amino acids in cystinuric patients could be explained by the activity of intact zwitterionic amino acid reabsorption systems in the renal tubule (e.g. system NBB).

Open questions and further perspectives

The proteins rBAT and 4F2hc introduce intriguing questions regarding amino acid transport. (1) How do these two proteins, which apparently do not act as pore-forming proteins, participate in specific amino acid transport activity? This question may be answered through the elucidation of the structure of rBAT and 4F2 proteins. First, the cloning and structural identification of the light subunit of 4F2 antigen and of the putative 'light subunit' of rBAT are required. Second, the topology of rBAT and 4F2hc should be determined experimentally. (2) Is *rBAT* the only gene involved in cystinuria? The hypothesis that rBAT acts as a component of the renal $b^{0,+}$ -like transporter suggests that other genes might also be involved in cystinuria. Recently, one case of a *de novo* balanced translocation (14;20) has been associated with cystinuria and mental retardation, suggesting that one of these breakpoints (14q22 or 20p13) might be involved in cystinuria (Sharland *et al.* 1992). In contrast, the linkage studies with chromosome 2p markers by Pras *et al.* (1994) suggest the chromosome 2 locus (i.e. *rBAT* gene) as the only genetic locus for cystinuria. A wider selection of cystinuria families for linkage studies, with attention to their cystinuria phenotypes (i.e. types I, II and III), will be needed to determine whether other genetic loci are involved in cystinuria. (3) What characteristics do the amino acid transport activities associated with rBAT and 4F2hc show in mammalian cells? At present, these have been investigated only in *Xenopus* oocytes. This question will most probably be answered following the study of cell models that naturally express rBAT (e.g. OK cells) and the 4F2 antigen (e.g. stimulated lymphocytes). (4) Finally, are there any genes homologous to *rBAT* and *4F2hc* to be identified? The related rBAT transcript present in neural tissues may foster studies directed to the cloning of a homologous *rBAT* gene in those tissues. In addition, homologous clones to rBAT and 4F2hc might be responsible for amino acid transport activities similar to those associated with these proteins (e.g. the blastocyst system $b^{0,+}$ and the sodium-dependent system $B^{0,+}$).

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