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,+L-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
significant amino acid transport activities have yet to be cloned (e.g. sodium-dependent systems, such as NBB, B\textsuperscript{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 \textit{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 \textit{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 \textit{Xenopus} oocytes was used in three laboratories to isolate cDNAs encoding putative transporters from rabbit, rat and human kidney (Bertran \textit{et al.} 1992b, 1993; Lee \textit{et al.} 1993; Tate \textit{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 \textit{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 \textit{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 \textit{et al.} 1989; Quackenbush \textit{et al.} 1987; Teixeira \textit{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 \textit{et al.} 1987; Teixeira \textit{et al.} 1987). Because 4F2hc shared sequence similarity with rBAT, synthetic 4F2hc RNA was tested in \textit{Xenopus} oocytes and found to induce amino acid transport activity in this system (Bertran \textit{et al.} 1992a; Wells \textit{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 \(\alpha\)-amylases and \(\alpha\)-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

<table>
<thead>
<tr>
<th>Family</th>
<th>Proteins</th>
<th>Probable related system</th>
<th>Number of transmembrane domains</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cationic amino acid transporters (CAT)</td>
<td>CAT1; CAT2; CAT2a</td>
<td>y+ isoforms</td>
<td>12–14</td>
</tr>
<tr>
<td>Na⁺- and Cl⁻-dependent transporters</td>
<td>GAT1; GAT2</td>
<td>GABA isoforms</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>GAT3; GAT4; Taut</td>
<td>GABA and beta isoforms</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GLYT BGT-1; PRO</td>
<td>Gly</td>
<td></td>
</tr>
<tr>
<td>Na⁺- and K⁺-dependent transporters</td>
<td>GLAST; GLT-1; EAAC-1</td>
<td>XₐG isoforms</td>
<td>6, 8, 10</td>
</tr>
<tr>
<td></td>
<td>SATT; ASCT1</td>
<td>ASC isoforms</td>
<td></td>
</tr>
<tr>
<td>Na⁺-dependent transporters for sugars,</td>
<td>SAAT1</td>
<td>A</td>
<td>12</td>
</tr>
<tr>
<td>amino acids, vitamins and nucleosides</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subunits of amino acid transporters</td>
<td>rBAT</td>
<td>b₀⁺⁺</td>
<td>1, 4</td>
</tr>
<tr>
<td></td>
<td>4F2hc</td>
<td>y⁺L</td>
<td>1</td>
</tr>
</tbody>
</table>

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 SAAT1 (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).
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~h~+~*-like activity in *Xenopus* oocytes. The b~h~+~* 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

![Hydropathy plots for human rBAT and human 4F2hc deduced proteins.](image-url)

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 γ-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 γ 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.
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-
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 μmol l$^{-1}$). This activity is reminiscent of the $y^+$ 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

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

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

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 pmol min$^{-1}$ 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). *Bertran et al. (1992b); †Wells and Hediger (1992); †Tate et al. (1992); †Bertran et al. (1993); †Lee et al. (1993).
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 ([35S]methionine labeling) of approximately 94kDa 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 72kDa (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 (Mr approximately 79’000). A similar size for the glycosylated protein (approximately 90kDa) 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) Immunolectron 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, b0,+,-like and y+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 $\alpha$-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 b0,+,-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 heteroligomeric 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 $\beta$ subunit (another type II membrane glycoprotein) which, upon injection of its RNA into oocytes, supports the maturation of active pumps containing the endogenous catalytic $\alpha$ subunit (Geering et al. 1989). If this hypothesis holds true for rBAT and 4F2hc, the structure of the mature b0,+,-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 b^0,+^-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

Proteins involved in amino acid transport

131
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,
Proteins involved in amino acid transport

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^\({\text{o}^+}\), 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...

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).

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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 b0,+‐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 b0,+ and the sodium‐dependent system B0,+).

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Proteins involved in amino acid transport

References


Proteins involved in amino acid transport


