Amino Acids (1996) 11: 193–208

Amino
Acids

CATs, a family of three distinct mammalian cationic amino acid transporters

Review Article

E. I. Closs

Department of Pharmacology, Johannes Gutenberg University, Mainz, Federal Republic of Germany

Accepted April 5, 1996

Summary. Three related mammalian carrier proteins that mediate the transport of cationic amino acids through the plasma membrane have been identified in murine and human cells (CAT for cationic amino acid transporter). Models of the CAT proteins in the membrane suggest they have 12 or 14 transmembrane domains connected by short hydrophilic loops and intracellular N- and C-termini. The transport activity of the CAT proteins is sensitive to trans-stimulation and independent of the presence of sodium ions. These features agree with the behaviour of carrier proteins mediating facilitated diffusion. The three CAT proteins, CAT-1, CAT-2A and CAT-2(B) are encoded by two different genes (*CAT-1 and CAT-2*). CAT-1 and CAT-2(B) exhibit transport properties consistent with system y^+, the principal mechanism for cellular uptake of cationic amino acids. In contrast, CAT-2A has tenfold lower substrate affinity, greater apparent maximal velocity and it is much less sensitive to trans-stimulation. In addition to structural and functional aspects, this review discusses the role of the CAT proteins for supplying substrate to NO synthases and the property of the rodent CAT-1 proteins to function as virus receptors.

Keywords: Cationic amino acid – Transporter – Carrier – System y^+ – Ecotropic virus – Nitric oxide

Abbreviations: CAT, cationic amino acid transporter; m, mouse; h, human; r, rat; Tea, T cell early activation protein; CAA, cationic amino acids; TM, transmembrane spanning domain; rBAT, related to $b^{0,+}$ amino acid transporter; 4F2hc, 4F2 heavy chain cell surface antigen; MuLV, murine leukemia viruses; K_m, Michaelis Menten constant

Mammalian cells exhibit a large variety of amino acid transport activities (for reviews see Christensen, 1990; Kilberg et al., 1993). In particular, several

transport systems mediate the uptake of the cationic amino acids (CAA) L-lysine, L-arginine and L-ornithine. These different systems can be distinguished by their affinity for CAA, by the dependence of their transport activity on the presence of sodium ions and by their ability to also transport non cationic amino acids (Table 1). The activity of each system is saturable and often stimulated by substrate on the trans-side of the membrane indicating that transport is mediated by a carrier protein. To date, it has not yet been elucidated if a transport system consists of only the carrier protein(s) itself or also of additional regulatory proteins associated with the carrier. Therefore, the various transport systems described in different cells could reflect the presence of diverse carrier and/or regulatory proteins. Only recently cDNAs encoding discrete transport proteins for CAA have been cloned from mammalian cells (for reviews see Van Winkle, 1993; Bertran et al., 1994). They can be classified into two unrelated groups of membrane proteins: First, the rBAT (D2)/4F2hc proteins (rBAT: related to $b^{0,+}$ amino acid transporter; 4F2hc: 4F2 heavy chain cell surface antigen), a family of type II glycoproteins with 1 or 4 predicted transmembrane spanning domains (TM) (for review see Palacin, 1994). This structure is unusual for carrier proteins and therefore it has been speculated that the rBAT/ 4F2 proteins may represent regulatory components rather than transport proteins. The second group comprises of three related cationic amino acid transporters (CATs) with 12 or 14 putative transmembrane spanning domains (Fig. 1). This review focuses on the structure and function of the CAT-proteins in different mammalian

Table 1. Transport systems described for cationic amino acids

Name	y^+	y^+L	$b1^+$	$b2^+$	$b^{0,+}$	$B^{+,0}$
Ref.	(Christensen, 1964; Christensen and Antonioli, 1969)	(Devés et al., 1993; Harvey et al., 1993)	(Van Winkle and Campione, 1990)	(Van Winkle and Campione, 1990)	(Van Winkle et al., 1988)	(VanWinkle et al., 1985)
Transport of cationic amino acids						
$+Na^+$	+	+	+	+	Lys uptake inhibited	+
$-Na^+$	+	+	+	+	+	−
Transport of small neutral amino acids						
$+Na^+$	+(low affinity)	+/−	−	−	+	+
$-Na^+$	−	−	−	−	+	−
Transport of large neutral amino acids						
$+Na^+$	−	+(high affinity)			+	+
$-Na^+$	−	−			+	−
Inhibition by N-ethylmaleimide (NEM)	+	−				
Inhibition by 2-aminobicyclo-(2,2,1)-heptane-2-carboxylic acid (BCH)	−	−	−	−	−	+

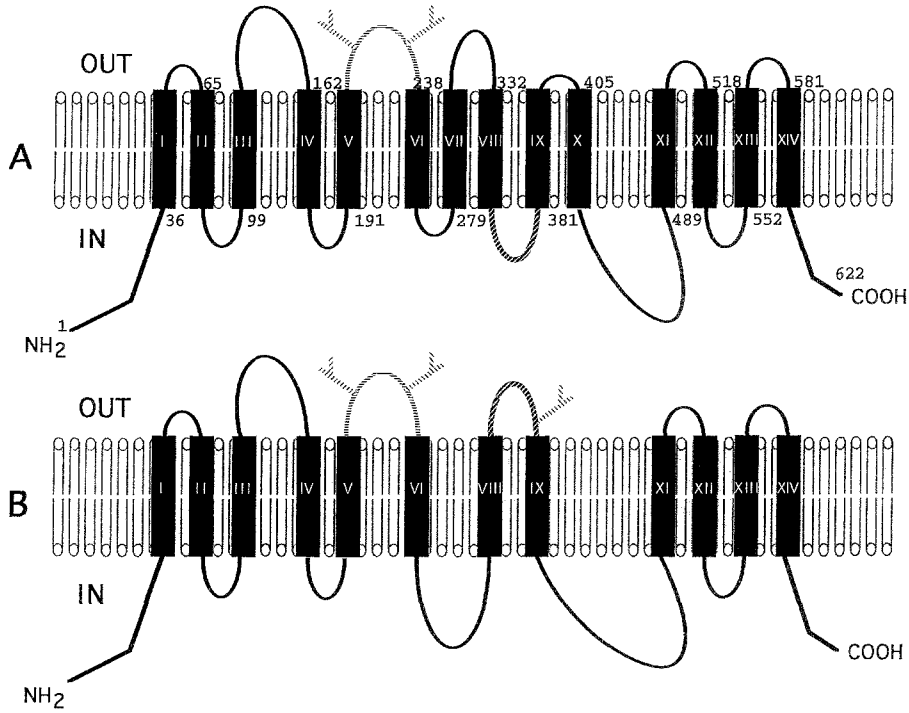

Fig. 1. Two alternative models for mCAT-1 in the membrane. **A** Model with 14 transmembrane spanning domains (TMs) based on the comparison of the five family members shown in Fig. 2. The numbering of the TMs corresponds to that in Fig. 2. **B** 12 TM model based on structural similarities with known permeases form bacteria and yeast. In this model, the sequences representing TM VII and X in **A** are not membrane spanning but belong to intracellular loops. Pale dashed line: third extracellular loop containing the MuLV binding site, Dashed forked lines: predicted N-linked glycosylation sites. (For mCAT-1 it has been shown that only the two N-linked glycosylation sites in the third extracellular domain are glycosylated.) Dark dashed line: domain shown to determine transport properties of the CAT proteins. Note that this domain is intracellular in **A** and extracellular in **B**

species, the role of the CAT proteins for supplying substrate to NO synthases and the property of the rodent CAT-1 proteins to function as virus receptors.

I mCAT-1, first described as a virus receptor

Retroviral infection requires binding of the viral envelope protein to a specific protein at the membrane of the host cell, the so called virus receptor. The binding of viral envelope to the cellular receptor determines the host range of the virus. For instance, infection of ecotropic murine leukemia viruses (MuLV) is restricted to rodent cells (for review see Weiss, 1993). In their cloning strategy, Albritton and co-workers took advantage of the narrow host range of these viruses. They introduced genomic DNA from mouse into human cells and obtained a human cell line susceptible for ecotropic MuLV

Table 2. The three mCAT-proteins

	mCAT-1	mCAT-2A	mCAT-2# (mCAT-2B)
Alternative names	REC-1, EcoR ERR, MLV-R, ATRC1, $y1^+$	mCAT-2# mCAT-2α, $y2^+$	Tea, mCAT-2β
First described as	receptor for ecotropic MuLV		T cell early activation protein
No. of amino acids	622	657	658
Predicted MW (kDa)	67	71.8	71.8
TMs*	12 or 14	12 or 14	12 or 14
N-glycosylation	yes	yes	yes
Principal sites of expression**	widely expressed (absent from liver)	liver, muscle, skin	activated T cells/ macrophages lung, testis

*Depending on the algorithm used to analyze the protein structure, 12 or 14 TMs (transmembrane domains) are predicted for the mCAT proteins (Fig. 1). #In the initial reports showing transport function of the mCAT-2 proteins, the term mCAT-2 was used for both the high and the low affinity isoforms (Closs et al., 1993a; Kakuda et al., 1993). The low affinity isoform is designated mCAT-2A. ** For a detailed analysis of CAT expression, see review by MacLeod and Kakuda (1996).

infection. This cell line allowed the isolation of a full length murine cDNA that codes for the receptor for ecotropic MuLVs (Albritton et al., 1989). The virus receptors known until then were found to have only one TM and a large extracellular entity. In contrast, analysis of the open reading frame of the cDNA encoding for the MuLV receptor predicted a hydrophobic protein of 622 amino acids with 14 putative TMs and only small extracellular regions. Although a search of the Genebank did not show any significant sequence homology with known proteins, we found similarities in the predicted secondary structure of the receptor and the L-histidine and L-arginine permeases of the yeast Saccharomyces cervisiae. By expressing the virus receptor in Xenopus oocytes and performing uptake studies with different amino acids we and others showed that the MuLV receptor mediates the transport of CAA (Kim et al., 1991; Wang et al., 1991). To reflect its cellular function we renamed the receptor mCAT-1 (for mouse cationic amino acid transporter). Expression of mCAT-1 has been found in all tissues investigated with the exception of the liver.

Several alternative names have been used for this carrier (Table 2). Rec-1 (receptor, ecotropic) and ATRC1 (amino acid transporter, cationic) are the symbols assigned respectively, to the genes encoding the mouse and human CAT-1 proteins but they have also been used to describe the gene products (Albritton et al., 1992). In this review, the name CAT-1 is used throughout.

II mCAT-2A and mCAT-2(B), two carriers related to mCAT-1

MacLeod and co-workers isolated several mouse cDNAs from a T-lymphoma cell line one of which was homologous to mCAT-1 that they named Tea for T-cell early activation (MacLeod et al., 1990; Reizer et al., 1993). In addition to activated T cells, they found expression of Tea mRNA in liver. Using the Tea cDNA clone provided by C. MacLeod, we isolated a full length cDNA from liver that is identical to Tea except for a stretch of 120 nucleotides encoding a putative intracellular loop between the Tms VIII and IX according to the 14 TM model (Fig. 1) (Closs et al., 1993a). In addition, we demonstrated that Tea transcripts expressed in activated macrophages are identical to the transcripts isolated from T cells and constructed a full length cDNA, using the original incomplete Tea cDNA clone (Closs et at., 1993c). The liver and T-cell/macrophage cDNAs encode proteins of 657 and 658 amino acids respectively that are 97% identical and differ only by 20 amino acids within a stretch of 42 amino acids. Expression in Xenopus oocytes showed that both protein mediate the transport of CAA (Closs et al., 1993a; Kakuda et al., 1993; Closs et al., 1993c). To reflect the transport function, the gene encoding both carriers was named *mCat-2*. Unfortunately, the term mCAT-2 was used by the MacLeod group and our group in papers published nearly simultaneously (Closs et al., 1993a; Kakuda et al., 1993) to designate the two distinct mCAT-2 isoforms. Here, mCAT-2A refers to the liver isoform as we later proposed (Closs et al., 1993c). The term mCAT-2 (Kakuda et al., 1993; MacLeod et al., 1994) or mCAT-2B (Closs et al., 1993c) refers to the T-cell/macrophage carrier. To indicate that mCAT-2 and mCAT-2B designate the same carrier, this isoform will be refered to as mCAT-2(B). A more recent report confirms the presence of the two different mCAT-2 transcripts in mouse liver and T-lymphocytes respectively (Kavanaugh et al., 1994b). Together the three carriers form a family of closely related proteins discovered by independent groups (Table 2).

III Transport properties of the mCAT proteins

The transport properties of each mCAT protein have been investigated individually by expression of the carriers in Xenopus oocytes and subsequent transport studies either using radiolabelled amino acids or measuring amino acid-induced membrane currents by the voltage clamp technique. Using both methods, mCAT-1 has been found to specifically mediate the transport of CAA, both into and out of cells. Transport is saturable with substrate, stereoselective and independent of the presence of sodium ions (Kim et al., 1991; Wang et al., 1991). The apparent K_m (Michaelis Menten constant) values for influx of L-arginine reported by different groups vary between 70 and 250μM (Table 3). K_m values for L-lysine and L-ornithine are similar to that for L-arginine, suggesting that all three substrates are recognized with similar affinity. CAA flux mediated by mCAT-1 is voltage dependent: hyperpolarization increases the V_{max} (maximal velocity) and decreases the apparent K_m for influx while it decreases the V_{max} and increases the apparent

K_m for efflux (Kavanaugh, 1993). Therefore, one explanation for the variation in K_m values found for mCAT-1 (Table 3) might result from differences in membrane potential of the oocytes under different experimental conditions. The V_{max} of mCAT-1-mediated influx or efflux is also dependent on the concentration of substrate at the trans-side of the membrane (Closs et al., 1993a, 1993c). In efflux experiments performed with no CAA at the outside (zero-trans experiments), the transport rate is up to 15-fold lower compared to efflux into 0.25 mM trans-substrate. It has not been investigated if the apparent K_m of mCAT-1 also depends on the substrate concentration at the trans-side. If true, this could also explain differences in K_m values determined by different groups. The selectivity of mCAT-1 for transport of dibasic amino acids is shown by the pH dependence of L-histidine transport. At pH 7.4, L-histidine is a poor substrate for mCAT-1, most likely because most of the L-histidine is dipolar (neutral). In contrast, at pH 5.5, when most of the L-histidine is protonated, the transport rate of mCAT-1 for L-histidine is similar to that for L-arginine (Kim et al., 1991). Using voltage-clamped Xenopus oocytes expressing mCAT-1, Wang and co-workers showed that mCAT-1 also transports small neutral amino acids with very low affinity. In contrast to CAA, transport of small neutral amino acids is sodium-dependent (Wang et al., 1991). The transport properties and tissue distribution of mCAT-1 are consistent with system y^+, the principal transport activity of mammalian cells, first described in Ehrlich cells (for review see White, 1985).

Like mCAT-1, mCAT-2(B) demonstrates "y^+"-like activity: transport of CAA is saturable, sodium-independent, stereoselective, and dependent on the substrate concentration at the trans-side (Kakuda et al., 1993; Closs et al., 1993c). A low affinity, sodium-dependent transport activity for neutral amino acids has also been reported (Kakuda et al., 1993; Table 3). The apparent K_m values of mCAT-2(B) reported for L-arginine range from 38 at 380 μM (Closs et al., 1993c; Kakuda et al., 1993; Kavanaugh et al., 1994b). This variability is probably also based on differences in the experimental conditions as discussed

Table 3. Substrate transport and apparent K_m values for mCATs

	L-Arg[1)]	L-Lys[1)]	L-Orn[1)]	L-His[2)]	L-Cys[3)]	Reference
mCAT-1	0.07 0.077 ± 0.002 0.140 – 0.25 0.206 ± 0.02	0.073 ± 0.008	0.105 ± 0.002	1.83 ± 0.07	24.7 ± 2.1	(Kim et al., 1991) (Wang et al., 1991) (Closs et al., 1993a) (Kakuda et al., 1993)
mCAT-2A	2.150 – 5.2 2.7	+	+	–	–	(Closs et al., 1993a) (Kavanaugh et al., 1994b)
mCAT-2	0.187 ± 0.028	0.203 ± 0.034	0.419 ± 0.05	3.89 ± 0.1	+	(Kakuda et al., 1993)
(mCAT-2B)	0.250 – 0.380 0.038	+ 0.051	+ 0.174	–	–	(Closs et al., 1993c) (Kavanaugh et al., 1994b)

+: significant transport activity reported, K_m not determined; –: no significant transport activity detected.
[1)] Na^+-independent; [2)] partially Na^+-dependent at pH 7.4; [3)] Na^+-dependent.

for mCAT-1. Interestingly, two groups found the apparent K_m for influx of L-ornithine to be two- to four-fold higher than for L-arginine or L-lysine (Kakuda et al., 1993; Kavanaugh et al., 1994b, Table 3). These results could reflect true differences in the recognition of the three substrates at the extracellular face of mCAT-2(B) because they were obtained under similar conditions in the two laboratories. It would be interesting to know if the same differences are found at the intracellular face of the transporter. When comparing L-arginine uptake by mCAT-1 and mCAT-2(B) directly in the same experiment, we find subtle, but reproducible differences between the two carriers: mCAT-1 has a higher apparent affinity for L-arginine, is more sensitive to trans-stimulation and accumulates less L-arginine when oocytes are incubated in 10 mM L-arginine for six hours (see also V). More experiments under defined conditions of the membrane potential and trans-substrate concentration will be necessary to illuminate the functional differences between these two "y^+"-like transporters.

mCAT-2A is highly expressed in liver (Closs et al., 1993a, 1993b). Similar to mCAT-1 and mCAT-2(B), CAA-transport mediated by mCAT-2A is saturable, sodium-independent, and stereoselective. However, mCAT-2A can be clearly distinguished from the two "y^+-carriers": it exhibits at least a ten-fold lower apparent substrate affinity (2–5 mM) and its activity is largely independent of substrate on the trans-side of the membrane (Closs et al., 1993a, 1993c; Kavanaugh et al., 1994b). Efflux mediated by mCAT-2A into a buffer containing no CAA is only 40% reduced when compared to efflux into 6.4 mM L-arginine. Although the initial rates of uptake mediated by all three carriers are similar, incubation of oocytes in 10 mM L-arginine for 6 hours leads to a ten-times greater accumulation of L-arginine in oocytes expressing mCAT-2A when compared to mCAT-1 or mCAT-2(B). This suggests that the apparent substrate affinity of mCAT-2A at the intracellular face is also an order of magnitude smaller than of mCAT-1 or mCAT-2(B). Therefore, in spite of its 97% identity with mCAT-2(B), mCAT-2A does not behave like a "y^+-carrier". The peculiarity of CAA-transport in the liver had already been recognized by White and Christensen who found only a low affinity transport of L-arginine in primary hepatocytes that is not subject to trans-stimulation. They concluded that $y^+$was absent or altered in hepatocytes (White and Christensen, 1982).

IV Structure of the mCAT proteins in the membrane

So far, little is known about the structure of the mCAT-proteins in the membrane. Most of the information available has been gained for mCAT-1. As mCAT-2A and mCAT-2(B) show almost identical hydrophilicity profiles to mCAT-1, it is likely that their protein domains are arranged in the same way. The amino termini have no signal sequences suggesting that both the amino and the carboxy termini are intracellular. Depending on the computer program used either 12 or 14 TMs are predicted for mCAT-1. For clarity, numbering of the TM and intra- and extracellular domains in this review will be according to the 14 TM domains suggested by Albritton et al. (Albritton et al., 1989) (Fig. 1A). This model is supported by the finding of similarities in

the localisation of TM I through VIII of mCAT-1 and protein permeases from yeast and fungi (Kim et al., 1991; Sophianopoulou and Diallinas, 1995). In contrast, MacLeod and colleagues point out that the mCAT proteins are members of a larger family of transport proteins (Reizer et al., 1993) with presumably 12 TM domains. In their model of mCAT-1 the hydrophobic domains VII and X are not membrane spanning but are located intracellularly. Accordingly, the loop between TM VIII and IX would be extracellular (Fig. 1B). Performing mutational analysis, Albritton, et al. showed that the viral binding site of mCAT-1 is located between TM V and VI, confirming the extracellular position of the connecting loop (Albritton et al., 1993). Evidence for the 14 TM structure also comes from immuno-staining of unpermealized cells with antibodies raised against peptides contained in the viral-binding (third) and also in the adjacent (fourth) extracellular loop (Woodard et al., 1994). If the latter would be intracellular as predicted in the 12 TM model, binding of antibody could only occur after permeabilization of the cell membrane.

Analyses of the N-glycosylation sites have brought further insight into the membrane topology of the mCAT proteins. N-glycosylation of mCAT-1 and mCAT-2A has been demonstrated by Western blots: mCAT-protein derived from N-glycosidase treated lysates of oocytes or of mammalian cells migrates as a distinct band on SDS PAGE. The sizes of the mRNA and cRNA-encoded deglycosylated proteins are indistinguishable and in agreement with the molecular mass predicted from the amino acid sequence (67 kDa for mCAT-1 and 71 kDa for mCAT-2A). In contrast, proteins from untreated lysates migrate slower, giving rise to a broad band between 70 and 80 kDa (Closs et al., 1993a; Kim and Cunningham, 1993). Two putative N-linked glycosylation sites (Asn^{223} and Asn^{229}) conserved in all CAT proteins characterized to date are present in the third extracellular loop. A third potential glycosylation site in the 12 TM model corresponds to Asn^{373} of mCAT-1. In the 14 TM model Asn^{373} is located intracellularly and therefore not glycosylated. Mutation of Asn^{223} and Asn^{229} to histidine in mCAT-1 results in a protein that migrates with the same mobility as the N-glycosidase-treated wild type mCAT-1 (Kim and Cunningham, 1993). Mutation of each asparagine residues individually results in proteins with molecular masses intermediate between the fully glycosylated and the deglycosylated mCAT-1. Together, the three mutant proteins demonstrate that both Asn^{223} and Asn^{229} are glycosylated and they represent the only glycosylated asparagine residues in mCAT-1. These experiments show that Asn^{373} is not glycosylated supporting the 14 TM model.

V Functional analysis of the mCAT proteins

The apparent substrate affinity and sensitivity to trans-stimulation of mCAT-2A and mCAT-2(B) differ markedly suggesting that these transport properties are determined by the region of 42 amino acids divergent between the two proteins. Exchange of the corresponding protein domains between mCAT-1 and mCAT-2A or -2(B) led to chimeric proteins with transport properties

Table 4. Comparison between chimeric and wild type mCAT-proteins

	Composition		L-Arginine transport properties			
			K_m (mM)	V_{max}	Trans-stimulation	Accumulation
mCAT-1	Wild type		0.14–0.25	1.10–1.60	8.3	1.0
mCAT-2A	Wild type		2.10–5.20	3.90–7.10	1.5	11.0
mCAT-2 (mCAT-2B)	Wild type		0.25–0.38	1.10–3.40	2.9	2.5
Chimeras	Backbone	*42 AA Domain				
mCAT-2/1	2	1	0.19–0.23	0.60–0.76	12.0	0.8
mCAT-1/2A	1	2A	1.43–1.57	8.70–9.50	1.1	8.0
mCAT-1/2(B)	1	2(B)	0.45–0.54	3.40–4.73	1.6	2.5

V_{max} nmol L-Arg/ oocyte/ h; *Trans-stimulation* Fold transport activity at high versus zero trans-L-arginine (high L-arginine for wild type mCATs 0.25 mM, for chimeras 0.1 mM); *Accumulation* nmol L-arginine taken up per oocyte after incubation in 10 mM L-arginine for 6 hours; **42 AA domain* region divergent between mCAT-2A and -2 (B); data are from Closs et al. (1993c).

corresponding to the donor of that region (Table 4). Interestingly, the chimeras mCAT-1/2(B) and mCAT-2/1 demonstrate the same subtle but reproducible differences observed between mCAT-1 and mCAT-2(B) (see III). The exchanged transport features include the apparent affinity for L-arginine, sensitivity to trans-stimulation and extent of L-arginine accumulation in an experiment with saturating substrate concentrations. These findings suggest that substrate recognition at both sides of the membrane as well as the mechanism of substrate translocation are established by this protein domain. Depending on the model, the domain is located either at the inside (14 TM model) or at the outside (12 TM model) of the membrane. It will be of great interest to find out if this domain contains the substrate binding site and if it is translocated from the cis to the trans-side of the membrane during a transport cycle as postulated for carrier-mediated transport.

Mutational analyses have shed further light on sites in the CAT proteins that are important for transport function. The transport activity of the unglycosylated AsnØHis double mutant of mCAT-1 is not altered indicating that N-linked glycosylation is not required for transport function (Kim and Cunningham, 1993). The presence of charged residues in TMs is unfavorable and hints at a possible involvement in substrate binding and translocation. Glu^{107} is located in TM III of all CAT-proteins characterized. The conservative replacement of Glu^{107} by Asp led to a loss of transport activity (Wang et al., 1994), whereas replacement by the uncharged Gln did not reduce transport activity (Kim and Cunningham, unpublished results) suggesting that the carbon backbone and not the negative charge of the amino acid is necessary to sustain function of the carrier.

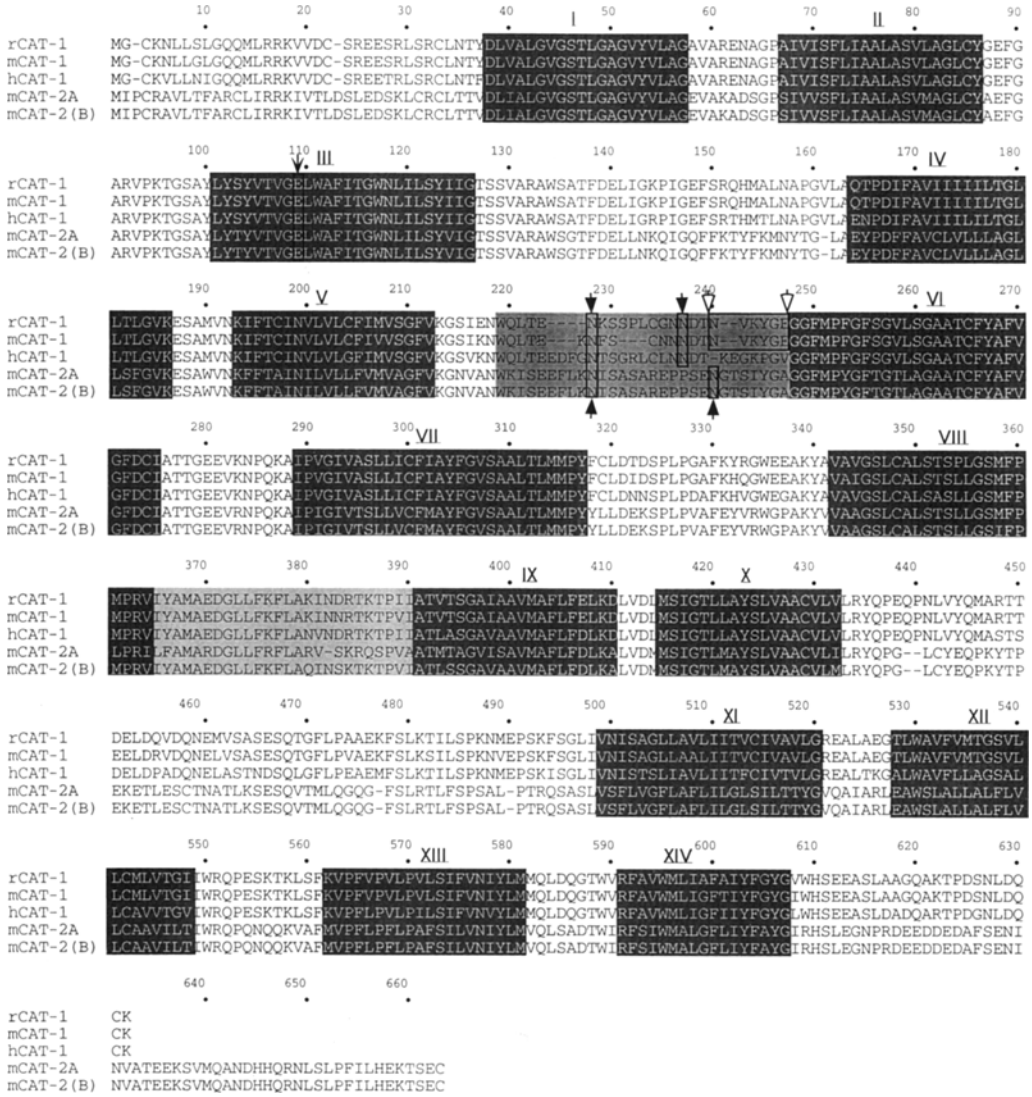

Fig. 2. Alignment of the amino acid sequences deduced from published cDNAs encoding mouse (*m*), rat (*r*) and human (*h*) CAT-proteins. Black boxes: indicate the location of the 14 predicted TMs (I through XIV) using five different algorithms (Bull and Breese, 1974; Manavalan and Ponnuswamy, 1978; Hopp and Woods, 1981; Kyte and Doolittle, 1982; Eisenberg et al., 1984); arrow points at charged residue (Glu) in TM III. Dark grey box: third extracellular loop, dark arrowheads point to the N-linked glycosylation sites identified in mCAT-1, white arrowheads point at the sequence in rat and mouse CAT-1 that is essential for virus binding and infection. Pale grey box: domain shown to determine the transport properties of the mCAT proteins

VI CATs in other mammalian species

Besides mouse CAT-1, cDNAs encoding the human (Yoshimoto et al., 1991; Albritton et al., 1992) and rat (Wu et al., 1994; Puppi and Henning, 1995) homologues have been isolated. An alignment of the deduced amino acids

demonstrates a high degree of identity between species: 86% between mouse and human and 95% between mouse and rat (Fig. 2). Transport of L-arginine by hCAT-1 has been shown (Albritton et al., 1993). In order to further characterize the CAA transporters in human cells, we cloned two cDNAs encoding the human homologues to mCAT-2A and mCAT-2(B) (Closs, E.I., Gräf, P., Habermeier, A., Cunningham, J., Förstermann, U. manuscript in preparation). Analyses of these cDNAs showed 90% identity between the deduced amino acid sequences of the murine and human proteins. Transport studies performed with oocytes injected with cRNAs for hCAT-1, hCAT-2A and hCAT-2(B) respectively demonstrated that, like the mouse homologues, hCAT-1 and hCAT-2(B) are high affinity "y^+"- like carrier proteins that are sensitive to trans-stimulation. In contrast, hCAT-2A exhibits a ten times lower substrate affinity and is largely independent of substrate at the trans-side of the membrane. The structural and functional similarities of the mouse and human carrier proteins suggest that transport of cationic amino acids is regulated in a similar way in both species.

VII Function of the rodent CAT-1 as an ecotropic MuLV receptor

Both murine and rat CAT-1 serve as receptors for ecotropic MuLV, whereas human CAT-1 does not. By domain swapping of murine and human CAT-1 and site directed mutagenesis, Albritton and co-workers identified the sequence $NVKYGE^{232-237}$ within the third extracellular loop as essential for both virus envelope binding and infection. This sequence is also contained in rCAT-1 but not in hCAT-1, mCAT-2A or mCAT-2 (B) (Wu et al., 1994) (Fig. 2). There seems to be no strict correlation between binding of the viral envelope protein (gp70) and permissivity to infection. Substitution of the seven amino acids $KEGKPGV^{238-244}$ in hCAT-1 by NVKYGE of mCAT-1 confers both gp70-binding and ecotropic receptor function. In contrast, a hCAT-1 mutant with only the three amino acids $PGV^{242-244}$ replaced by YGE functions as virus receptor, but does not demonstrate detectable gp70-binding. In the reciprocal mutant of mCAT-1 $YGE^{235-237}$ØPGV both receptor function and gp70-binding are lost (Albritton et al., 1993). The CAT-2 proteins can also function as ecotropic receptors when their third extracellular loop is exchanged by that of mCAT-1 (Kavanaugh et al., 1994b; Closs and Cunningham, unpublished observation). None of the mutations mentioned affects transport function.

Coexpression of mCAT-1 and the ecotropic envelope protein in Xenopus oocytes results in both an altered glycosylation of mCAT-1 and a 50 percent reduction of transport activity (Kim and Cunningham, 1993). These effects are not observed upon coexpression with envelope protein from non-ecotropic viruses. Glycosylation of Asn^{223} seems to be more affected than Asn^{229} indicating that it is in closer contact with the envelope protein. The unglycosylated Asn^{223}ØHis, Asn^{229}ØHis double mutant of mCAT-1 demonstrates the same gp70-binding activity as the wild type. Its transport activity is also reduced by 50 percent upon coexpression with gp70, suggesting that the altered glycosylation is not responsible for the reduced activity. A 50

percent reduction in L-arginine transport activity has also been found in MuLV infected cells (Wang et al., 1992). It is likely that gp70-binding to mCAT-1 represents a steric hindrance for the conformational change required for translocation of the substrate. Understanding this interference should shed light into the mechanism of the translocation step during the transport process.

Binding of the viral envelope protein to its receptor is the initial step in retroviral infection. Besides viral binding, the receptor might also be involved in downstream events that lead to the fusion of the viral and cellular membranes. Two other retroviral receptors have been found to be transport proteins: the receptors for amphotropic MuLV and for gibbon ape leukemia virus (GaLV). Both mediate the transport of inorganic phosphate (O'Hara et al., 1990; Kavanaugh et al., 1994a; Miller et al., 1994; Vanzeijl et al., 1994). An intriguing question is therefore if transport and receptor functions might be coupled. The Glu^{107}ØAsp mutant of mCAT-1 demonstrates no transport activity but mediates gp70-binding and susceptibility to ecotropic infection (Wang et al., 1994), suggesting that transport and receptor function can be discerned. However, the transport deficient carrier could still undergo a conformational change similar to that of mCAT-1 during a transport cycle. It needs to be elucidated if this movement is necessary for receptor function. Understanding the virus-receptor interaction should improve the design of new retroviral vectors with altered envelope proteins that allow tissue specific infection. Specific targeting is a desirable goal for use of retroviral vectors in gene therapy. For instance, introduction of a single chain immunoglobulin fragment specific for low density lipoprotein receptor into ecotropic gp70 results in a vector targeted to cells expressing the low density lipoprotein receptor (Somia et al., 1995).

VIII Physiological role of the CAT proteins

CAA are building blocks for proteins and are also precursors for a variety of bioactive metabolites. L-arginine for instance, is the substrate for the synthesis of urea and nitric oxide and L-ornithine for polyamines. What part do the CAT proteins play for the CAA supply of cells that have a different requirement for these amino acids depending on their differentiation and activation status? The distinct transport properties and tissue distribution of the CAT proteins gives a first hint on the physiological role of these carriers. The ubiquitous expression of CAT-1 suggests it is the principal y^+ carrier in most cells. However, not all of the Na^+-independent, high affinity CAA uptake observed in a given cell type must be mediated by CAT-1. Other transport proteins such as CAT-2(B), 4F2 or other still unknown carriers might also be involved (see below). At CAA concentrations below 200 μM at the trans-side of the membrane, the transport activity of CAT-1 is greatly reduced (Closs et al., 1993c). This might prevent CAA efflux from cells at low plasma concentrations.

White and Christensen had already observed that the plasma membrane of hepatocytes constitutes a barrier for CAA that protects the plasma L-

arginine from hydrolysis by hepatocyte arginase (White and Christensen, 1982). The low affinity and high capacity of CAT-2A allows hepatocytes to rapidly take up excess CAA at high plasma concentrations while leaving sufficient CAA in the circulation for cells expressing carriers with higher affinity. The transport activity of CAT-2A is largely independent of the substrate concentration at the trans-side of the membrane (Closs et al., 1993a; Kavanaugh et al., 1994b). This might facilitate uptake of CAA into hepatocytes that demonstrate very low intracellular CAA concentrations.

The expression of CAT-2(B) can be induced in T cells and macrophages – cells that also express CAT-1 constitutively. The transport properties of the two carriers are very similar and therefore difficult to distinguish when expressed simultaneously. Why do some cells need a second y^+ carrier under certain conditions? In the macrophage cell line RAW 264.7, CAT-2(B) is induced by bacterial lipopolysaccharide (LPS) (Closs et al., 1993c) in parallel with the inducible NO synthase (NOS II), an enzyme that uses L-arginine as its substrate. NOS II activity has been reported to be dependent on extracellular L-arginine (Hibbs et al., 1987; Bogle et al., 1992). The expression of CAT-2(B) probably accounts for the increased transport rate of L-arginine observed in LPS-activated macrophages (Bogle et al., 1992), but does it have a specific role for supplying substrate for NOS II? To answer this question specific inhibitor- and knock out-experiments will be required. Interestingly, NO synthesis in endothelial cells mediated by NOS III seems to be largely independent of extracellular L-arginine (Palmer et al., 1988). It is intriguing to speculate that the difference in L-arginine supply of NOS might be due to the expression of different CAA carriers in the two cell types.

IX Future aspects

In 1991, mCAT-1 represented the first mammalian amino acid transporter whose cDNA had been cloned. Since then, a large number of cDNAs encoding for mammalian amino acid transporters have been isolated. Analyses of the structure and function of these proteins have improved our knowledge of solute transport in mammalian cells. However, we are still far from understanding the cellular transport systems: their composition and association with other proteins, their compartimentation, as well as their regulation of expression and activity. Apprehending the physiological relevance of amino acid transporters will help to discern their involvement in disease states. Further, the elucidation of the three-dimensional structure of the carrier proteins and the mechanism of substrate translocation through the membrane remains a major scientific challenge.

References

Albritton LM, Tseng L, Scadden D, Cunningham JM (1989) A putative murine ectropic retrovirus receptor gene encodes a multiple membrane-spanning protein and confers susceptibility to virus infection. Cell 57: 659–666

Albritton LM, Bowcock AM, Eddy RL, Morton CC, Tseng L, Farrer LF, Cavalli-Sforza LL, Shows TB, Cunningham JM (1992) The human cationic amino acid transporter (ATRC1): physical and genetic mapping to 13q12–q14. Genomics 12: 430–434

Albritton LM, Kim JW, Tseng L, Cunningham J (1993) Envelope binding domain in the cationic amino acid transporter determines the host range of ecotropic murine retroviruses. J Virol 67: 2091–2096

Bertran J, Testar X, Zorzano A, Palacin M (1994) A new age for mammalian plasma membrane amino acid transporters. Cell Physiol Biochem 4: 217–241

Bogle RG, Baydoun AR, Moncada S, Pearson JD, Mann GE (1992) L-arginine transport is increased in macrophages generating nitric oxide. Biochem J 284: 15–18

Bull HB, Breese K (1974) Surface tension of amino acid solutions: a hydrophobicity scale of the amino acid residues. Arch Biochem Biophys 161: 665–670

Christensen HN (1964) A transport system serving for mono- and diamino acids. Proc Natl Acad Sci USA 51: 337–344

Christensen HN (1990) Role of amino acid transport and countertransport in nutrition and metabolism. Physiol Rev 70: 43–77

Christensen HN, Antonioli JA (1969) Cationic amino acid transport in the rabbit reticulocyte. J Biol Chem 244: 1497–1504

Closs EI, Albritton LM, Kim JW, Cunningham JM (1993a) Identification of a low affininty, high capacity transporter of cationic amino acids in mouse liver. J Biol Chem 268: 7538–7544

Closs EI, Borel Rinkes I, Bader A, Yarmush M, Cunningham J (1993b) Retroviral infection and the expression of cationic amino acid transporters in rodent hepatocytes. J Virol 67: 2097–2102

Closs EI, Lyons CR, Kelly C, Cunningham JM (1993c) Characterization of the third member of the MCAT family of cationic amino acid transporters – identification of a domain that determines the transport properties of the MCAT proteins. J Biol Chem 268: 20796–20800

Deves R, Angelo S, Chavez P (1993) N-Ethylmaleimide discriminates between two lysine transport systems in human erythrocytes. J Physiol-London 468: 753–766

Eisenberg D, Schwarz E, Komaromy M, Wall R (1984) Analysis of membrane and surface protein sequences with the hydrophobic moment plot. J Mol Biol 179: 125–142

Harvey CM, Muzyka WR, Yao SYM, Cheeseman CI, Young JD (1993) Expression of rat intestinal L-lysine transport systems in isolated oocytes of Xenopus laevis. Am J Physiol 265: G99–G106

Hibbs JJ, Vavrin Z, Taintor RR (1987) L-arginine is required for expression of the activated macrophage effector mechanism causing selective metabolic inhibition in target cells. J Immunol 138: 550–565

Hopp TP, Woods KR (1981) Prediction of protein antigenic determinants from amino acid sequences. Proc Natl Acad Sci USA 78: 3824–3828

Kakuda DK, Finley KD, Dionne VE, MacLeod CL (1993) Two distinct gene products mediate y^+ type cationic amino acid transport in Xenopus oocytes and show different tissue expression patterns. Transgene 1: 91–101

Kavanaugh MP (1993) Voltage dependence of facilitated arginine flux mediated by the system y^+ basic amino acid transporter. Biochemistry 32: 5781–5785

Kavanaugh MP, Miller DG, Zhang WB, Law W, Kozak SL, Kabat D, Miller AD (1994a) Cell-surface receptors for gibbon ape leukemia virus and amphotropic murine retrovirus are inducible sodium-dependent phosphate symporters. Proc Natl Acad Sci USA 91: 7071–7075

Kavanaugh MP, Wang H, Zhang Z, Zhang W, Wu YN (1994b) Control of cationic amino acid transport and retroviral receptor functions in a membrane protein family. J Biol Chem 269: 15445–15450

Kilberg MS, Stevens BR, Novak DA (1993) Recent advances in mammalian amino acid transport. Annu Rev Nutr 13: 137–165

Kim JW, Cunningham JM (1993) N-Linked glycosylation of the receptor for murine ecotropic retroviruses is altered in virus-infected cells. J Biol Chem 268: 16316–16320
Kim JW, Closs EI, Albritton LM, Cunningham JM (1991) Transport of cationic amino acids by the mouse ecotropic retrovirus receptor. Nature 352: 725–728
Kyte J, Doolittle RF (1982) A simple method for displaying the hydropathic character of a protein. J Mol Biol 157: 105–132
MacLeod CL, Finley K, Kakuda D, Kozak CA, Wilkinson MF (1990) Activated T cells express a novel gene on chromosome 8 that is closely related to the murine ectropic retroviral receptor. Mol Cell Biol 10: 3663–3674
Manavalan P, Ponnuswamy PK (1978) Hydrophobic character of amino acid residues in globular proteins. Nature 275: 673–674
Miller DG, Edwards RH, Miller AD (1994) Cloning of the cellular receptor for amphotropic murine retroviruses reveals homology to that for gibbon ape leukemia virus. Proc Natl Acad Sci USA 91: 78–82
O'Hara B, Johann SV, Klinger HP, Blair DG, Rubinson H, Dunn KJ, Sass P, Vitek SM, Robbins T (1990) A gene conferring sensitivity to infection by GALV. Cell Growth Differ 1: 119–127
Palacin M (1994) 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. J Exp Biol 196: 123–137
Palmer RM, Ashton DS, Moncada S (1988) Vascular endothelial cells synthesize nitric oxide from L-arginine. Nature 333: 664–666
Puppi M, Henning SJ (1995) Cloning of the rat ecotropic retroviral receptor and studies of its expression in intestinal tissues. Proc Soc Exp Biol Med 209: 38–45
Reizer J, Finley K, Kakuda D, MacLeod C, Reizer A, Saier M (1993) Mammalian integral membrane receptors are homologous to facilitators and antiporters of yeast, fungi and eubacteria. Prot Science 2: 20–30
Somia NV, Zoppe M, Verma IM (1995) Generation of targeted retroviral vectors by using single-chain variable fragment: an approach to in vivo gene delivery. Proc Natl Acad Sci USA 92: 7570–7574
Sophianopoulou V, Diallinas G (1995) Amino acid transporters of lower eukaryotes: regulation, structure and topogenesis. FEMS Microbiol Rev 16: 53–75
Van Winkle LJ (1993) Endogenous amino acid transport systems and expression of mammalian amino acid transport proteins in Xenopus oocytes. Biochem Biophys Acta 1154: 157–172
Van Winkle LJ, Campione AL (1990) Functional changes in cation-preferring amino acid transport during development of preimplantation mouse conceptuses. Biochim Biophys Acta 1028: 165–173
Van Winkle LJ, Christensen HN, Campione AL (1985) Na^+-dependent transport of basic, zwitterionic, and bicyclic amino acids by a broad scope system in mouse blastocytes. J Biol Chem 260: 12118–12123
Van Winkle LJ, Campione AL, Gorman JM (1988) Na^+-independent transport of basic and zwitterionic amino acids in mouse blastocytes by a shared system and by processes which distinguish between these substrates. J Biol Chem 263: 3150–3163
Vanzeijl M, Johann SV, Closs E, Cunningham J, Eddy R, Shows TB, O'Hara B (1994) A human amphotropic retrovirus receptor is a 2nd member of the gibbon ape leukemia virus receptor family. Proc Natl Acad Sci USA 91: 1168–1172
Wang H, Kavanaugh MP, North RA, Kabat D (1991) Cell-surface receptor for ecotropic murine retroviruses is a basic amino-acid transporter. Nature 352: 729–731
Wang H, Dechant E, Kavanaugh M, North RA, Kabat D (1992) Effects of ecotropic murine retroviruses on the dual-function cell surface receptor/basic amino acid transporter. J Biol Chem 267: 23617–23624
Wang H, Kavanaugh P, Kabat D (1994) A critical site in the cell surface receptor for ecotropic murine retroviruses required for amino acid transport but not for viral reception. Virology 202: 1058–1060

Weiss RA (1993) Cellular receptors and viral glycoproteins involved in retroviral entry. In: The retroviridae. Plenum Press, New York, pp 1–108

White MF (1985) The transport of cationic amino acids across the plasma membrane of mammalian cells. Biochim Biophys Acta 822: 355–374

White MF, Christensen HN (1982) Cationic amino acid transport into cultured animal cells: II. Transport system barely perceptible in ordinary hepatocytes, but active in hepatoma cell lines. J Biol Chem 257: 4450–4457

Woodard MH, Dunn WA, Laine RO, Malandro M, McMahon R, Simell O, Block ER, Kilberg MS (1994) Plasma membrane clustering of system $y(^+)$ (CAT-1) amino acid transporter as detected by immunohistochemistry. Am J Physiol 266: E817–E824

Wu JY, Robinson D, Kung HJ, Hatzoglou M (1994) Hormonal regulation of the gene for the type C ecotropic retrovirus receptor in rat liver cells. J Virol 68: 1615–1623

Yoshimoto T, Yoshimoto E, Meruelo D (1991) Molecular cloning and characterization of a novel human gene homologous to the murine ecotropic retroviral receptor. Virology 185: 10–15

Author's address: Dr. E. I. Closs, Pharmakologisches Institut, Obere Zahlbacher Strasse 67, D-55101 Mainz, Federal Republic of Germany.

Received January 10, 1996