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Na^+-INDEPENDENT TRANSPORT (UNIPORT) OF AMINO ACIDS AND GLUCOSE IN MAMMALIAN CELLS

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Summary

Recent advances have made possible the isolation of the genes and their cDNAs encoding Na^+-independent amino acid transporters. Two classes of amino acid 'uniporters' have been isolated. One class contains the *mCAT* (*m*urine *c*ationic *a*mino acid *t*ransporter) gene family that encodes proteins predicted to span the membrane 12–14 times and exhibits structural properties similar to the GLUT (*glu*cose *t*ransporter) family and to other well-known transporters. The other class consists of two known genes, *rBAT* (*r*elated to *B* system *a*mino acid *t*ransporters) and *4F2hc*, that share amino acid sequence similarity with α-amylases and α-glucosidases. They are type II glycoproteins predicted to span the membrane only once, yet they mediate the Na^+-independent transport of cationic and zwitterionic amino acids in *Xenopus* oocytes. Mutations in the human *rBAT* gene have been identified by Palacín and his co-workers in several families suffering from a heritable form of cystinuria. This important finding clearly establishes a key role for rBAT in cystine transport. The two classes of amino acid transporters are compared with the well-studied GLUT family of Na^+-independent glucose transporters.

Carrier-mediated secondary transport of nutrients

Transport proteins regulate the movement of lipid-insoluble nutrients across the hydrophobic domain of the plasma membrane. The capacity to transport amino acids and glucose is required by all cells and is essential for protein synthesis, for the supply of metabolic energy, for maintaining and replacing structural components and for other key physiological functions. This paper addresses amino acid (and to a lesser extent glucose) transport that is largely independent of co-substrate requirements (uniport).

Transport systems and transporters

Individual cells express a variety of distinct transport systems, each facilitating the flux of several different amino acid or sugar substrates. Furthermore, most amino acids can be transported by several different transport systems, some expressed in distinct organs and tissues. The broad substrate-specificity and overlapping tissue and cellular distribution

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Key words: facilitated transport, uniport, GLUT, glucose, arginine, cystine, lysine, mCAT, rBAT, 4F2hc, *Xenopus* expression.

Table 1. *Mammalian nutrient transport systems and transporters*

Transport system	Putative transporter	Substrates
Na^+-independent		
$b^{o,+}$	rBAT/D2/NBAT	Lys, Leu, Trp, Met
y^+	mCAT-1, -2, -2a	Arg, Lys, Orn
y^+L	4F2hc	Lys, Arg, NMM
L		Leu, Ile, Val, Phe, BCH
x_c^-		Glu and Cys (antiport)
GLUT	GLUT 1–7	Glucose and fructose
Na^+-dependent		
y^+	mCAT-1, -2, -2a	Homoserine, Gln
y^+L	4F2hc	Leu, Met, NMM
A	SAAT1	Ala, Gly, AIB (MeAIB)
N		Gln, His
$B^{o,+}$		Ala, Lys, Arg, BCO
ASC	ASCT1	Ala, Ser, Cys, Thr, Leu, Ile, Phe; Asp (<pH 5.5)
X_{AG}^-	EAAC1, GLAST, GLT1	Asp, Glu

MeAIB, α-amino isobutyrate (α-methylamino isobutyrate); BCO, 3-aminoendobicyclo-(3,2,1)-octane-3-carboxylate; BCH, 2-amino-(2,2,1) bicycloheptane-2-carboxylate; NMM, *N*-methymaleimide.

Only selected substrates are presented; the data were abstracted from Kilberg and Häussinger (1992); McGivan and Pastor-Anglada (1994); Bertran *et al.* (1994) and Mueckler (1994).

patterns of amino acid transporters posed an analytical challenge which was innovatively addressed beginning in the 1950s and 1960s in work pioneered by H. N. Christensen and investigated by many others (for reviews, see Christensen, 1989; Van Winkle, 1993; Kilberg and Häussinger, 1992; Kilberg *et al.* 1993; McGivan and Pastor-Anglada, 1994; Bertran *et al.* 1994). Numerous transport systems were identified and characterized (Table 1). This research established methods and chemical analogs useful for discriminating the transport systems and allowed the initial analysis of the newly cloned transporters to progress quickly. We use the terms *transport system* to designate a functionally distinct transport process and *transporter* to designate a protein that catalyzes amino acid transport across a biomembrane (after Van Winkle, 1993).

Energetics

Transport processes are classified according to structural or energetic aspects. Carrier-mediated transport can be distinguished from simple diffusion by the specific kinetic properties originally used to characterize enzymes (Krämer, 1994). It is limited by the number of available cell surface substrate binding sites and is demonstrated by transport rates measured when all binding sites are occupied (V_{max}); in contrast, simple diffusion is non-saturable and limited only by substrate concentration. The most widely accepted classification, introduced by Mitchell (1967), is based on the utilization of energy sources for transport. Primary transport involves vectorial solute translocation directly coupled to

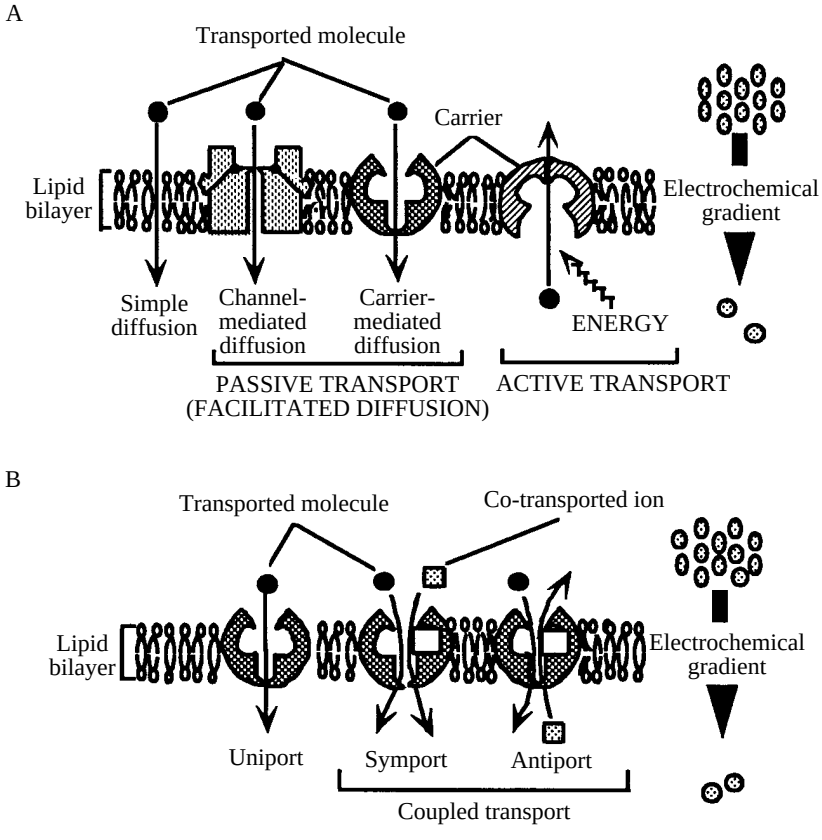

Fig. 1. Models of carrier-mediated transport across the lipid bilayer. These schematic diagrams show the several categories of carrier-mediated transport labeled according to the manner in which the solute flux is driven. (A) All the types of solute flow. Only small uncharged polar molecules can diffuse across the bilayer; other polar molecules flux at significant rates only when mediated by specific transporters. (B) Three types of facilitated transport. Symport and anitport require a co-substrate ion, whereas uniport is defined as ion-independent solute flux. Facilitated transport of all three classes frequently requires an electrochemical gradient. The figure is adapted from Alberts *et al.* (1989).

chemical or photochemical reactions. Secondary transport is driven solely by the electrochemical energy of a given solute, which can drive the uphill transport of some nutrients against their own concentration gradient and is achieved by cotransport (symport) or countertransport (antiport, Fig. 1).

The transport of cationic amino acids that is independent of co-substrate requirements (uniport) is driven simply by its own electrochemical gradient (Krämer, 1994; Kavanaugh, 1993) in a process often termed facilitated diffusion. This a misleading term since carrier-mediated transport is fundamentally different from diffusion (Krämer, 1994). Terms such as uniport and symport are confusing when describing amino acid transport systems because the transport of some substrates is ion-independent but that of

others is ion-dependent (see Table 1). For example, amino acid transport system y^+ requires Na^+ for zwitterionic amino acid influx (symport), but it mediates cationic transport (lysine and arginine) in a Na^+-independent manner (uniport; Christensen and Antonioli, 1969; White *et al.* 1982). The problem of naming cloned amino acid transporters in a manner recalling their precedent transport systems, substrate recognition and Na^+ requirements is discussed by Christensen *et al.* (1994).

The Na^+-independent transport of cationic amino acids is mediated by at least three distinct mCAT proteins (*m*urine *c*ationic *a*mino acid *t*ransporter) (for a review, see MacLeod *et al.* 1994) and also by a new class of proteins sometimes referred to as accessory proteins (for reviews, see Hediger *et al.* 1993; Bertran *et al.* 1994; Palacín, 1994). Cationic amino acid influx mediated by these transporters is driven by both substrate concentration and cellular voltage gradients. The negative resting membrane potential of cells favors the inward movement of cations. Kavanaugh (1993) used voltage-clamp experiments to determine the voltage- and concentration-dependence of arginine flux mediated by the mCAT-1 protein expressed in *Xenopus* oocytes. The voltage-sensitive behavior of the transporter was fitted with the iso-uni-uni kinetic model of facilitated transport (White and Christensen, 1982). In voltage-ramp experiments, arginine flux was examined. The I_{max} for arginine influx increased with hyperpolarization whereas the K_m value increased with depolarization. The data support the idea that the arginine binding site is located within the membrane electric field, resulting in voltage-dependent binding and unbinding. This model requires movement of negative charge from the inside to the outside during the transition of the unliganded transporter for the zero-*trans* influx cycle (Kavanaugh, 1993).

The GLUT (*glu*cose *t*ransport) proteins are encoded by six functional genes that mediate Na^+-independent uniport of glucose and fructose (for reviews, see Bell *et al.* 1993; Mueckler, 1994). For these hexoses, and zwitterionic amino acids that lack a net charge, their Na^+-independent transport is independent of membrane potential and is driven only by substrate concentration gradients. The GLUT proteins mediate the exchange of glucose between the blood and cytoplasm of cells in this manner. The direction of glucose transport is highly regulated and depends on the cell type and the metabolic status of the cell. However, the restricted expression of glucose-6-phosphatase endows only a subset of mammalian cells with the capacity to dephosphorylate, and hence export, glucose (e.g. liver and muscle, for a review, see Mueckler, 1994). Glucose transport is also mediated by a Na^+-dependent process that utilizes the electrochemical gradient to drive hexose influx (Wright *et al.* 1994).

A possible role for asymmetrical recognition in the bidirectionality of nutrient transport

Facilitated transport is bidirectional, and the direction of transport depends on several factors. In the case of cationic amino acids, cellular membrane potential strongly favors influx. Similarly, zwitterionic amino acid and glucose influx is favored when co-transported with Na^+. The asymmetrical recognition of substrate by a transporter may influence the preferred direction of transport. This property is exhibited by the y^+ transport system with the analog GPA (4-amino-1-guanylpiperidine-4-carboxlyate). GPA is recognized, transported and capable of lysine *trans*-stimulation only when present on the extracellular

face of the membrane (White *et al.* 1982; White and Christensen, 1982). *Trans*-stimulation is defined by an increase in transport when *trans*-side substrate concentration is elevated. *Trans*-stimulation by arginine was observed in mCAT-expressing *Xenopus* oocytes (Closs *et al.* 1993). Similarly, glucose transporters interact asymmetrically with ligands such as cytochalasin B (an inhibitor of hexose transport) and IAPS-forskolin (Wadzinski *et al.* 1988) or ATB-BMPA (Holman *et al.* 1990). Perhaps asymmetrical substrate recognition is a general property of transport proteins.

Essential roles and regulation of nutrient fluxes by uniporters

Amino acids

In addition to their constituent role in proteins, amino acids function as neurotransmitters, sources of metabolic energy and precursors to many biologically important molecules (Fig. 2). Nutrient homeostasis results from complex trafficking between organs and involves specific transport proteins (Christensen, 1982, 1990). Several different transporters mediate the flux of the cationic amino acids arginine, ornithine and lysine (Table 1). Although arginine is not an essential amino acid, only liver and kidney cells produce useful amounts. The kidney is the major arginine source *via* synthesis from citrulline (Barbul, 1990), whereas the high arginase activity in liver prevents its export (Herzfeld and Raper, 1976). All other cells import arginine from plasma for their metabolic requirements. Arginine is the immediate precursor in the synthesis of nitric oxide (NO), a short-lived metabolite involved in vasodilatation, neurotransmission, tumor immunity and non-specific host defense to foreign antigens (for

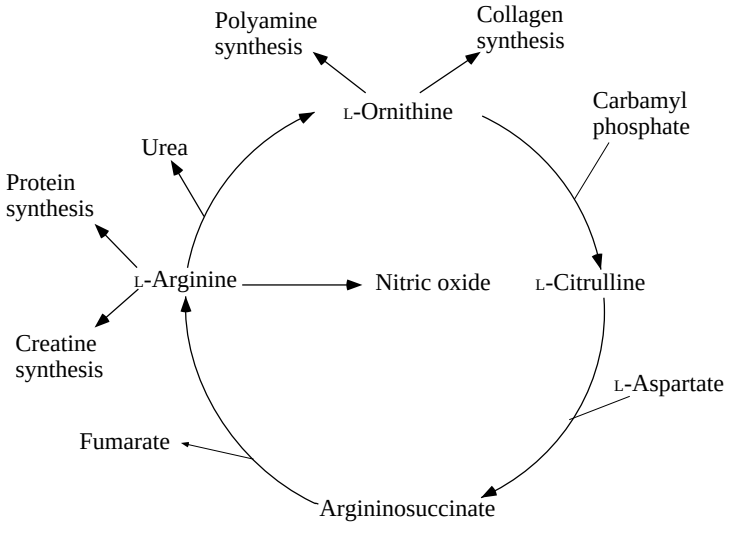

Fig. 2. Physiological roles of some amino acids. The sketch depicts the urea cycle combined with the functions of several of its substrate amino acids in various cellular biosynthetic reactions, showing the central role of arginine and ornithine in these processes.

a review, see Moncada and Higgs, 1993). Arginine is also the precursor of creatine, which functions as a high-energy phosphate carrier during muscle contraction. Ornithine is the precursor of polyamines (putrescine, spermidine and spermine), which are present in all cells and participate in cellular division and proliferation (Janne *et al.* 1991).

In mammals, lysine in an essential amino acid required for protein synthesis and is a precursor for trimethyllysine and carnitine synthesis, which are essential for the translocation of long-chain fatty acids into the mitochondrial matrix for β-oxidation. Since fatty acid oxidation is the major energy source used by cardiac and skeletal muscle, deficiencies in carnitine are associated with cardiac, liver and kidney disease (Borum, 1983; Bieber, 1988; Nyhan, 1988).

Cystine is not an essential amino acid. Nevertheless, the control of cystine distribution is interesting in that this amino acid is toxic to many cellular enzyme systems. Two cycles interchange cystine and cysteine within the cytoplasmic and lysosomal compartments and involve specific pH-dependent transport systems in lysosomes. Cystine transported into cells is used in protein or glutathione synthesis or is rapidly reduced to cysteine. The cysteine is transported into lysosomes, where it may react with disulfide bridges of proteins undergoing proteolysis. Cystine is exported from the lysosome back to the cytosol to complete the cycle. Glutathione is an essential antioxidant that plays a key role in oxidative stress responses (for a review, see Pisoni and Schneider, 1992). There are two common hereditary defects in cystine transport, Fanconi syndrome and hereditary cystinuria. Strong evidence for the role of rBAT in the latter condition is presented by Palacín (1994).

Amino acid reabsorption by the kidney is so efficient that more than 99 % is recovered in the glomerular filtrate. Heritable defects in intestinal and renal amino acid transport have been detected. For example, lysinuric protein intolerance appears to be a transport dysfunction in the basolateral excretion of lysine from kidney tubule cells (Rajantie *et al.* 1981) and intestinal mucosa (Rajantie *et al.* 1980). Patients with cystinuria hyper-excrete cystine, arginine, lysine and ornithine in their urine (Crawhall *et al.* 1967; Whelan and Scriver, 1968; Simell, 1989). Based on rBAT (*r*elated *B* system *a*mino acid *t*ransporter) expression in the microvilli of proximal straight tubules (Furriols *et al.* 1993), and the observation that anti-sense rBAT RNA reduced cystine uptake in *Xenopus* oocytes (Bertran *et al.* 1993), studies were undertaken to examine the *rBAT* gene in patients with cystinuria. Recent work from Palacín's group revealed one common and several other isolated missense mutations in the *rBAT* gene of individuals with heritable forms of cystinuria (Calonge *et al.* 1994; Palacín, 1994).

Glucose

The role of glucose and the regulation of its transport in metabolism and homeostasis are better understood than the more complex problem of transporting the wide variety of amino acids. The study of glucose transport benefited from the early cloning of the GLUT family of uniporters and the intense research effort directed to their analysis (over 1000 papers in 5 years; for a review, see Mueckler, 1994). Glucose is required by many cells for oxidative and non-oxidative ATP production. In mammalian cells, both a Na^+-

dependent cotransporter (Hediger *et al*. 1989; Wright *et al.* 1994) and at least six functional Na^+-independent uniporters regulate glucose fluxes.

Regulation

The appropriate distribution of whole-body glucose appears to be controlled by tissue-specific expression and regulation of several transporter isoforms with distinct kinetic properties that together play a major role in glucose homeostasis. Expression of GLUT1, GLUT2 and GLUT4 is regulated by numerous endogenous and xenobiotic factors. GLUT 4 regulation, the most intricate, illustrative and controversial of the GLUT isoforms, is discussed by Mueckler (1994).

GLUT4 is expressed only in adipocytes and muscle cells, both of which are highly 'insulin-sensitive' and respond to insulin with a rapid and reversible increase in glucose transport. The protein is sequestered intracellularly and, upon stimulation by insulin, it is recruited to the cell surface. This recruitment results in immediately increased glucose transport and occurs in the absence of *de novo* transcription or translation (for reviews, see Birnbaum, 1992; Bell *et al.* 1993). Skeletal muscle glucose transport accounts for 20 % of basal whole-body glucose uptake and for 75–95 % during hyper-insulinemia (Baron *et al.* 1988). GLUT4 regulation in these insulin-sensitive tissues is important for glucose homeostasis (Mueckler, 1994).

The intracellular sequestration of GLUT4 proteins presumably results from the presence of target sequences within the protein. Chimeric constructs formed between GLUT1 and GLUT4 were tested in several systems in combination with deletion mutations to identify the regions responsible for intracellular GLUT4 targeting. Results so far are directly conflicting. For example, Piper *et al.* (1992, 1993) have reported that the amino terminus of GLUT4 was necessary and sufficient for intracellular sequestration, whereas other groups report that the intracellular targeting sequence is within the carboxyl terminus (Verhey *et al.* 1993; Czech *et al.* 1993). To complicate matters further, data from Asano *et al.* (1992) support the idea that two *internal* domains are responsible for sequestering GLUT4 in the *trans*-Golgi. Unfortunately, no studies were performed in adipocyte or muscle cells; the investigations were carried out in Chinese hamster ovary, COS, NIH 3T3 cells or *Xenopus* oocytes. It is possible that tissue-specific factors involved in intracellular trafficking of GLUT4 may vary by cell type and modify the behavior of GLUT 4 in a cell-specific manner.

The study of amino acid transporter gene regulation is just beginning. The *mCAT*-2 gene encodes two distinct proteins that differ in their apparent K_m for arginine by greater than 10-fold (Table 2). These isoforms arise from alternate transcript splicing that involves a 40 amino acid internal domain segment. Additional variability in expression was revealed when mCAT-2 transcripts were found to contain at least four different 5′ untranslated regions (UTRs) which arise from at least three distinct promoters (Finley, 1993; K. Finley, A. Barrieux, J. Kleeman, P. Huynh and C. L. MacLeod, in preparation; for a review, see MacLeod *et al.* 1994). Hence, the *mCAT*-2 gene gives rise to two protein isoforms with different kinetic properties and the multiple promoters provide for specific gene regulation with the economy of a single gene. Such features may confer a similar

Table 2. *Comparison of the mCAT proteins*

Characteristics	mCAT-1	mCAT-2	mCAT-2a
Murine chromosome number	5	8	8
Percentage identity with mCAT-2	61	100	97
Predicted membrane-spanning domains	12–14	12	12
Protein length (aa)	622	658	657
K_m (Arg) in *Xenopus* oocytes (mmol l^{-1})	0.206	0.187	3.68
Na^+-independent substrates	Arg, Lys, Orn	Arg, Lys, Orn	Arg, Lys, Orn
Na^+-dependent substrates	Homoserine	Homoserine	NT

Several features of the three known mCAT proteins are compared.

Data are abstracted from MacLeod *et al.* (1990); Wang *et al.* (1991); Kakuda *et al.* (1993); Closs *et al.* (1993).

The term mCAT-2a denotes the high-capacity, low-affinity transporter used in Kakuda *et al.* (1993) and in Closs *et al.* (1993). mCAT-2 here denotes the name given in the first paper demonstrating the function of this cDNA, i.e. Kakuda *et al.* (1993).

The full-length sequence was reported in Reizer *et al.* (1993) (January); the sequence was later published again and termed mCAT-2B by Closs *et al.* (1993) (October).

NT, not tested.

degree of transport flexibility on mCAT-2-mediated amino acid transport as that provided by six different GLUT gene products on glucose transport.

The coregulation of *mCAT*-2 and *4F2hc* occurs in resting and activated lymphocytes and may prove to be useful in examining their possible functional interaction. Resting lymphocytes express mCAT-1 mRNA constitutively, yet they exhibit little arginine transport. Upon activation, T-cells show an absolute requirement for arginine that correlates with *mCAT*-2 and *4F2hc* gene induction and observed increases in arginine transport (Boyd and Crawford, 1992; Parmacek *et al.* 1989; Devés *et al.* 1992; Kilberg and Häussinger, 1992). Transcriptional and post-transcriptional regulation of the *mCAT*-2 gene has been observed in lymphoma cells that differ in mCAT-2 expression and in somatic cell hybrids formed among them (Wilkinson *et al.* 1991). The *4F2hc* gene has been isolated; its structure, sequence and expression have been used to define the promoter and other regulatory elements (Karpinski *et al.* 1989). The regulation of the *rBAT* gene has not been investigated.

The major challenge: topological structure of nutrient transporters

Precious little is known about the structure of vertebrate transporters in biomembranes. Major hurdles must be overcome before details of structure can be obtained. It is unfortunate that their hydrophobic properties, relatively large size and low abundance pose experimental difficulties that are confounded by the need to prepare large quantities of purified protein from expression systems such as bacteria or baculovirus. So far, these problems have prevented the successful resolution of their three-dimensional structure. Nevertheless, their similarity to the better-studied bacterial and yeast membrane proteins (Reizer *et al.* 1993), bacteriorhodopsin and porins has considerable value in approaching

this problem (for reviews, see Saier, 1994; Cowan and Rosenbusch, 1994). As the isolation of more transporters proceeds, family relationships among the proteins can be more firmly established and structural information on one family member may guide further analysis on other members. Family relationships may reveal common topological motifs among its members (for reviews, see Griffith *et al.* 1992; Saier, 1994).

The lack of precise information on the three-dimensional structure of transporters has forced researchers to rely heavily on computer modeling using algorithms. These aids are useful as a first approximation and can identify hydrophobic domains, putative α-helical regions and charged residues. But it is clear that computer programs cannot accurately predict membrane-spanning domains since some α-helical hydrophobic domains of greater than 18 residues are not embedded in membranes and the possible presence of β-barrels, for example, is often overlooked. Most researchers use computer predictions to make testable models to locate cytoplasmic and surface domains and to identify key residues for binding and transport.

Because positively charged amino acids in membrane proteins are often found on the intracellular face (von Heijne, 1992), their presence can assist in inside *versus* outside orientation modeling. An innovative strategy for determining the orientation of membrane proteins involves alkaline phosphatase as a reporter. Fusion constructs of alkaline phosphatase with the bacterial melibiose carrier exhibit enzymatic activity only when orientated towards the periplasm and not towards the cytoplasmic side of the bacterial membrane (Botfield *et al.* 1992; Manoil and Beckwith, 1985; Michaelis *et al.* 1983; Hoffman and Wright, 1985). Information on the tranporter orientation in membranes might be obtained by the placement of alkaline phosphatase coding sequences within sequences encoding various domains in the test protein (if their behavior in bacteria reflects their natural *in vivo* orientation). Site-directed mutagenesis has been profitably and extensively used to identify key residues in substrate recognition and/or transport functions (Kaback, 1994; Wright *et al.* 1994). Chimeric proteins can identify regions of substrate interaction, regulatory domains and binding sites. Protease treatment of membrane proteins has revealed sites protected by the substrate GABA (γ-aminobutyric acid) on the $GABA_1$ transporter (Kanner, 1994). The available information on the topology of GLUT transporters has been reviewed by Mueckler (1994).

Less is known about the structure of vertebrate amino acid facilitated transporters. Domain-swapping experiments among mCAT transporters located the mCAT-1 retrovirus binding site (Albritton *et al.* 1993). Hydrophobicity profiles and computer algorithms predict the mCAT proteins to contain 12–14 membrane-spanning domains (Closs *et al.* 1993; Reizer *et al.* 1993). Their nearly identical hydrophobicity profiles suggest they have similar structures.

The predicted single transmembrane structure of the type II glycoprotein rBAT family and 4F2hc suggests that they may be modulators rather than direct mediators of amino acid transport (see Palacín, 1994). Physical evidence regarding their structure is being sought, but strong evidence supports their important role in the regulation of amino acid transport. What is known of the unexpected structure of these proteins was recently reviewed (Hediger *et al.* 1993; Bertran *et al.* 1994; Palacín, 1994). Their features are compared with those of the mCAT transporters below.

Cationic amino acid transport is mediated by two distinct types of proteins

The mCAT family

The mCAT cDNAs were cloned serendipitously and their natural function was initially unknown (for a review, see MacLeod *et al.* 1994). Two known genes (*mCAT-1* and *mCAT-2*) constitute this family and encode three proteins which mediate the transport of cationic and dipolar amino acids when expressed in *Xenopus* oocytes (MacLeod *et al.* 1990; Wang *et al.* 1991; Kakuda *et al.* 1993; Closs *et al.* 1993). The *mCAT-2* gene encodes both the high-affinity mCAT-2 protein and the low-affinity high-capacity mCAT-2a isoform, as mentioned previously. The different K_m values of substrate exhibited by the isoforms are largely conferred by the alternately spliced exons, as shown by domain-swapping experiments (Closs *et al.* 1993). Some of their properties are summarized in Table 2 (see legend to Table 2, describing the confusion in the designations for these isoforms).

rBAT and 4F2hc

The second type of protein is represented by rBAT and 4F2hc. Here, the term rBAT is used to refer to the sequence and protein product variously named NAA-Tr (NBAT), D2 (Bertran *et al.* 1992*a*,*b*; 1994; Wells and Hediger, 1992; Tate *et al.* 1992; Hediger *et al.* 1993; Markovich *et al.* 1993; Mosckovitz *et al.* 1993). A related cDNA clone, 4F2hc, initially isolated as a T-cell marker (Parmacek *et al.* 1989), was later found also to mediate amino acid transport. Both *rBAT* and *4F2hc* genes encode type II membrane glycoproteins related to α-amylases and α-glucosidases (for reviews, see Hediger *et al.* 1993; Palacín, 1994). The rBAT protein, when expressed in *Xenopus* oocytes, exhibits properties similar to transport system $b^{o,+}$ (Table 1; Van Winkle *et al.* 1988). This transport system mediates the Na^+-independent movement of both cationic and dipolar amino acids. The rBAT protein also mediates the influx of cystine, an amino acid not associated with $b^{o,+}$ transport (Table 1). The 4F2hc protein expressed in oocytes mediates transport properties similar to those of system y^+L (Devéz *et al.* 1992; Table 1).

A possible association of mCAT and 4F2hc function may occur in activated lymphocytes. Quiescent lymphocytes express mCAT-1 but not mCAT-2 or 4F2hc and exhibit minimal lysine transport *via* systems y^+ and y^+L (Boyd and Crawford, 1992; Devés *et al.* 1992). After T-cells have been activated, the expression of mCAT-2/2a and 4F2hc are induced (Parmacek *et al.* 1989; MacLeod *et al.* 1990; Kakuda *et al.* 1993; Finley, 1993) in a time frame corresponding to an increased y^+ transport activity in activated T-cells (Boyd and Crawford, 1992). However, co-expression of mCAT proteins with 4F2hc in *Xenopus* oocytes has not yet revealed any transport synergy (M. Palacín, personal communication).

If 4F2hc and rBAT span the membrane only once, it is unlikely that they function independently in the transport of substrates. Perhaps these genes encode a family of proteins that function to modulate the predominant direction of transport, regulate cell surface localization and modify their substrate specificity in a tissue-specific or physiologically responsive manner; or protein oligomers may alter substrate specificity and/or affinity. Sequence and structural evidence of possible protein–protein interaction

exists. The rBAT protein appears to be disulfide-linked to a smaller 125 kDa subunit (Palacín, 1994); it also has a leucine zipper motif which could mediate dimerization. The 4F2hc protein is complexed with a light chain, which has not been isolated.

Recent immunohistochemical analyses of fibroblasts, endothelial cells and hepatoma cells have revealed mCAT-1 protein clustering associated with the membrane cytoskeleton (Woodard *et al.* 1994). The clustering of other membrane-associated proteins has been reported, including that of the *N*-methyl-D-aspartate receptor (Benke *et al.* 1993). The physiological significance of this clustering remains to be determined, but the association of several proteins into a complex might alter the substrate specificity and/or affinity and could thereby regulate substrate transport, depending on the availability of the subunits.

Properties of single membrane-spanning amino acid transporters

The rBAT and 4F2hc proteins contain conserved residues similar to the α-amylase and α-glycosidase sequences proposed as sites critical for calcium binding and catalytic activity. The rBAT and 4F2hc proteins do not exhibit detectable enzymatic activity (Wells and Hediger, 1992). Recently, an unrelated single membrane-spanning protein, aminopeptidase-N, was found to be associated with the Na^+-dependent transport of zwitterionic amino acids alanine, glutamine, leucine and phenlyalanine (Plakidou-Dymock *et al.* 1993). Aminopeptidase-N is both an enzyme and a mediator of amino acid transport. It has been postulated that, like rBAT and 4F2hc, the peptidase is associated with an unidentified transport protein. It is possible that the transport and enzymatic activities are not coincidental, but rather that the enzyme digestion products are the transported substrates, since aminopeptidase-N functions preferentially as a dipolar peptidase, which cleaves alanine, leucine and other bulky hydrophobic substrates (Kenny *et al.* 1987). The protein sequence similarity among rBAT, 4F2hc, α-amylase and α-glycosidase is intriguing in the light of the recent information that aminopeptidase-N is an active enzyme that mediates amino acid transport. Perhaps multiple membrane-spanning proteins form pathways for substrate flux whose substrate specificity and/or kinetic properties can be modulated in a tissue-specific manner by other less hydrophobic membrane glycoproteins responsive to cell signaling systems. This is an area of active investigation (see, for example, Palacín, 1994).

The power and limitations of *Xenopus laevis* oocyte expression analysis

The recent advances in mammalian amino acid transporter analysis have relied on their forced expression in *Xenopus* oocytes, since they have low levels of endogenous amino acid and glucose transport. Expression cloning in *Xenopus* oocytes was used to identify mRNA fractions responsible for specific transport functions and for isolating first the glucose transporters (for a review, see Wright *et al.* 1994) and later for those encoding amino acid transporters (Palacín, 1994). The *Xenopus* system was also used to identify the function of the *mCAT* genes for which the natural function of the isolated cDNAs was being sought (Kim *et al.* 1991; Wang *et al.* 1991; Kakuda *et al.* 1993).

Although the *Xenopus* oocyte expression has been invaluable for the identification and characterization of transporters, there are problems in using the system. Two groups have reported the detection of endogenous amino acid transport (Bertran *et al.* 1992*a*; Van Winkle, 1993). A second problem is illustrated by studies indicating that the *Xenopus* oocytes may be limited in their capacity to translate selected mRNAs or to modify their protein products correctly (Snutch, 1988). For example, translational selectivity was noted when only a subset of 5-hydroxytryptamine (5-HT) receptors and voltage-gated Ca^{2+} channels were detected after mRNA from cells expressing a wider set of proteins had been injected into oocytes (Leonard *et al.* 1987; Lübbert *et al.* 1987). Failure to modify transporters properly was evident when microinjected eel electroplax mRNA resulted in the production of only precursor forms of Na^+ channel protein (Thornhill and Levinson, 1987). A potential concern involves the frequent use of mRNA from tissues consisting of several cell types. When such mixtures of RNA are expressed in oocytes, the co-expression of their proteins could reveal non-physiological activities (Snutch, 1988).

Expression cloning or expression analysis may fail in situations were the functional protein unit is a hetero-oligomer and not all of their mRNA precursors are injected or expressed as proteins. Conversely, the protein expressed from injected mRNA might interact with an endogenous *Xenopus* oocyte protein and modify transport, resulting in transport activity that may not reflect the normal physiological function of this protein (Bertran *et al.* 1992*a*,*b*; Markovich *et al.* 1993; Tate *et al.* 1992; Wells and Hediger, 1992). To be confident that the function of the cloned gene product measured in *Xenopus* oocytes reflects its normal activity, other experimental verification is desirable, although sometimes quite difficult to obtain. Antisense or other tests of specificity have been employed in *Xenopus* and might be profitably used in somatic cells.

Conclusion

The uniporters discussed here are important for nutrient homeostasis and further research will yield exciting new information on their structure, the mechanisms of their transport and regulation, and their role in health and disease.

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