

**REVIEW
ARTICLE****Families of transmembrane transporters selective for amino acids and their derivatives**

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Keywords: transmembrane transport, phylogeny, evolution, amino acids, polyamines**Overview**

Twenty-one families of secondary [proton-motive force (PMF)-driven] carriers are herein described, which, in addition to 13 of the currently recognized 48 families within the ATP-dependent ATP-binding cassette (ABC) superfamily and three families of channel proteins, largely account for the transport of amino acids and their derivatives (excluding proteins) into and out of all living cells on Earth. Family characteristics as well as structural and functional properties of the family constituents are described. By reference to our web site (<http://www-biology.ucsd.edu/~msaier/transport/>), sequences, phylogenetic relationships, detailed substrate-specificity information and the distribution of members of each family in 20 fully sequenced genomes of bacteria, archaea and eukaryotes are available. This review provides a comprehensive guide to the diversity of carriers that mediate the transport of amino acids and their derivatives across cell membranes.

Background

Amino acids are the building blocks of proteins. They also provide raw materials for a variety of important cellular processes such as energy generation, nitrogen metabolism, cell-wall synthesis, antibiotic production and intercellular communication. Amino acids can be zwitterionic, anionic or cationic, and their side chains can be hydrophobic, semipolar or strongly hydrophilic. These side chains may be aliphatic or aromatic, and they sometimes serve as physiologically important proton donors and acceptors in enzymes with pK_a values

ranging from about 4 to 12. Amino acids in proteins can be derivatized by methylation, acetylation, formylation and phosphorylation; the free compounds can be decarboxylated to amines, and L-aminoacyl derivatives can be condensed enzymically with other L-amino acids, with amino acids of the D configuration and with hydroxy acids to yield peptides and depsipeptides of unusual composition. These aminoacyl residue derivatives serve a wide variety of biological functions including those of biological warfare, quorum sensing, intercellular signalling and regulation of gene expression. It is therefore not surprising that several transporter families have evolved to control and facilitate exchange of these substances between cells and their environment, between cells and their organelles, and between cells and other living entities.

Over two dozen transporter families and superfamilies are currently recognized that include members that transport amino acids and their derivatives (Paulsen *et al.*, 1998a, b; Saier, 1998, 1999a, b, c; see also <http://www-biology.ucsd.edu/~msaier/transport/>). A number of interesting questions arise related to these families. For example (1) what are the ranges of substrates transported by members of each of these families? (2) What energy-coupling mechanisms do they employ to accumulate or expel their substrates within or from living cells? (3) What is/are the polarity/polarities of transport allowed by members of each such family? (4) In what range of organisms are members of each of these families found? (5) When, during evolutionary history, did each family appear and via what route? (6) What are the mechanisms used to effect transmembrane transport? (7) Do homologues of these transport proteins function in capacities other than in transport, and if so, in what capacities? (8) Are certain families concerned primarily with a particular physiological function (e.g. uptake, secretion or intercellular communication), or do they all serve comparable and broadly overlapping physiological functions? (9) How are the syntheses, activities, intracellular localizations and proteolytic degradation processes of transporter proteins regulated, and in response to what stimuli? (10)

The information presented in this review was initially prepared for presentation at the FASEB meeting on amino acid transport held in Copper Mountain, Colorado, June 26–July 1, 1999 and was updated in January 2000 following the meeting of the Transport Nomenclature Panel of the International Union of Biochemistry and Molecular Biology (IUBMB) in Geneva, November 28–30, 1999. The system of classification described in this review reflects the recommendations of that panel.

What transport characteristics are readily altered during evolution and which characteristics remain relatively immutable? These and other questions will be systematically addressed in the following section, and information that will allow the reader to personally evaluate the answers to these questions, relevant to specific families, will be provided with greater insight in the subsequent sections.

Characteristics of transporters and their homologues

Careful examination of the properties of the functionally characterized members of currently recognized transporter families selective for amino acids and their derivatives reveals tentative answers to the ten questions posed above.

(1) The range of substrates transported by an individual member of a family can be narrow or broad, depending on the transporter, and an entire family can often transport a wide variety of structurally related compounds. Seldom, however, do members of a single family catalyse the transport of structurally divergent types of compounds (i.e. sugars versus amino acids versus inorganic anions).

(2) Only two forms of energy are generally used for the active uptake or expulsion of amino acids and their derivatives: electrochemical energy stored in the gradients of H^+ , Na^+ and structurally related substrates (utilized by secondary active carriers), and chemical energy in the form of ATP (utilized by ABC-type uptake and efflux systems as well as by several macromolecular transport systems). Channel proteins usually do not couple transmembrane translocation to energy, although a few interesting exceptions are known.

(3) With very few exceptions (i.e. the major-facilitator superfamily and the ABC superfamily) all members of a phylogenetically defined family function with strongly preferential inwardly directed or outwardly directed polarity. Seldom do different members of a single transporter family transport solutes with opposite polarity, and no permease is currently known to actively take up its substrates under one set of experimental conditions but actively extrude them under another set of conditions.

(4) Some families of sequence-related transport proteins are found ubiquitously in all major groups of organisms (i.e. among the bacteria, archaea and eukaryotes), while others are restricted to just one of these three domains. In some cases, all members of a family derive from just one kingdom within one of these domains. The former proteins may represent ancient families of transporters while the latter proteins are those which have more recently appeared, either *de novo* or by extensive sequence divergence from a pre-existing family.

(5) As noted above and discussed in several reviews

(Saier, 1994, 1996, 1998), it appears that new families have appeared throughout evolutionary history although with variable frequencies. Moreover, they probably underwent sequence divergence at rates that were neither constant with time nor constant for all members of the family. Most of the families that appeared after bacteria, archaea and eukaryotes separated from each other during the 'great split' are apparently still restricted to the domain in which they arose. These proteins have often arisen from much simpler peptides or proteins as a result of internal gene-duplication and gene-fusion events. Little lateral transfer of the resultant genetic information between the three domains of organisms has occurred, at least during the past two billion years.

(6) The primary modes of transport have historically been thought to involve, and are still considered to involve, channels and carriers. While the mechanistic details of the former type of transporters are well understood, in part due to the availability of high-resolution three-dimensional structural data for these proteins, carrier mechanisms are still poorly understood (West, 1997). These carrier mechanisms represent the primary types responsible for the transmembrane translocation of organic nutrients.

(7) Homologues of transporters almost never function in a primary capacity other than transport. Those that do usually serve as simple receptors, influencing a single aspect of cellular physiology. We have consequently proposed that integral transmembrane transporters evolved as a distinct class of proteins, and that they did not appear by modification of other protein types (i.e. enzymes, structural proteins or regulatory proteins) (Saier & Tseng, 1999).

(8) Some families or subfamilies of transporters selective for amino acids and their derivatives seem to have evolved to serve a highly specific physiological function such as that of auxin secretion for the purpose of promoting gravitropism in plants, performed by members of the auxin efflux carrier (AEC) family [transporter classification (TC) no. 2.A.69], or that of promoting dormant spore germination in *Bacillus* species, performed by members of the spore germination protein (SGP) family (TC no. 2.A.3.9) of the amino acid/polyamine/organocation (APC) superfamily (TC no. 2.A.3). Other families selectively catalyse either uptake or efflux of specific amino acids and/or their derivatives for the purpose of nutrition, cellular protection or intercellular signalling. Thus, it is clear that members of a family may have evolved to provide specific physiological functions. Nevertheless, specialization for a specific physiological function appears to be a late-evolving characteristic and therefore is of minimal importance for purposes of classifying transporters (Saier & Tseng, 1999).

(9) In general, the syntheses, activities and degradation of transport proteins are stringently regulated, employing the same or similar universal mechanisms (i.e.

ligand binding, covalent modification, protein–protein interactions) that are operative and well established for cytoplasmic enzymes. Similarity, subcellular localization is determined by targeting sequences in the proteins of both prokaryotes and eukaryotes, and by complex, reversible, hormone-regulated processes in higher eukaryotes. These topics are in general beyond the scope of this review but are the topics of numerous other reviews, to which the reader is referred (Saier *et al.*, 1989; Knutson, 1991; Fekkes & Driessen, 1999; Koch *et al.*, 1999).

(10) Finally, the classification and characterization of transporter families have revealed that transport mode and energy-coupling mechanism are highly conserved evolutionary traits, that protein topology, polarity of transport and substrate specificity are traits conserved to an intermediate degree, and that the regulatory mechanisms imposed on transporters are late evolving, poorly conserved traits. The utilization of these various traits for purposes of transport-protein classification has been the topic of discussion in several recently published reviews (Saier, 1999a, b, c; Saier & Tseng, 1999).

Below, the transporter families concerned with transmembrane transport of amino acids and their derivatives are discussed. Groups of functional types will first be considered according to class and transport mechanism, and the unique features of each such family will be presented.

Most amino acid transporters are secondary carriers

We currently recognize about 120 families of channel/pore proteins, about 30 families of primary active transport systems and about 80 families of secondary carriers (Saier, 1998, 1999a, b, c; see also <http://www-biology.ucsd.edu/~msaier/transport/>). Additionally, dozens of families include transporters with undefined, or ill-defined, transport mechanisms. Of the former families, members of only one recognized family of channel proteins, the phospholemman (PLM) family (TC no. 1.A.27; Kirk & Strange, 1998), in addition to nonspecific outer-membrane porins of Gram-negative bacteria and eukaryotic organelles (TC classification division 1.B), and only one recognized family of primary carriers, the ABC superfamily (Saurin *et al.*, 1999), can transport amino acids and their structurally related derivatives. All other families apparently consist of secondary carriers that function by PMF or SMF (sodium-ion motive force) -driven uptake, by PMF-driven efflux, by solute–solute exchange or by uniport. Secondary carriers will therefore be the main focus of this review.

ABC (ATP-binding cassette) -type transporters

ABC-type transporters fall into 48 currently recognized families within the ABC superfamily (Table 1). Of these, 19 include members that are exclusively uptake systems

(all found in prokaryotes); 19 include members that are prokaryotic-specific efflux systems and 10 include members that are primarily eukaryotic-specific efflux systems. The uptake and efflux systems cluster separately on the phylogenetic tree for this superfamily (Saurin *et al.*, 1999).

Of the 19 ABC-type uptake-system families, two are primarily concerned with polar and non-polar amino acid transport (TC 3.A.1.3 and 4, respectively); a third is largely concerned with peptide transport (TC 3.A.1.5); a fourth is primarily concerned with polyamine and opine uptake (TC 3.A.1.11); a fifth is concerned with quaternary amine uptake (TC 3.A.1.12); and a sixth functions to take up taurine (the decarboxylation product of cysteic acid) (TC 3.A.1.17). Many the ABC uptake systems appear to catalyse irreversible vectorial reactions, but one system, the polar amino acid transporter (Aap) of *Rhizobium leguminosarum*, has been shown to catalyse both active uptake and passive efflux. This system is unusual not only because of its reversibility but also because of its broad specificity (Walshaw & Poole, 1996; Walshaw *et al.*, 1997). Whether the capacity to catalyse efflux of solutes will prove to be a general characteristic of ABC uptake systems or a specific trait of only a few of these systems is currently an unanswered question requiring further experimental work.

Of the prokaryotic ABC-type exporter families, three are concerned with drug efflux, five catalyse peptide export and two function in protein transport. The five peptide-exporter families are the Pep1–3E families (TC 3.A.1.101–103) and the two microcin-exporter families (the McbE and McjD families; TC 3.A.1.106 and 108 respectively), but some of the drug-export permeases may be capable of transporting certain peptides. Of the currently recognized eukaryotic families, two function primarily in peptide export (a-factor sex pheromone exporter, STE, and MHC peptide transporter, TAP; TC 3.A.1.206 and 209 respectively) and three function to export drugs (including some peptides). However, no eukaryotic ABC exporter is known to transport proteins. Eukaryotes export macromolecules by exocytosis rather than by a permease-mediated molecular-transport process. Thus, the functional diversity of prokaryotic ABC transporters far exceeds that of the eukaryotic ABC transporters. Of the 48 recognized families, 13 (27%) are concerned primarily with the transport of amino acids and their derivatives including peptides, and only two of these families derive from eukaryotes. In bacteria, another two families are concerned with protein export.

Secondary carriers

Table 2 tabulates the secondary carrier families that are known to function in transport of (a) amino acids and their conjugates, (b) amines, amides and polyamines and (c) peptides. Altogether, 19 families fall into category a, eight fall into category b, and four fall into category c. Nevertheless, there is a total of only 21 families. All

Table 1. The ATP-binding cassette (ABC) superfamily (TC 3.A.1)

TC no.	Name
ABC-type uptake permeases (prokaryotes)	
3.A.1.1	Carbohydrate uptake transporter 1 (CUT1) family
3.A.1.2	Carbohydrate uptake transporter 2 (CUT2) family
3.A.1.3	Polar amino acid uptake transporter (PAAT) family
3.A.1.4	Hydrophobic amino acid uptake transporter (HAAT) family
3.A.1.5	Peptide/opine/nickel uptake transporter (PepT) family
3.A.1.6	Sulfate uptake transporter (SulT) family
3.A.1.7	Phosphate uptake transporter (PhoT) family
3.A.1.8	Molybdate uptake transporter (MolT) family
3.A.1.9	Phosphonate uptake transporter (PhnT) family
3.A.1.10	Ferric iron uptake transporter (FeT) family
3.A.1.11	Polyamine/opine/phosphonate uptake transporter (POPT) family
3.A.1.12	Quaternary amine uptake transporter (QAT) family
3.A.1.13	Vitamin B ₁₂ uptake transporter (VB ₁₂ T) family
3.A.1.14	Iron chelate uptake transporter (FeCT) family
3.A.1.15	Manganese/zinc/iron chelate uptake transporter (MZT) family
3.A.1.16	Nitrate/nitrite/cyanate uptake transporter (NitT) family
3.A.1.17	Taurine uptake transporter (TauT) family
3.A.1.18	Putative cobalt uptake transporter (CoT) family
3.A.1.19	Thiamin uptake transporter (ThiT) family
ABC-type efflux permeases (bacterial)	
3.A.1.101	Capsular polysaccharide exporter (CPSE) family
3.A.1.102	Lipooligosaccharide exporter (LOSE) family
3.A.1.103	Lipopolysaccharide exporter (LPSE) family
3.A.1.104	Teichoic acid exporter (TAE) family
3.A.1.105	Drug exporter (DrugE1) family
3.A.1.106	Putative lipid A exporter (LipidE) family
3.A.1.107	Putative haem exporter (HemeE) family
3.A.1.108	β -Glucan exporter (GlucanE) family
3.A.1.109	Protein-1 exporter (Prot1E) family
3.A.1.110	Protein-2 exporter (Prot2E) family
3.A.1.111	Peptide-1 exporter (Pep1E) family
3.A.1.112	Peptide-2 exporter (Pep2E) family
3.A.1.113	Peptide-3 exporter (Pep3E) family
3.A.1.114	Probable glycolipid exporter (DevE) family
3.A.1.115	Na ⁺ exporter (NatE) family
3.A.1.116	Microcin B17 exporter (McbE) family
3.A.1.117	Multidrug exporter (DrugE2) family
3.A.1.118	Microcin J25 exporter (McyjD) family
3.A.1.119	Drug/siderophore exporter 3 (DrugE3) family
ABC-type efflux permeases (mostly eukaryotic)	
3.A.1.201	Multidrug-resistance exporter (MDR) family
3.A.1.202	Cystic fibrosis transmembrane conductance exporter (CFTR) family
3.A.1.203	Peroxisomal fatty acyl CoA transporter (FAT) family
3.A.1.204	Eye pigment precursor transporter (EPP) family
3.A.1.205	Pleiotropic drug resistance (PDR) family
3.A.1.206	α -Factor sex pheromone exporter (STE) family
3.A.1.207	Conjugate transporter 1 (CT1) family
3.A.1.208	Conjugate transporter 2 (CT2) family
3.A.1.209	MHC peptide transporter (TAP) family
3.A.1.210	Heavy metal transporter (HMT) family

families that fall into category b are also represented in category a and only two families of peptide transporters (oligopeptide transporter, OPT, and peptide-uptake

permease, PUP) have not been shown to include members that transport simple amino acids. Below we examine these families in greater detail.

Table 2. Families of secondary active transporters specific for amino acids and their derivatives

Substrate*	No. families	Families†
Amino acids and conjugates	19	AAAP, AEC, AGCS, APC, BCCT, DAACS, Dcu, GltS, HAAAP, LIVCS, LysE, MC, MFS, NSS, POT, RhtB, SSS, TRAP-T, CAAT
Amines, amides and polyamines	8	AAAP, AEC, APC, BCCT, MC, MFS, NSS, SSS
Peptides	4	MFS, OPT, POT, PUP
	Total 21	

* These compounds fall into transport substrate category III as described by Saier (1999c).

† The full names of these families are provided in Tables 3, 4 and 6.

Secondary amino acid transporter families found exclusively in bacteria

Table 3 lists and provides characteristics of the ten prokaryotic-specific families of secondary transporters that include members exhibiting specificity for amino acids and their derivatives. These families are almost without exception small, usually with less than 20 currently sequenced members. At least one of these families, the hydroxy and aromatic amino acid porter (HAAAP) family, is probably distantly related to the largest known superfamily of amino acid transporters, the APC superfamily (Young *et al.*, 1999; Jack *et al.*, 2000).

The first two bacterial-specific families listed in Table 3 (C_4 -dicarboxylate uptake, Dcu, and betaine/carnitine/choline transporter, BCCT) have recently been described in some detail and characterized phylogenetically (Saier *et al.*, 1999a). Six of the ten families listed in Table 3 exhibit properties that are generally characteristic of secondary solute-uptake transporters: they function by H^+ or Na^+ symport exclusively in the uptake of their nutrient substrates and they usually consist of single polypeptide chains exhibiting about 12 transmembrane α -helical spanners (TMSs). In most cases, they function in the absence of auxiliary subunits. One exceptional family is the tripartite ATP-independent periplasmic transporter (TRAP-T) family of dicarboxylate/amino acid uptake permeases (Table 3). TRAP-T family transporters function by a typical H^+ symport mechanism, characteristic of secondary carriers, for the uptake of dicarboxylates including the amino acid glutamate. However, in contrast to other secondary carriers, they are normally heterotrimeric with three dissimilar, non-homologous subunits/domains (Forward *et al.*, 1997; Rabus *et al.*, 1999). In contrast, the characterized proteins of the L-lysine exporter (LysE) and resistance to homoserine and threonine (RhtB) families export amino acids, probably by a proton antiport mechanism (Vrljic *et al.*, 1999; Zakataeva *et al.*, 1999). These two families are probably constituents of a

single superfamily (Vrljic *et al.*, 1999). The available evidence suggests that a single gene product participates in the transport process. Another family, the carboxylate/amino acid/amine transporter (CAAT) family, also includes members that export amino acids and their derivatives. A number of additional prokaryotic-specific families of (putative) transporters included under TC classification category 9 and not presented in Tables 2 and 3 are likely to prove to consist of efflux pumps.

The 10 families listed in Table 3 will be discussed individually below. The descriptions provided reflect our knowledge of these families as of July 1999.

The Dcu (C_4 -dicarboxylate uptake) family (TC 2.A.13)

Several proteins of the Dcu family have been sequenced, all from Gram-negative bacteria (Engel *et al.*, 1994; Six *et al.*, 1994; Uden & Bongaerts, 1997; Golby *et al.*, 1998). The two best characterized systems (DcuA and DcuB) are from *Escherichia coli*. The fully sequenced proteins of the Dcu family are of fairly uniform size (434–446 residues). They possess 12 putative TMSs, but DcuA of *Escherichia coli* has 10 experimentally determined TMSs with both the N and C termini localized to the periplasm. For DcuA, the 'positive inside' rule (von Heijne, 1992) is obeyed and two putative TMSs are localized to a cytoplasmic loop between TMS 5 and 6 and in the C-terminal periplasmic region.

The *Escherichia coli* DcuA and DcuB proteins transport aspartate, malate, fumarate and succinate, and function as antiporters with any two of these substrates. The two proteins exhibit 36% identity with 63% similarity, and both transport fumarate in exchange for succinate with about the same affinity (30 μ M). Since DcuA is encoded near the gene for aspartase, and DcuB is encoded in an operon with the gene for fumarase, their physiological functions have been hypothesized to involve aspartate:fumarate and fumarate:malate exchange during the anaerobic utilization of aspartate and fumarate,

Table 3. Secondary active amino acid transporter families found exclusively in prokaryotes

TC no.	Family	Substrate	Size range (residues)	No. TMSs	Organism*	No. members	Example
2.A.13	C ₄ -dicarboxylate uptake (Dcu) family	C ₄ -dicarboxylates	440	12	B	> 10	Dicarboxylate uptake porter A, DcuA of <i>Escherichia coli</i>
2.A.15	Betaine/carnitine/choline transporter (BCCT) family	Glycine betaine, carnitine, choline, proline	480–680	12	B	> 10	Carnitine transporter, CaiT of <i>Escherichia coli</i>
2.A.25	Alanine/glycine:cation symporter (AGCS) family	Alanine, glycine	440–540	8–12	B	> 10	Alanine/glycine transporter, DagA of <i>Alteromonas haloplanktis</i>
2.A.26	Branched chain amino acid:cation symporter (LIVCS) family	Branched chain amino acids	~ 440	12	B	> 10	Branched-chain-amino-acid transporter, BraB of <i>Pseudomonas aeruginosa</i>
2.A.27	Glutamate:Na ⁺ symporter (GltS) family	Glutamate	~ 400	12	B	> 10	Glutamate:Na ⁺ symporter, GltS of <i>Escherichia coli</i>
2.A.42	Hydroxy/aromatic amino acid permease (HAAAP) family	Hydroxy- and aromatic amino acids	400–450	11	B	> 20	Tyrosine permease, TyrP of <i>Escherichia coli</i> ; serine permease, SdaC of <i>Escherichia coli</i>
2.A.56	Tripartite ATP-independent periplasmic transporter (TRAP-T) family	C ₄ -dicarboxylates, acidic amino acids, sugars (?)	~ 1000 (three components)	12 + 4	B, A	> 20	Dicarboxylate transporter, DctPQM of <i>Rhodobacter capsulatus</i>
2.A.75	L-Lysine exporter (LysE) family	Basic amino acids	190–240	5 or 6	B, A	> 10	Lysine/arginine exporter, LysE of <i>Corynebacterium glutamicum</i>
2.A.76	Resistance to homoserine and threonine B (RhtB) family	Neutral amino acids and their derivatives (export)	190–240	5 or 6	B, A	> 20	Homoserine/threonine exporter, RhtB of <i>Escherichia coli</i>
2.A.78	Carboxylate/amino acid/amine transporter (CAAT) family	Carboxylates, amino acids and amines	260–320	10	B, A	> 100	Putative acetate exporter, MadN of <i>Malonomonas rubra</i>

* B, bacteria; A, archaea.

respectively. However, the electroneutral antiport of fumarate for succinate during anaerobic fumarate respiration has been demonstrated, and both permeases are induced under anaerobic conditions and subject to catabolite repression. The two transporters can apparently substitute for each other under certain physiological conditions. A third dicarboxylate transporter, DcuC, exhibits slight sequence similarity with DcuA (optimized comparison score of 5 SD; 23% identity for a segment of 100 residues). This degree of similarity is

insufficient to establish homology (Saier, 1994, 1996) and consequently, DcuC is assigned to a distinct family (TC 2.A.61).

The BCCT (betaine/carnitine/choline transporter) family (TC 2.A.15)

Proteins of the BCCT family are found in Gram-negative and Gram-positive bacteria (Lamark *et al.*, 1991; Eichler *et al.*, 1994; Kappes *et al.*, 1996; Kempf & Bremer, 1998;

Peter *et al.*, 1998). Their common functional feature is that they all transport molecules with a quaternary ammonium group [R-N⁺(CH₃)₃]. BCCT family proteins vary in length between 481 and 677 aminoacyl residues, possess 12 putative TMSs and are energized by PMF-driven proton symport.

The alanine:glycine:cation symporter (AGCS) family (TC 2.A.25)

Members of the AGCS family transport alanine and/or glycine in symport with a monovalent cation, Na⁺ and/or H⁺ (see Reizer *et al.*, 1994). The proteins are 445–542 aminoacyl residues in length and possess 8–12 putative TMSs. They are found in Gram-positive and Gram-negative bacteria. Only two members of the family have been functionally characterized. These proteins show minimal sequence similarity to members of the APC family (TC 2.A.3; see below; Jack *et al.* 2000), but the significance of this observation is not known.

The branched chain amino acid:cation symporter (LIVCS) family (TC 2.A.26)

Characterized members of this family transport all three of the branched chain aliphatic amino acids [leucine (L), isoleucine (I) and valine (V)] (Reizer *et al.*, 1994; Stucky *et al.*, 1995; Tauch *et al.*, 1998). They are found in Gram-negative and Gram-positive bacteria and function by a Na⁺ or H⁺ symport mechanism. They possess about 440 aminoacyl residues and display 12 putative TMSs.

The glutamate:Na⁺ symporter (GltS) family (TC no. 2.A.27)

A single member of this family has been functionally characterized (Deguchi *et al.*, 1990). This permease (GltS of *Escherichia coli*) catalyses glutamate:Na⁺ symport. It exhibits 401 aminoacyl residues with 12 putative helical TMSs. Homologues are found in other bacteria including *Haemophilus influenzae*, *Helicobacter pylori* and *Synechocystis*.

The HAAAP (hydroxy and aromatic amino acid porter) family (TC 2.A.42)

The HAAAP family includes three well characterized aromatic amino acid:H⁺ symport permeases of *Escherichia coli*: a high-affinity tryptophan-specific permease, Mtr, a low affinity tryptophan permease, TnaB, and a tyrosine-specific permease, TyrP (Wookey & Pittard, 1988; Sarsero *et al.*, 1991; Sarsero & Pittard, 1995). These proteins possess 403–405 aminoacyl residues with 11 TMSs. The HAAAP family also includes two well characterized permeases specific for hydroxy amino acids: one is the serine permease, SdaC, of *Escherichia coli*, the other is the threonine permease, TdcC, of *Escherichia coli* (Goss *et al.*, 1988; Shao *et al.*, 1994). These permeases are 429 and 443 aminoacyl

residues long, respectively, and each possesses 11 putative TMSs. Homologues are present in a variety of Gram-negative bacteria. They show topological features common to members of the eukaryotic amino acid/auxin permease (AAAP) family (TC 2.A.18), and they display limited sequence similarity with some of them (Young *et al.*, 1999). Since members of the AAAP family show limited sequence similarity with members of the large APC superfamily (TC no. 2.A.3) (Young *et al.*, 1999; Jack *et al.*, 2000), all of these proteins are probably related.

The TRAP-T (tripartite ATP-independent periplasmic transporter) family (TC 2.A.56)

TRAP-T family permeases generally consist of three components (Forward *et al.*, 1997; Rabus *et al.*, 1999). The best characterized of these systems is the DctMQP system of *Rhodobacter capsulatus* (Forward *et al.*, 1997). DctM is a typical 12 TMS protein with weak sequence and motif similarity to several other families of secondary carriers (Rabus *et al.*, 1999). However, DctQ is a 4 TMS integral membrane protein, and DctP is a periplasmic binding protein. Database searches and phylogenetic analyses have revealed that unlike most of the other families included in Table 3, homologous TRAP-T systems are found in archaea as well as bacteria. In some of these permeases, including all currently recognized archaeal systems, the M homologue is fused to the Q homologue yielding a 16 TMS protein, and in a few cases, the Q homologue is fused to the P homologue. Thus, in the TRAP-T family, as for the ABC superfamily, domain fusion/splicing has occurred repeatedly during evolution of the family.

All three Dct proteins are required for the uptake of dicarboxylates in *Rhodobacter capsulatus*, suggesting that these subunits function together, employing a concerted mechanism. Moreover, phylogenetic analyses reveal that all TRAP-T systems probably depend on the structural equivalent of the three proteins, DctMQP. The family appears to be an ancient but functionally unified family that evolved in parallel with the ABC superfamily, with little or no shuffling of constituents between families (Rabus *et al.*, 1999). The TRAP-T family is the only family currently known for which an extracytoplasmic receptor functions in conjunction with a secondary carrier.

Many members of the TRAP-T family are functionally uncharacterized. An operon encoding a *Synechocystis* system includes a protein homologous to the glutamine-binding protein of an *Escherichia coli* ABC-type permease, and biochemical evidence has suggested that a glutamate transporter from *Rhodobacter sphaeroides* is a periplasmic binding protein-dependent, PMF-dependent, secondary carrier (Jacobs *et al.*, 1996). *Escherichia coli* homologues may be involved in the uptake of pentoses and/or pentitols (Reizer *et al.*, 1996; Sanchez *et al.*, 1994). Thus, members of the TRAP-T family of permeases may take up widely divergent compounds.

The LysE (L-lysine exporter) family (TC 2.A.75)

One member of the LysE family (LysE of *Corynebacterium glutamicum*) is functionally well characterized, but functionally uncharacterized or partially characterized homologues are encoded within the genomes of a variety of bacteria and archaea (Bröer & Krämer 1991a, b; Vrljic *et al.*, 1996, 1999; Zakataeva *et al.*, 1999). All of these proteins are 190–240 amino acid residues in length and possess six hydrophobic regions. PhoA fusion analyses provided evidence that LysE of *Corynebacterium glutamicum* exhibits a 5 TMS topology, with the N terminus inside and the C terminus outside (Vrljic *et al.*, 1999). LysE appears to catalyze unidirectional efflux of L-lysine and other basic amino acids such as L-arginine and L-ornithine, and it provides the sole route for L-lysine excretion in this bacterium. The energy source is believed to be the PMF (H⁺ antiport or OH⁻ symport).

The RhtB (resistance to homoserine and threonine) family (TC 2.A.76)

Distant homologues of LysE have been shown to expel threonine. They may catalyze efflux of homoserine and homoserine lactones as well (Aleshin *et al.*, 1999; Zakataeva *et al.*, 1999). Because homoserine lactones are signalling molecules in Gram-negative bacteria, this observation has special significance (Fuqua *et al.*, 1996; Swift *et al.*, 1996). The family that includes these amino acid efflux pumps has been termed the RhtB family, and in the transporter-classification system, it was given the TC number 2.A.76. Evidence has been presented that it and the LysE family (above), together with the cadmium-resistance (CadD) family (TC no. 2.A.77), compose a single superfamily, the LysE superfamily (Vrljic *et al.*, 1999). All characterized members of this superfamily catalyze solute export, presumably by a proton-antiport mechanism.

The CAAT (carboxylated amino acid/lamine transporter) family (TC 2.A.78)

The CAAT family is a large family of integral membrane proteins with sizes ranging from 287 to 310 amino acid residues and exhibiting 10 putative TMSs. These proteins are derived from phylogenetically divergent bacteria and archaea, and *Escherichia coli*, *Bacillus subtilis* and *Aspergillus fulgidus* have multiple paralogues. With one PSI-BLAST iteration, they show low degrees of sequence similarity with members of the ubiquitous L-rhamnose transporter (RhaT) family (TC 2.A.9) and with the eukaryotic triose phosphate transporter (TPT) family (TC 2.A.50), found in plant chloroplasts and plastids as well as in yeast. One distant plant homologue is the Medicago nodulin N21-like protein (gbAC004218; 374 aa) of *Arabidopsis thaliana*.

Proteins of the CAAT family evidently arose by an internal gene-duplication event as the first halves of these proteins are homologous to the second halves. Although none of these prokaryotic proteins is

functionally characterized, several members of the CAAT family have been implicated in solute transport. Thus, the MttP protein of the archaeon *Methanosarcina barkeri* may transport methylamine (Ferguson & Krzycki, 1997) and the YtFF protein of *Chlamydia trachomatis* may transport basic amino acids (Stephens *et al.*, 1998). Additionally, MadN is encoded within the malonate utilization operon of *Malonomonas rubra* and PecM is encoded within a locus of *Erwinia chrysanthemi* controlling pectinase, cellulase and blue-pigment production. MadN may be an acetate-efflux pump while PecM might export the pigments produced by gene products encoded in the *pecM* operon (Berg *et al.*, 1997; Reverchon *et al.*, 1994).

Amino acid transporter families that have been found in eukaryotes

While Table 3 lists the properties of the ten families of amino acid transporters found exclusively in prokaryotes, Table 4 provides comparable information for the nine families that occur in eukaryotes. Of these families, the AAAP and mitochondrial carrier (MC) families are found only in eukaryotes. The MC family undoubtedly arose in eukaryotes in response to a need for a new type of communication between the matrix of the mitochondrion and the cytoplasm of the eukaryotic cell (Kuan & Saier, 1993; Saier, 1994, 1996). However, we have presented evidence (Young *et al.*, 1999) that the AAAP family is distantly related to the ubiquitous APC superfamily, and this claim has recently been further substantiated (Jack *et al.*, 2000).

The AEC family is of particular physiological interest. It is apparently a ubiquitous family found in bacteria, archaea and eukaryotes, but only its members from plants have been characterized. These plant proteins serve a single function: to catalyze polarized auxin efflux for the purpose of promoting gravitropism. This therefore represents an example where a specific subfamily of a larger family has become specialized for a particular physiological function.

Below, the nine families described in Table 4 are discussed.

The APC (amino acid/polyamine/organocation) family (TC 2.A.3)

The APC family of transport proteins includes nearly 250 currently sequenced members that function as solute:cation symporters and solute:solute antiporters (Closs *et al.*, 1993; Reizer *et al.*, 1993a; Sophianopoulou & Diallinas, 1995; Isnard *et al.*, 1996; Kashiwagi *et al.*, 1997; Brechtel & King, 1998; Hu & King, 1998a, b; Sanders *et al.*, 1998; Sato *et al.*, 1999). All functionally characterized members thus catalyze active solute uptake or exchange transport. These proteins occur in bacteria, archaea, yeast, fungi, unicellular eukaryotic protists, plants and animals, and are thus essentially ubiquitous. They vary dramatically in length, being as

Table 4. Secondary active amino acid transporter families found in eukaryotes

These families, all represented in eukaryotes, may be found in the bacterial and archaeal kingdoms as well.

TC no.	Family	Substrate	Size range (residues)	No. TMSs	Organism*	No. members	Example
2.A.3	Amino acid/polyamine/organocation (APC) family	Amino acids, polyamines, organocations	440–630	12	B, A, E	> 100	Lysine permease, LysP of <i>Escherichia coli</i>
2.A.18	Amino acid/auxin permease (AAP) family	Amino acids, auxin and their derivatives	400–710	11	E (An, Pl, Y, F)	> 30	Amino acid/auxin:H ⁺ symporter, Aux-1 of <i>Arabidopsis thaliana</i>
2.A.1	MHS family of the MFS	Various small molecules	400–600	12 or 14	B, A, E	> 1000	Proline permease, PutP of <i>Escherichia coli</i>
2.A.17	Proton-dependent oligopeptide (POT) family	Peptides, nitrates, amino acids	450–600	12	B, E	> 30	Dipeptide transporter, DtpT of <i>Lactococcus lactis</i>
2.A.21	Solute:sodium symporter (SSS) family	Sugars, amino acids, vitamins, nucleosides, inositols, iodide, urea	400–700	12–15	B, A, E	> 30	Pantothenate:Na ⁺ symporter, PanF of <i>Escherichia coli</i>
2.A.22	Neurotransmitter:sodium symporter (NSS) family	Neurotransmitters, amino acids, osmolytes, taurine, creatine	600–700	12	B, A, E (An)	> 50	Serotonin:Na ⁺ symporter of <i>Homo sapiens</i>
2.A.23	Dicarboxylate/amino acid:cation (Na ⁺ or H ⁺) symporter (DAACS) family	C ₄ -dicarboxylates, acidic and neutral amino acids	420–580	10–12	B, A, E	> 20	Glutamate/aspartate permease, GltP of <i>Escherichia coli</i>
2.A.29	Mitochondrial carrier (MC) family	ATP/ADP, P _i , organic anions, H ⁺ , carnitine/acyl carnitine, basic amino acids, FAD	300	6	E (mitochondria, peroxisomes)	> 100	ATP/ADP exchanger of <i>Homo sapiens</i>
2.A.69	Auxin efflux carrier (AEC) family	Auxin (efflux)	600–700	8–12	B, A, E	~ 20	Auxin efflux carrier, PIN1

* B, bacteria; A, archaea; E, eukaryote; An, animal; Pl, plant; Y, yeast; F, fungi.

small as 350 residues and as large as 800 residues. The smaller proteins are generally of prokaryotic origin while the larger ones are of eukaryotic origin. Most of them possess 12 TMSs, but predicted topologies of 10 or 14 TMSs are also sometimes observed (Cosgriff & Pittard, 1997; Hu & King, 1998c; Jack *et al.*, 2000). The larger eukaryotic proteins have N- and C-terminal extensions that may serve regulatory functions. At least some animal proteins such as ASUR4 (gbY12716) and SPRM1 (gbL25068) associate with a type 1 transmembrane glycoprotein that is essential for insertion, stability or activity of the permease and forms a disulfide

bridge with it (Verrey *et al.*, 1999). These glycoproteins include the CD98 heavy-chain protein of *Mus musculus* (gbU25708) and the 4F2 cell surface antigen heavy chain of man (Mastroberardino *et al.*, 1998). These homologues are members of the rBAT family of mammalian transporter-accessory proteins (TC 8.A.9). All of the members of the rBAT family are believed to associate with members of the L-type amino acid transporter (LAT) family (TC 2.A.3.8) within the APC superfamily to form heterodimeric complexes.

One APC family member, Hip1 of *Saccharomyces*

cerevisiae, which clearly functions in amino acid transport, has also been implicated in heavy metal transport (Farcasanu *et al.*, 1998). Another member of the family, Ssy1 of *Saccharomyces cerevisiae*, appears to be a transcriptional regulatory sensor (Didion *et al.*, 1998). It is possible, but not demonstrated that APC family homologues in *Bacillus subtilis* that promote spore germination may function as receptors rather than transporters. Thus, a few APC family members may serve functions other than that of the transport of amino acids and their derivatives.

The AAAP (amino acid/auxin permease) family (TC 2.A.18)

The AAAP family includes over four dozen sequenced proteins from plants, animals, yeast and fungi (Fischer *et al.*, 1995; Bennett *et al.*, 1996; Rentsch *et al.*, 1996; McIntire *et al.*, 1997; Young *et al.*, 1999). Individual permeases of the AAAP family transport auxin (indole-3-acetic acid), γ -aminobutyric acid, a single L-amino acid or multiple amino acids. Some of these permeases exhibit very broad specificities, transporting all of the L-amino acids naturally found in proteins. There are 16 AAAP paralogues in *Saccharomyces cerevisiae*, 13 in *Caenorabditis elegans* and at least 9 in *Arabidopsis thaliana*. These proteins, all from eukaryotes, vary from 376 to 713 aminoacyl residues in length, but most are 400–500 residues. Most of the size variation occurs as a result of the presence of long N-terminal hydrophilic extensions in some of the proteins, but size variation in the loops and the C termini is sometimes observed. These proteins exhibit 11 putative TMSs and show limited sequence similarity with members of the large APC superfamily. Thus, the AAAP family is probably part of the APC superfamily (Jack *et al.*, 2000).

The major facilitator superfamily (MFS) (TC 2.A.1)

The MFS is a very old, large and diverse superfamily that includes over a thousand sequenced members (Pao *et al.*, 1998; Saier *et al.*, 1999b). These permeases catalyse uniport, solute:cation (H^+ or Na^+) symport and/or solute: H^+ or solute:solute antiport. Most are of 400–600 aminoacyl residues in length and possess either 12 or 14 putative or established TMSs. They exhibit specificity for sugars, polyols, drugs, neurotransmitters, Krebs-cycle metabolites, phosphorylated glycolytic intermediates, amino acids, peptides, osmolites, nucleosides, organic anions, inorganic anions, iron siderophores, etc. They are found ubiquitously in all three domains of living organisms. Each of the 29 currently recognized families is in general specific for one class of compounds (see <http://www-biology.ucsd.edu/~msaier/transport/>). While one family in the MFS includes permeases that catalyse amino acid (proline) uptake [the metabolite: H^+ symporter (MHS) family (TC 2.A.1.6)], another, the peptide-acetyl-CoA transporter (PAT) family (TC 2.A.1.25), functions in peptide uptake in bacteria (see below) but in acetyl-CoA:CoA antiport in the endoplasmic reticular membrane of higher eukaryotes.

The proton-dependent oligopeptide transporter (POT) family (TC 2.A.17)

The POT family [also called the PTR (peptide transport) family] is primarily a peptide-transporter family, but one member is a nitrate/chlorate permease, and one has been reported to transport histidine as well as nitrate and peptides (Frommer *et al.*, 1994; Zhou *et al.*, 1998). Because this family is primarily concerned with peptide transport, it will be described below in the section entitled ‘families of peptide transporters’.

The solute:sodium symporter (SSS) family (TC 2.A.21)

All functionally characterized members of the SSS family catalyse solute uptake via Na^+ symport (Reizer *et al.*, 1994). The solutes transported may be sugars, amino acids, nucleosides, inositols, vitamins, urea or anions, depending on the transport system (Eskandari *et al.*, 1997; Prasad *et al.*, 1998; Sarker *et al.*, 1997). The broad substrate specificity but uniform energy-coupling mechanism exhibited by members of this family are particularly unusual and noteworthy traits of a single family.

Members of the SSS family have been identified in bacteria, archaea and animals. They vary in size from about 400 residues to about 700 residues and possess 12–15 putative TMSs, often sharing a core of 13 TMSs. A 13 TMS topology with a periplasmic N terminus and a cytoplasmic C terminus has been experimentally determined for the proline: Na^+ symporter, PutP, of *Escherichia coli* (Turk & Wright, 1997; Jung *et al.*, 1998).

The neurotransmitter:sodium symporter (NSS) family (TC 2.A.22)

Members of the large NSS family catalyse uptake of a variety of neurotransmitters, amino acids, osmolites and related nitrogenous substances by a solute: Na^+ symport mechanism (Beckman & Quick, 1998; Kavanaugh, 1998; Berfield *et al.*, 1999). Sometimes Cl^- is co-transported, and some of the NSS family members exhibit a K^+ dependency. For example, human dopamine, glutamate and γ -aminobutyric acid transporters co-transport their positively charged or zwitterionic substrates with 2 or 3 Na^+ , and 1 Cl^- is probably co-transported, at least with dopamine and γ -aminobutyric acid. The glutamate system may counter-transport K^+ (Clark & Amara, 1993). Most sequenced members of the NSS family are from animals, and these proteins are generally 600–700 aminoacyl residues in length with 12 TMSs. Bacterial and archaeal homologues have been sequenced, but few functional data are available for these proteins.

Recently, several members of the NSS family have been shown to exhibit channel-like properties under certain experimental conditions. Sizeable unitary ionic currents have been reported for membrane patches containing either the γ -aminobutyrate, noradrenaline or serotonin transporter (Galli *et al.*, 1998). Channel-like currents

have also been measured for mammalian $\text{Na}^+/\text{H}^+/\text{K}^+$ -coupled glutamate transporters of the dicarboxylate/amino acid:cation symporter (DAACS) family (TC no. 2.A.23; see below). Evidence suggests that these channels can accommodate neurotransmitters as well as inorganic ions. These observations suggest that, as has been demonstrated for carriers of a few other families, neurotransmitter transporters can be manipulated to function as voltage-gated channels. Whether or not this observation is of physiological relevance has yet to be determined.

The DAACS [dicarboxylate/amino acid:cation (Na^+ or H^+) symporter] family (TC 2.A.23)

Members of the DAACS family catalyse Na^+ and/or H^+ symport together with (a) a Krebs-cycle dicarboxylate (malate, succinate, or fumarate), (b) a dicarboxylic amino acid (glutamate or aspartate), (c) a small, semipolar, neutral amino acid (Ala, Ser, Cys, Thr) (Arriza *et al.*, 1993; Ogawa *et al.*, 1998), (d) both neutral and acidic amino acids or (e) most zwitterionic and dibasic amino acids (Reizer *et al.*, 1994; Palacín *et al.*, 1998; Zerbiv *et al.*, 1998). The bacterial members are of about 450 (420–491) aminoacyl residues in length while the mammalian proteins are of about 550 (503–574) residues. These proteins possess between 10 and 12 putative TMSs (Slotboom *et al.*, 1999).

All of the bacterial proteins cluster together on the phylogenetic tree as do the mammalian proteins (Saier *et al.*, 1999a). The mammalian permeases that transport neutral amino acids cluster separately from those that are specific for acidic amino acids. Among the mammalian proteins are neuronal excitatory amino acid neurotransmitter permeases. One of these, the Glt-1 L-glutamate/L-aspartate/D-aspartate transporter co-transporters the neurotransmitter with 3 Na^+ and 1 H^+ and countertransports it against 1 K^+ (Clark & Amara, 1993).

The MC (mitochondrial carrier) family (TC 2.A.29)

Permease protein subunits of the MC family possess six TMSs and exist in the mitochondrial membrane as homodimers (Aquila *et al.*, 1987; Walker & Runswick, 1993; Palmieri, 1994; Palmieri *et al.*, 1997, 1999; Tzagoloff *et al.*, 1996; Echtay *et al.*, 1998; Fiermonte *et al.*, 1998; Schroers *et al.*, 1998). The proteins are of fairly uniform size, about 300 residues (Kuan & Saier, 1993; Indiveri *et al.*, 1997). They arose by tandem intragenic triplication such that a genetic element encoding two TMSs gave rise to one encoding six TMSs (Saraste & Walker, 1982; Walker & Runswick, 1993). This event may have occurred when mitochondria first developed their specialized but permanent organellar functions within eukaryotic cells from endosymbiotic bacteria (Kuan & Saier, 1993; Indiveri *et al.*, 1997). Members of the MC family are found exclusively in eukaryotic organelles although they are nuclearly encoded. Most are found in mitochondria, but some are found in peroxisomes of animals and in amyloplasts of plants

(McCammon *et al.*, 1990; Sullivan *et al.*, 1991; see Kuan & Saier, 1993 for a review). Many of them preferentially catalyse the exchange of one solute for another (antiport); but two of them, the aspartate/glutamate exchanger and the ADP/ATP exchanger can function as anion-selective channels after chemical treatment with thiol reagents (Dierks *et al.*, 1990a, b). Thirty-four paralogues of the MC family are encoded within the genome of *Saccharomyces cerevisiae*, and 35 are encoded within the *Caenorhabditis elegans* genome (Paulsen *et al.*, 1998b; I. T. Paulsen, S. R. Goldman, W. S. Barnes and M. H. Saier, Jr, unpublished).

The AEC (auxin efflux carrier) family (TC 2.A.69)

Plants possess tissue-specific, PMF-driven, polarly localized cellular auxin efflux systems (Gälweiler *et al.*, 1998; Luschnig *et al.*, 1998). These carriers are saturable, auxin specific and localized to the basal ends of auxin-transport-competent cells. They may be found in various plant tissues including vascular tissues and roots. They are responsible for the polar (downwards) transport of auxins from the leaves to the roots and function in gravitropism. A single plant such as *Arabidopsis thaliana* may possess over six genes encoding such systems. Two isoforms, one in vascular tissue (PIN1) and one in roots (REH1) have been functionally characterized as has a homologue from *Oryza sativa*. These plant proteins are 600–700 aminoacyl residues long and exhibit 8–12 TMSs.

Homologues of the AEC family are found in bacteria (*Escherichia coli*, *Klebsiella pneumoniae*, *Synechocystis*, *Aquifex aeolicus*, *Bacillus subtilis* and *Rickettsia prowazekii*) as well as in archaea (*Methanococcus jannaschii* and *Methanobacterium thermoautotrophicum*). The *Klebsiella pneumoniae* homologue (MdcF, 319 aa) has been suggested to function in malonate uptake (Hoenke *et al.*, 1997; Dimroth & Hilbi, 1997). The bacterial proteins are generally of 300–400 aa in length.

Yeasts also possess homologues of the AEC family. *Saccharomyces cerevisiae* has three functionally characterized AEC family members (YL52, spP54072, 64.0 kDa; YNJ5, spP53930, 71.2 kDa; and YB8B, spP38355, 47.5 kDa), and *Schizosaccharomyces pombe* also has at least one sequenced homologue. It is thus clear that members of the AEC family are widespread, being found in bacteria, archaea, fungi and plants. *Caenorhabditis elegans*, however, appears to lack identifiable homologues of the AEC family. Based on PSI-BLAST results, the AEC family may be distantly related to the bile acid: Na^+ symporter (BASS) family (TC 2.28), which is represented in animals.

Families of secondary transporters specific for amines, amides and polyamines

In addition to the ABC superfamily, which includes members that transport various amines, amides and polyamines, several of the amino acid transporter families described above have been shown to include

members that are capable of transporting these compounds. These families include the MFS, APC, BCCT, AAAP, SSS, NSS, MC and AEC families (see Table 2). Additionally, the CAAT family has been implicated in amine transport (Ferguson & Krzycki, 1997). Most of these families are described in Table 4, but the BCCT family and the CAAT family, being prokaryote-specific, are described in Table 3. There are no families of secondary carriers in which all members transport simple amines and/or amides exclusively, but not amino acids. Thus, none of the families listed above needs be described here. The fact that most families of permeases which include members that transport amino acids and their derivatives are exclusive for these compounds and do not include members that transport other classes of compounds (i.e. sugars, nucleobases and their derivatives, vitamins, etc.) emphasizes the value of the phylogenetic approach to the functional characterization of recognized permease homologues.

Families of channel-forming proteins capable of transporting amines and amides

Three families of channel-forming proteins appear to be capable of transporting simple amines and/or amides. These families are listed in Table 5 and are described below.

The major intrinsic protein (MIP) family (TC 1.A.8)

The MIP family is large and diverse, possessing over 100 sequenced members that all form transmembrane channels. These channel proteins function in the transport of water, small carbohydrates (e.g. glycerol), urea and other small neutral molecules by an energy-independent mechanism (Li *et al.*, 1997; Calamita *et al.*, 1998; Deen & van Os, 1998; Dean *et al.*, 1999). They are

found ubiquitously in bacteria, archaea and eukaryotes. Phylogenetic clustering of the proteins is largely according to the phylum of the organism of origin, but one to three clusters are observed for each phylogenetic kingdom (plants, animals, yeast, bacteria and archaea) (Park & Saier, 1996). The known aquaporins cluster loosely together as do the known glycerol facilitators.

MIP family proteins are believed to form aqueous pores that selectively allow passive transport of their solute(s) across the membrane with minimal apparent recognition (Chrispeels & Maurel, 1994; Shukla & Chrispeels, 1998). Aquaporins selectively transport water (but not glycerol), while glycerol facilitators selectively transport glycerol but not water (Maurel *et al.*, 1993). Glycerol facilitators function as solute nonspecific channels, and may transport glycerol, dihydroxyacetone, propanediol, urea and other small straight-chain neutral molecules in physiologically important processes (Heller *et al.*, 1980; Maurel *et al.*, 1994). A few members of the family, including the yeast FPS protein (TC 1.A.8.5.1), transport both water and glycerol. Reports of MIP family proteins transporting ions may or may not be physiologically significant. However, demonstration of the involvement of the cyanobacterial channel protein (TC 1.A.8.4.1) in copper homeostasis suggests that it may transport Cu^{2+} (Kashiwagi *et al.*, 1995).

The physiological functions of many MIP family proteins are unknown. They probably consist of homodimers (GlpF of *Escherichia coli*; TC 1.A.8.1.1) or tetramers (MIP of *Bos taurus*; TC 1.A.8.8.1). Each subunit spans the membrane six times as putative α -helices and arose from a three-spanner-encoding genetic element by a tandem, intragenic duplication event (Reizer *et al.*, 1993b). Consequently, the two halves of the protein are in opposite orientations in the membrane, an unusual feature of a transport protein.

Table 5. Channel protein families capable of transporting amines and amides

TC no.	Family	Substrate	Size range	No. TMSs	Organism*	No. members	Example
1.A.8	Major intrinsic protein (MIP) family	H ₂ O, glycerol, urea, polyols	220–310	(6) _{2 or 4}	B, A, E	> 100	Aquaporins, Aqp1 of <i>Homo sapiens</i> ; glycerol facilitators, GlpF, of <i>Escherichia coli</i>
1.A.44	Urea transporter (UT) family	Urea, water	380–400	10	E (An)	> 10	Kidney vasopressin-regulated urea transporter, UT2
1.A.27	Phospholemman (PLM) family	Cl ⁻ (anion selective), taurine, lactate, glutamate, isethionate, gluconate	70–100	(1) _n	E (An)	> 10	Phospholemman; Cl ⁻ -conductance inducer protein, Mat-8

*B, bacteria; A, archaea; E, eukaryote; An, animal.

The urea transporter (UT) family (TC 1.A.44)

Members of the UT family are found only in mammals and amphibians (Olives *et al.*, 1994; Couriaud *et al.*, 1998). In a single species (i.e. rat or human) there may be at least three isoforms. One of the human UT family members is the Kidd (JK) blood group glycoprotein (Lucien *et al.*, 1998). Most of these proteins vary in size from 380–400 residues and exhibit 10 putative TMSs, but one of them, a rat urea transporter (gbU77971), is reported to be 929 residues long (Shayakul *et al.*, 1996). At least one of these proteins (UT3) can transport water as well as urea (Yang & Verkman, 1998). A channel-type mechanism is probable. Two members of this family, UT1 and UT2, may be derived from a single gene by alternative splicing.

The PLM (phospholemman) family (TC 1.A.27)

The PLM family includes mammalian phospholemmans of 8–10 kDa size (Chen *et al.*, 1997; Foskett, 1998; Kirk & Strange, 1998). The human, rat and dog proteins have been sequenced and characterized. They span the membrane once with their N termini outside. These proteins induce a hyperpolarization-activated chloride current in *Xenopus* oocytes. They are found in muscle and many body tissues and are targets of protein kinases A and C. Other possible members include the chloride-conductance inducer protein, Mat8, and the Na⁺/K⁺ ATPase-subunit ‘proteolipid’ (TC 3.A.3). These proteins are smaller, but they exhibit the same orientation in the membrane.

PLM forms anion-selective channels when reconstituted in planar lipid bilayers. These channels display a linear current–voltage relationship, have a unitary conductance and are open most of the time at voltages between –70 and +70 mV. The PLM channel is permeable to both organic and inorganic anions including chloride, taurine, lactate, glutamate, isethionate and gluconate (Kirk & Strange, 1998).

Families of peptide transporters

In addition to the ATP-dependent ABC superfamily, in which members can transport peptides with either inward or outward polarity, depending on the family to which the transporter belongs, four families of PMF-driven transporters are primarily concerned with peptide uptake. These families are listed in Table 6 and are described below.

The PAT (peptide-acetyl-CoA transporter) family of the MFS (TC 2.A.1.25)

Two members of the PAT family of the MFS have been partially characterized from physiological standpoints, but the precise biochemical functions of these proteins are not certain. One of these proteins is the putative acetyl-CoA transporter found in the endoplasmic reticular and golgi membranes of man (Kanamori *et al.*, 1997). It is homologous to proteins in *Caenorhabditis*

elegans, *Saccharomyces cerevisiae* and several Gram-negative bacteria. The other of these proteins, the homologous *Escherichia coli* AmpG protein, probably brings peptides, including cell-wall degradative peptides, and inducers of β -lactamase synthesis into the cell (Lindquist *et al.*, 1993; Jacobs *et al.*, 1994; Park *et al.*, 1998). Thus, AmpG may transport cell-wall-derived peptides and glycopeptides. In *Haemophilus influenzae*, the gene encoding a PAT homologue is found in a gene cluster concerned with lipopolysaccharide synthesis. A homologue from *Neisseria gonorrhoeae* has also been sequenced. These proteins are of 425–632 aminoacyl residues in length and exhibit 12 putative TMSs as is characteristic of most MFS permeases.

The mechanism of energy coupling exhibited by members of the PAT family is not established, but the topology of these proteins and their established inclusion in the MFS suggest that they are secondary carriers. The acetyl-CoA transporter of mammals is expected to function by acetyl-CoA:CoA antiport while the AmpG protein of *Escherichia coli* is most likely energized by substrate:H⁺ symport. Of the PAT family members, prokaryotic proteins are smaller than the eukaryotic proteins by about 100 aminoacyl residues (408–491 residues versus 538–560 residues). Since acetyl-CoA contains several secondary amide (peptide-like) bonds, the inclusion of a substrate such as acetyl-CoA in a family of peptide transporters is not entirely surprising.

Rickettsia prowazekii encodes three AmpG-like paralogues within its small (1.1 Mbp) genome (Andersson *et al.*, 1998) although other bacteria (*Escherichia coli* and *Haemophilus influenzae* and the two sequenced eukaryotic genomes, *Saccharomyces cerevisiae* and *Caenorhabditis elegans*), all with much larger genomes, only encode one. Most of the bacteria for which fully sequenced genomes are available, and all of the four archaea with sequenced genomes do not encode a recognizable PAT family member. An analysis of this family has appeared in a recently updated description of the MFS (Saier *et al.*, 1999b).

The POT (proton-dependent oligopeptide transporter) family (TC 2.A.17)

Proteins of the POT family (Paulsen & Skurray, 1994) [also called the PTR (peptide transport) family (Steiner *et al.*, 1995)] are found in animals, plants, yeast and both Gram-negative and Gram-positive bacteria (Hagting *et al.*, 1994; Steiner *et al.*, 1994; Daniel, 1996; Leibach & Ganapathy, 1996; Miyamoto *et al.*, 1996; Döring *et al.*, 1998; Fei *et al.*, 1998). Several of these organisms possess multiple POT family paralogues. The proteins are of about 450–600 aminoacyl residues in length, with the eukaryotic proteins in general being longer than the bacterial proteins (Saier *et al.*, 1999a). They exhibit 12 putative or established TMSs (Hagting *et al.*, 1997; Covitz *et al.*, 1998). Some members of the POT family exhibit limited sequence similarity to protein members of the MFS (comparison scores of up to 8 SD for segments in excess of 60 residues

Table 6. Secondary active peptide transporter families

TC no.	Family	Substrate	Size range (residues)	No. TMSs	Organism*	No. members	Example
2.A.1.25	Peptide-acetyl-CoA transporter (PAT) family of the MFS	Cell wall peptides, glycopeptides, acetyl-CoA	400–600	12	B, E	> 20	AmpG of <i>Escherichia coli</i> ; acetyl-CoA transporter of <i>Homo sapiens</i>
2.A.17	Proton-dependent oligopeptide transporter (POT) family	Peptides, nitrates, amino acids	450–600	12	B, E	> 30	Dipeptide transporter, DtpT of <i>Lactococcus lactis</i>
2.A.67	Oligopeptide transporter (OPT) family	Peptides	600–900	12–15	B, A, E	> 20	Oligopeptide transporter, OPT1, of <i>Candida albicans</i>
9.A.18	Peptide uptake permease (PUP) family	Peptides, antibiotics (uptake)	406	7	B	2	Microbin uptake permease, SbmA of <i>Escherichia coli</i>

* B, bacteria; A, archaea; E, eukaryote.

in length). Thus the POT family is probably a family within the MFS (Pao *et al.*, 1998).

While most members of the POT family catalyse peptide transport, one is a nitrate/chlorate permease (Tsay *et al.*, 1993; Frommer *et al.*, 1994), and one (or more) can transport histidine as well as nitrate and peptides (Frommer *et al.*, 1994; Zhou *et al.*, 1998). Some of the peptide transporters can also transport antibiotics. They function by proton symport, but the substrate:H⁺ stoichiometry is variable: the high-affinity rat PepT2 carrier catalyses uptake of 2 and 3 H⁺ with neutral and anionic dipeptides, respectively, while the low-affinity PepT1 carrier catalyses uptake of 1 H⁺ per neutral peptide (Chen *et al.*, 1999).

The OPT (oligopeptide transporter) family (TC 2.A.67)

The OPT family consists of transporters for oligopeptides of 4–6 aminoacyl residues (Lubkowitz *et al.*, 1997, 1998). Two transporters from *Saccharomyces cerevisiae*, one from *Schizosaccharomyces pombe* and one from *Candida albicans* have been functionally characterized, and all are peptide-uptake systems. *Saccharomyces cerevisiae* possesses three paralogues of the OPT family while *Schizosaccharomyces pombe* has at least two. One of the *Schizosaccharomyces pombe* homologues is the sexual differentiation process (ISP4) protein. Homologues are also found in plants, and distant homologues may be present in bacteria and archaea as well. The prokaryotic homologues are very distant, being revealed only upon PSI-BLAST iterations, and they are uncharacterized functionally. Energy coupling probably involves H⁺ symport. The full-length yeast proteins are reported to be 700–900 residues long and exhibit up to 12 TMSs. A putative bacterial

homologue from *Haemophilus influenzae* is 633 aminoacyl residues long and exhibits 15 putative TMSs.

The PUP (peptide-uptake permease) family (TC 9.A.18)

Two partially functionally characterized proteins, the SbmA protein (406 aa) of *Escherichia coli* and the BacA protein (420 aa) of *Rhizobium meliloti*, define the PUP family (Glazebrook *et al.*, 1993; Salomón & Farías, 1995; Ichige & Walker, 1997). SbmA catalyses uptake of thiazole ring-containing peptide antibiotics such as microcin B17 and microcin J25 as well as the non-peptide antibiotic, bleomycin. BacA is a nodulation protein essential for bacterial development when *Rhizobium* is in symbiosis with a leguminous plant such as alfalfa. These two proteins exhibit 64% identity and are functionally interchangeable in both *Escherichia coli* and *Rhizobium meliloti*. *Rhizobium meliloti* bacA null mutants show increased resistance to bleomycin and certain aminoglycosides as well as increased sensitivity to ethanol and detergents. The latter properties are not characteristic of *Escherichia coli* sbmA mutants. It is hypothesized that BacA may take up peptide substances required for developmental progression towards bacteroid formation. Based on the mutant analyses, BacA (but not SbmA) may also play a role in the maintenance of membrane integrity.

Proteins of the PUP family are homologous, but distantly related, to a few putative ABC-type transporters of Gram-negative and Gram-positive bacteria. Unlike SbmA and BacA, the latter proteins possess ABC-containing domains. SbmA and BacA also differ from these putative ABC proteins in possessing seven rather than six putative TMSs per polypeptide chain. It is possible that ATP-hydrolysing protein constituents (ABC proteins) of the PUP family transporters will be

Table 7. Protein secretory pathways (PSP) in living organisms

PSP no.	Type	Name	TC no.	Bacteria	Archaea	Eukarya	No. proteins	Energy	
I	ABC	ATP-binding cassette translocase	3.A.1	+	+	+	1–2	ATP	
II	Sec	General secretory translocase	3.A.5	+	+	+	~12	GTP or ATP+PMF	
III	Vir	Virulence-related translocase	3.A.6	+	–	–	>10	ATP	
IV	Conj	Conjugation-related translocase	3.A.7	+	–	–	>6	ATP	
V	Tat	Twin arginine targeting translocase	2.A.64	+	+	+	5	PMF	
VI	MPT	Mitochondrial protein translocase	3.A.8	–	–	+	~20	ATP	
VII	CEPT	Chloroplast envelope protein translocase	3.A.9	+	–	+	3	GTP	
VIII	MTB	Main terminal branch of the Sec translocase (Gram-negative bacterial outer membranes)	3.A.5	+	–	–	~14	ATP?	
IX	α -Type channels	Cytoplasmic membrane channels							
		(a) MscL	Large conductance mechano-sensitive channel family	1.A.22	+	–	–		None
		(b) Bcl-2	Bcl-2 family	1.A.21	–	–	+		None
	(c) Holins	Holin functional superfamily	1.A.28–43	+	–	–		None	
X	OMPs	Gram-negative bacterial outer membrane channel-forming translocases							
		(a) FUP	Fimbrial usher protein	1.B.11	+	–	–	1	None
		(b) AT	Auto transporter	1.B.12	+	–	–	1	None
		(c) OMR	Outer-membrane receptor	1.B.14	+	–	–	1	None
		(d) OMF	Outer-membrane factor	1.B.17	+	–	–	1	None
		(e) Secretins	Secretin	1.B.22	+	–	–	1	None
XI	Toxins	Channel-forming toxins							
		(a) DT*	Diphtheria toxin family	1.C.7	+	–	–		None
		(b) BTT*	Botulinum and tetanus toxin family	1.C.8	+	–	–		None
	(c) IIITCP*	Bacterial type III-target cell pore family	1.C.36	+	–	–		ATP	

* Made by bacteria but inserted into host animal or plant cell membranes.

found. If so, these systems may prove to be energized by ATP hydrolysis. However, the mechanism of energy coupling to PUP family permeases is currently unknown. It is for this reason that the PUP family is placed into the '9' category of the TC system. If ABC proteins prove not to be associated with these permeases, they will provide one of the few examples of secondary carriers that are phylogenetically related to primary active-transport systems.

Protein-transport systems

Twenty transporter families are known to include members that function in the transmembrane transport of proteins (Table 7). Many of these (TC category 3.A) are primary active-transport systems driven by either ATP or GTP hydrolysis although protein export via several of these systems is stimulated by the PMF. One family (twin arginine targeting translocase, Tat; TC

2.A.64) exports proteins in a process that is energized exclusively by the PMF, implying that protein export is coupled to proton import. Transport systems of the Tat family therefore fall into the category of secondary transporters. Finally, many channel-forming proteins (categories IX and X in Table 7) have the capacity to transport proteins, most of them probably in an energy-independent fashion. These families will not be described here but the interested reader will find descriptions of these families at <http://www-biology.ucsd.edu/~msaier/transport/>.

Conclusion

Over two dozen families of transporters are currently known to be responsible for the transmembrane transport of amino acids, small amines, polyamines, amides and peptides. Three of these are families of channel-forming proteins, and they generally transport urea and other small nitrogen-containing compounds. Of the 21 currently recognized families of secondary carriers specific for amino acids and their derivatives, 11 are found only in prokaryotes. Nine of these families include members that have so far been found only in bacteria, but two are also represented in archaea. All but one of the prokaryote-specific transporter families are capable of transporting amino acids, but the one exceptional family consists of transporters that seem to be highly selective for peptides and glycopeptides. An additional ten families are either ubiquitous (eight families) or restricted to eukaryotes (two families). Eight of the 19 amino acid-transporting families of secondary carriers include members that also transport small amines and amides, and two of these families include members that exhibit the capacity to transport peptides. Only two families include members that appear to transport peptides specifically, lacking the capacity to transport simple amino acids.

Numerous permeases within the ABC superfamily catalyse either uptake or extrusion of amino acids, amines and/or peptides (Table 1). For the uptake of amino acids, one family (TC 3.A.1.3) is generally selective for polar amino acids while a second (TC 3.A.1.4) is selective for nonpolar amino acids. Only one uptake family (TC 3.A.1.5) within the ABC superfamily includes members that transport peptides. Three families include members that take up polyamines (TC 3.A.1.11), quaternary amines (TC 3.A.1.12) and taurine (TC 3.A.1.17). Amines are often the decarboxylation products of amino acids or the methylated derivatives of these products, while peptides and decapeptides (peptide-like molecules synthesized by enzymes in nonribosome-dependent processes) are condensation products of amino acids. All of these compounds can therefore be thought of as amino acid derivatives.

The ABC uptake permeases segregate on a phylogenetic tree from the efflux systems (Saurin *et al.*, 1999). Of the efflux systems (Table 1), the peptide transporters are found primarily in seven families, five prokaryote-

specific families (TC 3.A.1.111–113, 116, 118) and two eukaryote-specific families (TC 3.A.1.206 and 3.A.1.208). Two ABC families (TC 3.A.1.109 and 110) function in the secretion of proteins from bacteria, but no ABC-type eukaryotic exporters have been shown to function in protein secretion. This may be due to the fact that most proteins and complex carbohydrates are secreted in eukaryotes primarily, if not exclusively, by exocytosis. We note, therefore that of the 48 recognized families of ABC transporters, 15, or nearly one-third of all recognized ABC transport families, are primarily concerned with transport of amino acids and their derivatives. Taken together, about 10% of all currently recognized families of transporters include members that are known to transport low-molecular-mass amines, amides, amino acids and peptides, with another 10% functioning in the export of proteins. These large percentages illustrate the importance of amino acids and their derivatives to the normal function of all biological cells.

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