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A Functional-Phylogenetic System for the Classification of Transport Proteins

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Abstract Twenty completely sequenced genomes of bacteria, archaea, and eukaryotes have been surveyed for the presence of genes encoding homologues of known solute transport proteins. These analyses and others have demonstrated the presence of nearly 250 families of sequence-related transporters. All such proteins have been classified according to the system we call the transporter classification system of the transport commission (TC). This short summary article describes the main features of this system; the families are presented in tabular form. Detailed information concerning these families and their constituent transporters is available on our web sites. J. Cell. Biochem. Suppls. 32/33:84–94, 1999. © 1999 Wiley-Liss, Inc.

Biological organisms consist of cells built of proteins, carbohydrates, lipids, and nucleic acids. Virtually all the catalytic activities of the cell are performed by proteins. About one-third of the proteins of a cell are embedded in biological membranes, and about one-third of these function to catalyze the transport of molecules from one side of the membrane to the other (Paulsen et al., 1998a,b]. While many of these proteins couple their vectorial reactions to changes in cellular energy state, others catalyze the vectorial process without the expenditure of energy.

THE TC SYSTEM

Our research group has undertaken the task of classifying all biological transmembrane molecular transport systems [Saier, 1998, 1999a,b,c]. In the proposed classification system, each functionally dissimilar transporter is classified by a 4-digit TC number. The first of these four numbers refers to the mode of transport and energy coupling mechanism; the second refers to the family or superfamily; the third refers to a phylogenetic cluster within the family (or a family within the superfamily), and the fourth number refers to the substrate specificity of the individual permease. Thus, the number 2.1.4.5 refers to a secondary carrier (2) of the major facilitator superfamily (MFS; 2.1), in the fourth family of the MFS, called the organophosphate:P_i antiporter (OPA) family (2.1.4), with a specificity for glucose 6-P (2.1.4.5). The example selected to illustrate this particular TC number is the microsomal glucose 6-P transporter (glycogen storage or Gierke's disease protein) of man. The entire TC system can be found on our web site 1 (http://www-biology.ucsd.edu/~msaier/transport/titlepage.html). The genome analyses upon which this system was originally based can be found on our web site 2 (http://www-biology.ucsd.edu/~ipaulsen/transport/titlepage.html). Web site 3 (http://www.biology.ucsd.edu/~msaier/transport/software. html) provides useful programs developed in our lab and elsewhere for protein and DNA analyses, and web site 4 (http://www-biology.ucsd.edu/~msaier/transport/phylo.html) presents representative multiple alignments, phylogenetic trees, and other analyses for some of the families included within the TC systems.

All recognized transport proteins and peptides are classified into 15 different groups of systems. All families of the sequenced and func-

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tionally characterized systems are placed into classes 1–11, corresponding to TC numbers 1–11. Those families that are uncharacterized or partially characterized in various ways are placed into classes 96–99 with the same TC numbers (see below).

As noted above, transport systems are classified on the basis of four criteria, and each of these criteria corresponds to one of the four numbers within the TC number for a particular type of permease. For example, a TC number has four components as follows: W.X.Y.Z. W corresponds to the transporter type and energy source (if any) used to drive transport; X corresponds to the permease family (or superfamily); Y corresponds to the subfamily in which a permease is found, and Z corresponds to the substrate(s) transported. Any two transport proteins in the same subfamily of a permease family that transport the same substrate(s) are given the same TC number, regardless of whether they are orthologues (e.g., arose in distinct organisms by speciation) or paralogues (e.g., arose within a single organism by gene duplication). Sequenced homologues of unknown or unsuspected function are not assigned a TC number. Classification according to permease type and energy source is as follows:

1. *Channel-type transporters*: Transmembrane channel proteins of this class are found ubiquitously in the membranes of all types of organisms from bacteria to higher eukaryotes. Transport systems of this type catalyze facilitated diffusion (by an energy-independent process) by passage through a transmembrane aqueous pore or channel without evidence for a carrier-mediated mechanism. These channel proteins usually consist largely of α-helical spanners, although β-strands may also be present and may even comprise part of the channel. However, outer membrane porin-type channel proteins of bacterial and eukaryotic organelles are excluded from this class and are instead included in class 9.
2. *Carrier-type transporters*: Transport systems are included in this class if they use a carrier-mediated process to catalyze uniport (a single species is transported by facilitated diffusion), antiport (two or more species are transported in opposite directions in a tightly coupled process, not coupled to a direct form of energy other than chemiosmotic energy), and/or symport (two or more species are transported together in the same direction in a tightly coupled process, not coupled to a direct form of energy other than chemiosmotic energy).
3. *Pyrophosphate bond hydrolysis-driven active transporters*: Transport systems are included in this class if they hydrolyze pyrophosphate or the terminal pyrophosphate bond in ATP or another nucleoside triphosphate to drive the active uptake and/or extrusion of a solute or solutes. The transport protein may or may not be transiently phosphorylated, but the substrate is not phosphorylated.
4. *PEP-dependent, phosphoryl transfer-driven group translocators*: Transport systems of the bacterial phosphoenolpyruvate:sugar phosphotransferase system are included in this class. The product of the reaction, derived from extracellular sugar, is a cytoplasmic sugar-phosphate.
5. *Decarboxylation-driven active transporters*: Transport systems that drive solute (e.g., ion) uptake or extrusion by decarboxylation of a cytoplasmic substrate are included in this class.
6. *Oxidoreduction-driven active transporters*: Transport systems that drive transport of a solute (e.g., an ion) energized by the flow of electrons (or hydride ions) from a reduced substrate to an oxidized substrate are included in this class.
7. *Light-driven active transporters*: Transport systems that use light energy to drive transport of a solute (e.g., an ion) are included in this class.
8. *Mechanically driven active transporters*: Transport systems are included in this class if they drive movement of a cell or organelle by allowing the flow of ions (or other solutes) through the membrane down their electrochemical gradients.
9. *Outer membrane porins (of* β*-structure)*: These proteins form transmembrane pores or channels that usually allow the energy independent passage of solutes across a membrane. The transmembrane portions of these proteins consist exclusively of β-strands, which form β-barrels. These porin-type proteins are found in the outer membranes of gram-negative bacteria, mitochondria, and eukaryotic plastids, and possibly in the outer membranes of gram-positive, acid-fast bacteria as well.

10. *Methyltransferase-driven active transporters*: A single characterized multicomponent family of transporters currently falls into this category, the Na^+-transporting methyltetrahydromethanopterin:coenzyme M methyltransferases.
11. *Nonribosome-synthesized, channel-forming or carrier-forming peptides, or peptide-like molecules*: These molecules, usually chains of L- and D-amino acids as well as other small molecular building blocks such as lactate, often form oligomeric transmembrane ion channels (e.g., gramicidin). Voltage may induce channel formation by promoting assembly of the transmembrane channel. Alternatively, these peptides may act as carriers to shuffle ions across the membrane (e.g., valinomycin). These peptides are often made by bacteria or fungi as agents of biological warfare.
96. *Functionally characterized transporters for which sequence data are lacking*: Transporters of particular physiological significance are included in this category even though a family assignment cannot be made. They will be transferred to another category when their sequences become available.
97. *Putative transporters in which no family member is an established transporter*: Putative transport protein families are grouped under this number and will either be classified elsewhere when the transport function of a member becomes established or will be eliminated from the TC classification system if the proposed transport function is disproved. These families include at least one member for which a transport function has been suggested, but evidence for such a function is not compelling.
98. *Auxiliary transport proteins*: Proteins that in some way facilitate transport across one or more biological membranes but do not themselves participate directly in transport are included in this class. These proteins always function in conjunction with one or more transport proteins. They may provide a function connected with energy coupling to transport, play a structural role in complex formation, serve a regulatory function, or provide a function related to biogenesis or stability of the transporter.
99. *Transporters of unknown classification*: Recognized transport protein families are grouped under this number when their mode of transport or energy coupling mechanism is unknown. They will be classified elsewhere (i.e., classes 1–11) when the transport process and energy coupling mechanism become characterized. These families include at least one member for which a transport function has been established.

FAMILIES OF TRANSPORT SYSTEMS

Table I lists all the currently recognized families of transporters. Channel-type transporters of class 1 (42 families represented) include a variety of types including channel-forming proteins and peptides that generally form transmembrane α-helix-lined pores. These proteins include (1) cellular pores in which the protein is made in the cell in which it functions, often as an ion channel; (2) channel-forming toxins in which the protein is made by a cell other than the one in which it generates the transmembrane pore; (3) α-helical proteic peptides that function in the membrane of the cell that produces them as agents of programmed cell death (e.g., holins); and (4) α-helical proteic peptides that function as toxins in cells other than the producing cell.

Carrier-type transporters of class 2 include uniporters, symporters, and antiporters. This is the largest class of transporters within the TC system with 77 families (Table I). Some of these families are large superfamilies. The largest one is the major facilitator superfamily (MFS) (TC 2.1). It includes 29 currently recognized families, each specific for a different class of compounds. Table I presents the 29 families of the MFS [Pao et al., 1998] by TC number, family name, and family abbreviation. A brief examination of the list of families reveals the broad range of substrates transported.

Distant probable family members of the MFS include the GPH family (TC 2.2), the POT family (TC 2.17), the OAT family (TC 2.60), and the FBT family (TC 2.71) (Table I). These families have not been established to be members of the MFS by classical statistical methods of sequence analysis, but refined search tools as well as motif analyses provided the evidence suggesting that they are phylogenetically related to this superfamily. Because it has not been possible to establish their MFS affiliation rigorously, these families have their own TC numbers.

TABLE I. Families of Transport Proteins Included in the Transporter Classification (TC) System of the Transport Commission

1. Channel-type transporters
   - 1.1 The major intrinsic protein (MIP)
   - 1.2 The epithelial Na^+ channel (ENaC)
   - 1.3 Large conductance mechanosensitive ion channel (MscL)
   - 1.4 ATP-gated cation channel (ACC)
   - 1.5 Voltage-gated ion channel (VIC) (superfamily)
   - 1.6 Ligand-gated ion channel (LIC) (family of neurotransmitter receptors)
   - 1.7 Glutamate-gated ion channel (GIC) (family of neurotransmitter receptors)
   - 1.8 Channel-forming Amphipathic Peptide (CAP) (functional superfamily)
   - 1.9 The ryanodine-inositol 1,4,5-triphosphate receptor Ca^{2+} channel (RIR-CaC)
   - 1.10 Chloride channel (CIC)
   - 1.11 Holin (Holin) (functional superfamily)
   - 1.12 Channel-forming colicin (Colicin)
   - 1.13 Channel-forming δ-endotoxin insecticidal crystal protein (ICP)
   - 1.14 α-Hemolysin channel-forming toxin (αHL)
   - 1.15 Aerolysin channel-forming toxin (aerolysin)
   - 1.16 Animal inward rectifier K^+ channel (IRK-C)
   - 1.17 Organellar chloride channel (O-ClC)
   - 1.18 Channel-forming colicin V (colicin V)
   - 1.19 Channel-forming ϵ-toxin (ϵ-toxin)
   - 1.20 Transient receptor potential Ca^{2+} channel (TRP-CC)
   - 1.21 Yeast killer toxin K1 (YKT-K1)
   - 1.22 Urea transporter (UT)
   - 1.23 Gap junction-forming connexin (connexin)
   - 1.24 Phospholemman (PLM)
   - 1.25 Diphtheria toxin (DT)
   - 1.26 Symbiotic ammonium transporter (SAT)
   - 1.27 Nonselective cation channel-1 (NSCC1)
   - 1.28 Nonselective cation channel-2 (NSCC2)
   - 1.29 Influenza virus matrix-2 channel (IVC)
   - 1.30 Bcl-2 (Bcl-2)
   - 1.31 The $gp91^{phox}$ phagocyte NADPH oxidase-associated cytochrome b_{558} (CybB) H^+-channel
   - 1.32 Cytohemolysin (CHL)
   - 1.34 Small conductance mechanosensitive ion channel (MscS)
   - 1.35 Botulinum and tetanus toxin (BTT)
   - 1.36 Vacuolating cytotoxin (VacA)
   - 1.37 Pore-forming hemolysin E (HlyE)
   - 1.38 Pore-forming RTX toxin (RTX-toxin)
   - 1.39 Thiol-activated cytolysin (TAC)
   - 1.40 Bacterial type III-target cell pore (IIITCP)
   - 1.41 Channel-forming leukocidin cytotoxin (Ctx)
   - 1.42 Whipworm stichosome porin (WSP)

2. Carrier-type transporters (Uni-, Sym-, and antiporters)
   - 2.1 The major facilitator superfamily (MFS)
     - 2.1.1 Sugar porter (SP)
     - 2.1.2 Drug:H^+ Antiporter-1 (12 spanner) (DHA1)
     - 2.1.3 Drug:H^+ Antiporter-2 (14 spanner) (DHA2)
     - 2.1.4 Organophosphate:P_i Antiporter (OPA)
     - 2.1.5 Oligosaccharide:H^+ symporter (OHS)
     - 2.1.6 Metabolite:H^+ symporter (MHS)
     - 2.1.7 Fucose:H^+ symporter (FHS)
     - 2.1.8 Nitrate/nitrite porter (NNP)
     - 2.1.9 Phosphate:H^+ symporter (PHS)
     - 2.1.10 Nucleoside:H^+ symporter (NHS)
     - 2.1.11 Oxalate:formate antiporter (OFA)
     - 2.1.12 Sialate:H^+ Symporter (SHS)

TABLE I. (continued)

2.1.13 Monocarboxylate porter (MCP)
2.1.14 Anion:cation symporter (ACS)
2.1.15 Aromatic Acid:H^+ Symporter (AAHS)
2.1.16 Siderophore-iron transporter (SIT)
2.1.17 Cyanate permease (CP)
2.1.18 Polyol permease (PP)
2.1.19 Organic cation transporter (OCT)
2.1.20 Sugar efflux transporter (SET)
2.1.21 Drug:H^+ antiporter-3 (12 spanner) (DHA3)
2.1.22 Vesicular neurotransmitter transporter (VNT)
2.1.23 Conjugated bile salt transporter (BST)
2.1.24 Unknown major facilitator-1 (UMF1)
2.1.25 Peptide-acetyl-coenzyme A transporter (PAT)
2.1.26 Unknown major facilitator-2 (UMF2)
2.1.27 Phenyl propionate permease (PPP)
2.1.28 Unknown major facilitator-3 (UMF3)
2.1.29 Unknown major facilitator-4 (UMF4)
2.2 Glycoside-pentoside-hexuronide (GPH):cation symporter
2.3 Amino acid-polyamine-organocation (APC) (superfamily)
2.3.1 Amino acid transporter (AAT)
2.3.2 Basic amino acid/polyamine antiporter (APA)
2.3.3 Cationic amino acid transporter (CAT)
2.3.4 Amino acid/choline transporter (ACT)
2.3.5 Ethanolamine transporter (EAT)
2.3.6 Archaeal/bacterial transporter (ABT)
2.3.7 Glutamate:GABA antiporter (GGA)
2.3.8 L-type amino acid transporter (LAT)
2.3.9 Spore germination protein (SGP)
2.3.10 Yeast amino acid transporter (YAT)
2.4 Cation diffusion facilitator (CDF)
2.5 Zinc (Zn^{2+})-Iron (Fe^{2+}) permease (ZIP)
2.6 Resistance-nodulation-cell division (RND) (Superfamily)
2.6.1 Heavy metal efflux (HME)
2.6.2 (Largely gram-negative bacterial) hydrophobe/amphiphile efflux-1 (HAE1)
2.6.3 Putative nodulation factor exporter (NFE)
2.6.4 SecDF (SecDF)
2.6.5 (Gram-positive bacterial putative) hydrophobe/amphiphile efflux-2 (HAE2)
2.6.6 Eukaryotic (putative) sterol transporter (EST)
2.6.7 (Largely archaeal putative) hydrophobe/amphiphile efflux-3 (HAE3)
2.7 Small multidrug resistance (SMR)
2.8 Gluconate:H^+ symporter (GntP)
2.9 L-Rhamnose transporter (RhaT)
2.10 2-Keto-3-deoxygluconate transporter (KDGT)
2.11 Citrate-Mg^{2+}:H^+ (CitM)-citrate:H^+ (CitH) symporter (CitMHS)
2.12 ATP:ADP antiporter (AAA)
2.13 C_4-Dicarboxylate uptake (Dcu)
2.14 Lactate permease (LctP)
2.15 Betaine/carnitine/choline transporter (BCCT)
2.16 Telurite-resistance/dicarboxylate transporter (TDT)
2.17 Proton-dependent oligopeptide transporter (POT)
2.18 Amino acid/auxin permease (AAAP)
2.19 Ca^{2+}:cation antiporter (CaCA)
2.20 Inorganic phosphate transporter (PiT)
2.21 Solute:sodium symporter (SSS)
2.22 Neurotransmitter:sodium symporter (NSS)
2.23 Dicarboxylate/amino acid:cation (Na^+ or H^+) symporter (DAACS)
2.24 Citrate:cation symporter (CCS)
2.25 Alanine or glycine:cation symporter (AGCS)

TABLE I. (continued)

2.26 Branched chain amino acid:cation symporter (LIVCS)
2.27 Glutamate:Na^+ Symporter (GltS)
2.28 Bile acid:Na^+ symporter (BASS)
2.29 Mitochondrial carrier (MC)
2.30 Cation-chloride cotransporter (CCC)
2.31 Anion exchanger (AE)
2.32 Silicon transporter (Sit)
2.33 NhaA Na^+:H^+ antiporter (NhaA)
2.34 NhaB Na^+:H^+ antiporter (NhaB)
2.35 NhaC Na^+:H^+ antiporter (NhaC)
2.36 Monovalent cation:proton antiporter-1 (CPA1)
2.37 Monovalent cation:proton antiporter-2 (CPA2)
2.38 K^+ transporter (Trk)
2.39 Nucleobase:cation symporter-1 (NCS1)
2.40 Nucleobase:cation symporter-2 (NCS2)
2.41 Concentrative nucleoside transporter (CNT)
2.42 Aromatic amino acid permease (ArAAP)
2.43 Serine/threonine permease (STP)
2.44 Formate-nitrite transporter (FNT)
2.45 Metal ion transporter (MIT)
2.46 Benzoate:H^+ symporter (BenE)
2.47 Divalent anion:Na^+ symporter (DASS)
2.48 Reduced folate carrier (RFC)
2.49 Ammonium transporter (Amt)
2.50 Triose phosphate translocator (TPT)
2.51 Nucleotide-sugar transporter (NST)
2.52 Ni^{2+}-Co^{2+} transporter (NiCoT)
2.53 Sulfate permease (SulP)
2.54 Mitochondrial tricarboxylate carrier (MTC)
2.55 Metal ion (Mn^{2+}-iron) transporter (Nramp)
2.56 Tripartite ATP-independent periplasmic transporter (TRAP-T)
2.57 Equilibrative nucleoside transporter (ENT)
2.58 Phosphate:Na^+ symporter (PNaS)
2.59 Arsenical resistance-3 (ACR3)
2.60 Organo anion transporter (OAT)
2.61 C_4-dicarboxylate uptake C (DcuC)
2.62 NhaD Na^+:H^+ antiporter (NhaD)
2.63 Monovalent cation (K^+ or Na^+):proton antiporter-3 (CPA3)
2.64 Type V secretory pathway (VSP) or twin arginine targeting (Tat)
2.65 Bilirubin transporter (BRT)
2.66 Multi antimicrobial extrusion (MATE)
2.67 Oligopeptide transporter (OPT)
2.68 p-Aminobenzoyl-glutamate transporter (AbgT)
2.69 Auxin efflux carrier (AEC)
2.70 Malonate:Na^+ symporter (MSS)
2.71 Folate-biopterin transporter (FBT)
2.72 K^+ uptake permease (KUP)
2.73 Inorganic carbon (HCO_3^-) transporter (ICT)
2.74 4 TMS multidrug endosomal transporter (MET)
2.75 L-Lysine exporter (LysE)
2.76 Resistance to homoserine/threonine (RhtB)
2.77 Cadmium resistance (CadD)

3. Pyrophosphate bond (ATP; GTP; P_2) hydrolysis-driven active transporters
3.1 The ATP-binding Cassette (ABC) (Superfamily)
ABC-type Uptake Permeases (All from Prokaryotes (Bacteria and Archaea))
3.1.1 Carbohydrate uptake transporter-1 (CUT1)
3.1.2 Carbohydrate uptake transporter-2 (CUT2)

TABLE I. (continued)

3.1.3 Polar amino acid uptake transporter (PAAT)
3.1.4 Hydrophobic amino acid uptake transporter (HAAT)
3.1.5 Peptide/opine/nickel uptake transporter (PepT)
3.1.6 Sulfate uptake transporter (SulT)
3.1.7 Phosphate uptake transporter (PhoT)
3.1.8 Molybdate uptake transporter (MolT)
3.1.9 Phosphonate uptake transporter (PhnT)
3.1.10 Ferric iron uptake transporter (FeT)
3.1.11 Polyamine/opine/phosphonate uptake transporter (POPT)
3.1.12 Quaternary amine uptake transporter (QAT)
3.1.13 Vitamin B_{12} uptake transporter ($VB_{12}T$)
3.1.14 Iron chelate uptake transporter (FeCT)
3.1.15 Manganese/zinc/iron chelate uptake transporter (MZT)
3.1.16 Nitrate/nitrite/cyanate uptake transporter (NitT)
3.1.17 Taurine uptake transporter (TauT)
3.1.18 Putative cobalt uptake transporter (CoT)
3.1.19 Thiamin uptake transporter (ThiT)

ABC-type Efflux Permeases (Bacterial)
3.1.31 Capsular polysaccharide exporter (CPSE)
3.1.32 Lipo-oligosaccharide exporter (LOSE)
3.1.33 Lipopolysaccharide exporter (LPSE)
3.1.34 Teichoic acid exporter (TAE)
3.1.35 Drug exporter (DrugE)
3.1.36 Putative lipid A exporter (LipidE)
3.1.37 Putative heme exporter (HemeE)
3.1.38 β-Glucan exporter (GlucanE)
3.1.39 Protein-1 exporter (Prot1E)
3.1.40 Protein-2 exporter (Prot2E)
3.1.41 Peptide-1 exporter (Pep1E)
3.1.42 Peptide-2 exporter (Pep2E)
3.1.43 Peptide-3 exporter (Pep3E)
3.1.44 Probable glycolipid Exporter (DevE)
3.1.45 Na^+ exporter (NatE)
3.1.46 Microcin B17 exporter (McbE)
3.1.47 Multidrug exporter (MDE)
3.1.48 Microcin J25 exporter (McjD)
3.1.49 Drug/siderophore exporter-3 (DrugE3)

ABC-type efflux permeases (mostly eukaryotic)
3.1.61 Multidrug resistance exporter (MDR)
3.1.62 Cystic fibrous transmembrane conductance exporter (CFTR)
3.1.63 Peroxysomal fatty acyl CoA transporter (FAT)
3.1.64 Eye pigment precursor transporter (EPP)
3.1.65 Pleiotropic drug resistance (PDR)
3.1.66 a-Factor sex pheromone exporter (STE)
3.1.67 Conjugate transporter-1 (CT1)
3.1.68 Conjugate transporter-2 (CT2)
3.1.69 MHC peptide transporter (TAP)
3.1.70 Heavy metal transporter (HMT)

3.2 H^+- or Na^+-translocating F-type, V-type and A-type ATPase (F-ATPase) (Superfamily)
3.3 P-type ATPase (P-ATPase) (Superfamily)
3.4 Arsenite-antimonite (Ars) efflux
3.5 Type II (general) secretory pathway (IISP)
3.6 Type III (virulence-related) secretory pathway (IIISP)
3.7 Type IV (conjugal DNA-protein transfer or VirB) secretory pathway (IVSP)
3.8 Mitochondrial protein translocase (MPT)
3.9 Chloroplast envelope protein translocase (CEPT)
3.10 Plant vacuolar and bacterial H^+-translocating pyrophosphatase (H^+-PPase)
3.11 Bacterial competence-related DNA transformation transporter (DNA-T)

TABLE I. (continued)

4. Phosphotransferase systems:Phosphoenolpyruvate (PEP)-dependent, sugar-transporting phosphotransferase system (PTS) (functional superfamily)
   - 4.1 PTS glucose-glucoside (Glc)
   - 4.2 PTS fructose-mannitol (Fru)
   - 4.3 PTS lactose-N,N′-diacetylchitobiose-β-glucoside (Lac)
   - 4.4 PTS glucitol (Gut)
   - 4.5 PTS galactitol (Gat)
   - 4.6 PTS mannose-fructose-sorbose (Man)
5. Decarboxylation-driven active transporters
   - 5.1 Na^+-transporting Carboxylic Acid Decarboxylase (NaT-DC)
6. Oxidoreduction-driven active transporters
   - 6.1 Proton-translocating NADH dehydrogenase (NDH)
   - 6.2 Proton-translocating transhydrogenase (PTH)
   - 6.3 Proton-translocating quinol:cytochrome c reductase (QCR) (superfamily)
   - 6.4 Proton-translocating cytochrome oxidase (COX) (Superfamily)
   - 6.5 Na^+-translocating NADH:quinone dehydrogenase (Na-NDH)
   - 6.6 Putative ion (H^+ or Na^+)-translocating NADH:ferredoxin oxidoreductase (NFO)
   - 6.7 H_2:heterodisulfide oxidoreductase (HHO)
   - 6.8 Na^+- or H^+-pumping formyl methanofuran dehydrogenase (FMF-DH)
7. Light-driven Active Transporters
   - 7.1 Ion-translocating fungal/archaeal rhodopsin (FAR)
   - 7.2 Proton-translocating photosynthetic reaction center (PRC)
8. Mechanically-driven Active Transporters
   - 8.1 H^+- or Na^+-translocating bacterial flagellar motor (Mot)
9. Outer membrane porins (β-structure):Outer membrane porin (OMP) functional superfamily
   - 9.1 General bacterial porin (GBP)
   - 9.2 Chlamydial porin (CP)
   - 9.3 Sugar porin (SP)
   - 9.4 *Brucella-Rhizobium* porin (BRP)
   - 9.5 *Pseudomonas* OprP porin (POP)
   - 9.6 OmpA-OmpF porin (OOP)
   - 9.7 *Rhodobacter* PorCa porin (RPP)
   - 9.8 Mitochondrial and plastid porin (MPP)
   - 9.9 FadL outer membrane protein (FadL)
   - 9.10 Nucleoside-specific channel-forming outer membrane porin (Tsx)
   - 9.11 Outer membrane fimbrial usher porin (FUP)
   - 9.12 Autotransporter (AT)
   - 9.13 Alginate export porin (AEP)
   - 9.14 Outer membrane receptor (OMR)
   - 9.15 Raffinose porin (RafY)
   - 9.16 Short-chain amide and urea porin (SAP)
   - 9.17 Outer membrane factor (OMF)
   - 9.18 Outer membrane auxiliary (OMA)
   - 9.19 Glucose-selective OprB porin (OprB)
   - 9.20 Bacterial toxin export channel (TEC)
   - 9.21 OmpG porin (OmpG)
   - 9.22 Outer bacterial membrane secretin (secretin)
   - 9.23 Cyanobacterial porin (CBP)
   - 9.24 Mycobacterial porin (MBP)
   - 9.25 Outermembrane porin (Opr)
10. Methyltransfer-driven Active Transporters
    - 10.1 The Na^+-transporting methyltetrahydromethanopterin:coenzyme M methyltransferase (NaT-MMM)
11. Non-ribosome-synthesized channel- and carrier-forming peptides
    - 11.1 Gramicidin A (gramicidin) channel
    - 11.2 Valinomycin carrier (valinomycin)
    - 11.3 Syringomycin channel-forming (syringomycin)
    - 11.4 Syringopeptin channel-forming (syringopeptin)
    - 11.5 Tolaasin channel-forming (tolaasin)
    - 11.6 Alamethicin channel-forming (Alamethicin)

TABLE I. (continued)

96. Functionally characterized permeases for which sequences are not available
    - 96.1 Lysosomal sialic acid transporter (LST)
    - 96.2 Volume-sensitive anion channels (VAC)

97. Putative transporters of unknown classification
    - 97.1 Metal homeostasis protein (MHP)
    - 97.2 Ca^{2+} Homeostasis protein (CHP)
    - 97.3 Putative bacterial murein precursor exporter (MPE)
    - 97.4 Putative efflux transporter (PET)
    - 97.5 KX blood-group antigen (KXA)
    - 97.6 Toxic Hok/Gef protein (Hok/Gef)
    - 97.7 Putative bacteriochlorophyll delivery (BCD)
    - 97.8 Canalicular bile acid transporter (C-BAT)
    - 97.9 Urate transporter (UAT)
    - 97.12 (Salt or low temperature) stress-induced hydrophobic peptide (SHP)
    - 97.13 Putative pore-forming entericidin (ECN)
    - 97.14 Putative heme exporter protein (HEP)
    - 97.15 Putative chloroquine resistance Na^+/H^+ exchanger of *Plasmodium falciparum* (CQR)
    - 97.16 Putative ductin channel (Ductin)
    - 97.17 Putative fatty acid transporter (FAT)
    - 97.18 SecDF-associated single transmembrane protein (SSTP)
    - 97.19 Mn^{2+} homeostasis protein (MnHP)
    - 97.20 Putative Mg^{2+} transporter-C (MgtC)
    - 97.21 MadN-YedA-PecM (MYP)
    - 97.22 Putative permease (PerM)

98. Auxiliary transport proteins
    - 98.1 Membrane fusion protein (MFP)
    - 98.2 Secretin auxiliary lipoprotein (SAL)
    - 98.3 Cytoplasmic membrane-periplasmic auxiliary-1 (MPA1) protein with cytoplasmic (C) domain (MPA1−C or MPA1+C)
    - 98.4 Cytoplasmic membrane-periplasmic auxiliary-2 (MPA2)
    - 98.5 Voltage-gated K^+ channel β-subunit (VICβ)
    - 98.6 TonB-ExbB-ExbD/TolA-TolQ-TolR (TonB) family of auxiliary proteins for energization of outer membrane receptor (OMR)-mediated active transport
    - 98.7 Phosphotransferase system enzyme I (EI)
    - 98.8 Phosphotransferase system HPr (HPr)
    - 98.9 rBAT transport accessory protein (rBAT)
    - 98.10 Slow voltage-gated K^+ channel accessory protein (MinK)
    - 98.11 Phospholamban (Ca^{2+}-ATPase regulator) (PLB)
    - 98.12 ABC Bacteriocin exporter accessory protein (BEA)

99. Transporters of unknown classification
    - 99.1 Polysaccharide transporter (PST)
    - 99.2 MerTP mercuric ion (Hg^{2+}) permease (MerTP)
    - 99.3 MerC mercuric ion (Hg^{2+}) uptake (MerC)
    - 99.4 Nicotinamide mononucleotide (NMN) uptake permease (PnuC)
    - 99.5 Nephropathic cystinosin protein (NCP)
    - 99.6 Intracellular nucleoside transporter (INT)
    - 99.7 Chromate ion transporter (CHR)
    - 99.8 Ferrous iron uptake (FeoB)
    - 99.9 Low-affinity Fe^{2+} transporter (FeT)
    - 99.10 Oxidase-dependent Fe^{2+} transporter (OFeT)
    - 99.11 Copper transporter-1 (Ctr1)
    - 99.12 Copper transporter-2 (Ctr2)
    - 99.13 Short chain fatty acid transporter (scFAT)
    - 99.14 Nuclear pore complex (NPC)
    - 99.15 Putative amide transporter (Ami)
    - 99.16 Septal DNA translocation pore (DTP)
    - 99.18 Peptide uptake permease (PUP)
    - 99.19 Mg^{2+} transporter-E (MgtE)
    - 99.20 Low-affinity cation transporter (LCT)

Other superfamilies under TC classification 2 are the APC superfamily (TC 2.3) [Jack et al., 1999] with 10 currently recognized constituent families, and the RND superfamily (TC 2.6) with seven recognized families [Tseng et al., 1999]. While the APC superfamily is primarily concerned with amino acid uptake, the RND superfamily appears to be primarily concerned with drug and heavy metal efflux [Tseng et al., 1999]. Many other families represented under TC class 2 include transporters that function in the maintenance of cellular ionic homeostasis, in the uptake of essential nutrients, and in the efflux of metabolites and toxic substances. (Table I).

TC classification 3 includes all ATP or pyrophosphate hydrolysis-driven transporters. TC family 3.1 is the ABC superfamily with 46 currently recognized families (Table I). However, 10 other major families are found under TC classification 3. These include the F-type ATPases (TC 3.2) that interconvert chemiosmotic and chemical energy, and P-type ion-transporting ATPases (TC 3.3) such as the Na^+,K^+-ATPase of mammals, primarily pumps for ions. A variety of families of protein secretion systems (TC 3.5–3.9), a family of DNA uptake systems (TC 3.11) and a family of pyrophosphate-hydrolysis driven H^+ pumps (TC 3.10) are also included under TC 3.

TC classification 4 includes the permeases of the PEP-dependent phosphotransferase system (PTS). This bacterial-specific system includes six families, each permease of which actually represents a composite of protein domains. While one of these domains functions to transport sugar substrates across the membrane, the other PTS protein/domain constituents function to couple transport to substrate phosphorylation. This group translocation system thus modifies the substrate during transport. Like the proteins of TC class 3, the permeases of TC class 4 are true enzymes in the classical sense, even though they catalyze vectorial reactions.

TC class 5 includes a single family of Na^+-transporting carboxylic acid decarboxylases, all of which are heteroligomeric biotin-dependent enzyme complexes found only in prokaryotes. These enzymes use a unique type of energy coupling mechanism to extrude Na^+ (decarboxylation of any one of several carboxylates) for the purpose of generating a Na^+ electrochemical gradient.

TC class 6 includes eight different families of electron-transfer (or hydride transfer) oxidoreductase protein complexes. These complexes can transport H^+, Na^+ or both, depending on the system. In all cases, ion transport seems to be tightly coupled to the oxidoreduction reaction catalyzed. Most of these systems are prokaryote-specific although several are found in eukaryotic organelles that were derived from bacteria after the eukaryotic lines split off from the prokaryotic lines. The last two entries (TC 6.7 and 6.8) are found exclusively in archaea.

TC class 7 includes two well-characterized light absorption-driven ion transporter families. These are the fungal/archaeal rhodopsin (FAR) family and the photosynthetic reaction center (PRC) family. While a single protein of the FAR family catalyzes H^+ or Cl^- transport, a complex of PRC proteins initiates H^+ transport. Proteins of the FAR family include putative non transport chaperone proteins from fungi.

TC class 8 includes the bacterial flagellar motor, a mechanically driven active transporter. This complex system normally functions with reverse polarity so that transport of H^+ or Na^+ down its electrochemical gradient drives flagellar rotation. Although few such systems are currently recognized, more are likely to be discovered as novel mechanisms of prokaryotic motility are investigated.

TC class 9 includes a total of 25 currently recognized families of outer membrane porins, of β-structure, mostly found in the outer membranes of gram-negative bacteria. Although many of these are known to be fairly nonspecific with respect to the substrate transported, others exhibit a high degree of specificity. Several are specific for macromolecules, either proteins or complex carbohydrates.

TC class 10 includes a single family of methyl transfer-driven Na^+ pumps. These proteins, exhibiting a unique mode of energy coupling, are found exclusively in methylotrophic archaea.

Finally, TC class 11 includes non-ribosome-synthesized channel- or carrier-forming peptides or peptide-like molecules. The best characterized examples of these toxic substances are the cation-transporting channel-forming gramicidin and the K^+-transporting carrier-forming valinomycin. Many other less well-known substances that belong to TC class 11 have been identified and characterized.

The above-described 11 categories include all the currently recognized, well-characterized

transport systems with established energy coupling mechanisms. However, many supposed transporters are insufficiently well characterized to be included in one of these classes. They are are therefore given a TC classification number of their own. TC 96 includes functionally characterized permeases for which amino acid sequence data are not available, TC 97 includes sequenced, putative transporter families of unknown classification, TC 98 includes auxiliary transport proteins that function in conjunction with recognized transporters, and TC 99 includes established transporter families for which the mode of transport or energy coupling mechanism is unknown.

PERSPECTIVE

The Enzyme Commission (EC) established a system for the classification of enzymes many years ago, but their system did not (and still does not) include most permease proteins that function to facilitate vectorial reactions. The TC system has been designed to correct this deficiency. In November 1999, an International Nomenclature Committee will meet in Geneva, Switzerland, to consider the TC system for adoption. Although modifications and improvements are likely to be incorporated, it seems clear that the TC system will be adopted in some form. It is hoped that this short review provides the reader with sufficient exposure to the TC system to prompt him or her to examine our web sites for more detailed information. In this way, we hope that the maze of transport data, which is expanding in magnitude at a rapid rate due primarily to genome sequencing, can be placed into a rational framework for intellectual conceptualization and evaluation. Thus, I would like to encourage the reader to consult our web sites for further information. These web sites will be continuously updated as more information becomes available and as the system becomes modified. It is hoped that it will be adopted as the standard of transport system classification worldwide.

Together with the SmithKline-Beecham Bioinformatics group under Dr. Andrei Lupas, we are creating a user-friendly search tool for identifying transport proteins. This tool will permit identification of such proteins by gene name, protein name, protein abbreviation, family name, TC, protein sequence, sequence motif, and other details in a tool similar to that provided by BLAST. We hope that this tool will be of general value to the scientific community, particularly for purposes of identifying and annotating new transporter protein sequences as these become available.

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