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REVIEW

Comparative molecular biological analysis of membrane transport genes in organisms

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Abstract Comparative analyses of membrane transport genes revealed many differences in the features of transport homeostasis in eight diverse organisms, ranging from bacteria to animals and plants. In bacteria, membrane-transport systems depend mainly on single genes encoding proteins involved in an ATP-dependent pump and secondary transport proteins that use H^+ as a co-transport molecule. Animals are especially divergent in their channel genes, and plants have larger numbers of P-type ATPase and secondary active transporters than do other organisms. The secondary transporter genes have diverged evolutionarily in both animals and plants for different co-transporter molecules. Animals use Na^+ ions for the formation of concentration gradients across plasma membranes, dependent on secondary active transporters and on membrane voltages that in turn are dependent on ion transport regulation systems. Plants use H^+ ions pooled in vacuoles and the apoplast to transport various substances; these proton gradients are also dependent on secondary active transporters. We also compared the numbers of membrane transporter genes in Arabidopsis and rice. Although many transporter genes are similar in these plants, Arabidopsis has a more diverse array of genes for multi-efflux transport and for response to stress signals, and rice has more secondary transporter genes for carbohydrate and nutrient transport.

Keywords Membrane transporter · Comparative molecular analysis · *Oryza sativa*

Introduction

Cells maintain their biological activities by importing and exporting various substances. Provision of energy and nutrients and efflux of salts, biochemicals, and ions are necessary to maintaining biological activity in prokaryotic and eukaryotic cells. Environmental situations within cells differ among organisms: unicellular organisms cannot control the ion concentrations outside the cell, but multicellular eukaryotes (especially animals) can precisely regulate the ion concentrations of their cellular environments within micromolar ranges. Therefore, we can expect organisms to differ in gene number, structure, and function according to their biological abilities and environmental situations. Recent sequence analyses of entire genomes have made it possible to confirm the existence of homologous genes by computer data analysis. It is also possible to reveal the overall patterns of gene networks. In plants, complete genomic sequences are available for Arabidopsis and rice, but gene annotation programs are not yet sufficiently accurate to determine the function of all genes Instead, full-length cDNA data are useful for precise analysis of genes. Because transport activities are required at distinct levels in most tissues, we expected that the transcripts of most transmembrane transporters would be represented in full-length cDNA libraries from plants at various developmental stages, various plant tissues, and plants exposed to various treatments.

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We searched for orthologs of known membrane transport genes by using the 35,180 full-length rice cDNA sequences (Rice Full-Length cDNA Consortium 2003; Satoh et al. 2007) and genomic sequence data from Arabidopsis (Arabidopsis Genome Initiative 2000) and japonica rice (Goff et al. 2002; International Rice Genome Sequencing Project 2005) and global functional gene annotation in Arabidopsis and rice (Munich Information Center for Protein Sequences (MIPS) data service (http://mips.gsf.de/proj/plant/jsf/) (Schoof et al. 2004; Karlowski et al. 2003)) (RAP-DB = Rice Annotation Project Data Base: http://rapdb.lab.nig.ac.jp/ (Rice Annotation Project 2007, 2008)); the TIGR Rice Genome Annotation: http://www.tigr.org/tdb/e2k1/osa1/index.shtml (Ouyang et al. 2007). Transmembrane proteins have a hydrophobic structure, a pore-forming sequence, and molecule-binding sites. Because of these specific structural features, the identification of membrane transport orthologs is clear from computer calculations. Previous reports have characterized individual transporter protein families but have not extended to whole transport systems in general (Eng et al. 1998; Pao et al. 1999; Mäser et al. 2001; Sánchez-Fernández et al. 2001). In a more general analysis of various organisms, the features of prokaryotes were contrasted with those of eukaryotes (Ren and Paulsen 2005). However, differences among eukaryotes—especially animals and plants—were not a focus of that report. We also searched for orthologs of membrane transport gene in various organisms database (The Human Gene Nomenclature Database Search Engine (http://www.genenames.org/cgi-bin/hgnc_search.pl) (Wain et al. 2004); Genomic comparison of membrane transport systems (TransportDB: http://www.membranetransport.org/index.html) (Ren et al. 2004)), the functional genomics of plant transporters (PlantsT: http://plantst.genomics.purdue.edu/ (Tchieu et al. 2003) and ARAMEMNON (http://aramemnon.botanik.uni-koeln.de/) (Schwacke et al. 2003)). Here, we compare total membrane transport systems from diverse organisms and conclude that membrane transport genes exemplify evolutionary diversity of homeostatic systems. Evolutionary changes in gene families indicate the dynamics of alterations in biological systems and gene networks. Therefore, analysis of large categories of gene families may reveal many basic concepts of biological systems.

General comparisons of membrane transport genes

Membrane transport protein carries various materials for homeostasis. There are many clear features of domains (ex. Transmembrane, pore-forming, ATP binding, molecular capture), and functional features in the transport proteins. According to the structure and its functional systems, the membrane transport proteins have divided to three categories-pump, channel and secondary transporter. The pump system is the slowest ($1–10^3$ molecules/s) but environmental-independent system which consume energy (mainly ATP) for transport. The channel is the most rapid ($10^7–10^8$ molecules/s) and non-energy consuming systems but its need concentration gradients previously (transport directions are only according to the gradients). The secondary transport system adapts co-transport molecules movement energy to carry molecules. Therefore, it needs co-transport molecules and the transport directions have depended on environmental conditions and the speeds ($10^2–10^4$ molecules/s) are the middle of pump and channel. We summarized all three categories (pump, channel and secondary transporter) of genes and compared the total numbers of membrane transport genes in *Escherichia coli*, *Arabidopsis thaliana*, *Oryza sativa*, *Caenorhabditis elegans*, *Drosophila melanogaster*, *Homo sapiens*, *Neurospora crassa*, and *Saccharomyces cerevisiae*. The genome sizes among these organisms were diverse (4.6–3150 megabases), and the numbers of transmembrane genes ranged from 300 to 350 in *E. coli*, fungi, and yeast to about 1000–1200 in Arabidopsis and rice (Table 2). This indicates that a minimum number of about 300 gene species may be required to retain cell homeostasis. The greater numbers of transmembrane transport genes in Arabidopsis and rice may indicate additional redundancy as well as the modification of genes for new roles (e.g. addition of new substances, adaptation of systems for regulating transport, divergence of stage- and tissue-specific material transport), specialization for the various tissues and cells of multicellular organisms, and increased complexity of cells, which in eukaryotes have many additional organelles.

The greater relative increase (plants versus bacterium, fungus, and yeast) in the numbers of membrane transport genes was less than has been reported for other gene categories, such as transcription factor genes and metabolic enzyme genes, in higher eukaryotes (Wray et al. 2003). This may indicate that adaptations in membrane transport are critical for the survival of organisms during evolution. The total numbers of membrane transport genes in higher plants (Arabidopsis, about 1000; rice, 1200; Table 2) are 1.2–2.0 times those in animals (fly, 600; nematode, 650; human, 750; Table 2). These differences in numbers of transporter genes may be related to differences in the need for efflux and influx systems in restricted habitation environments. Because of their immobility and the simplicity of their uptake systems, plant cells have more opportunity to absorb inappropriate substances and greater amounts of substrates and to synthesize larger amounts of secondary products than do animals.

In accordance with their structures and mechanisms of action, transport proteins are classified into three classes:

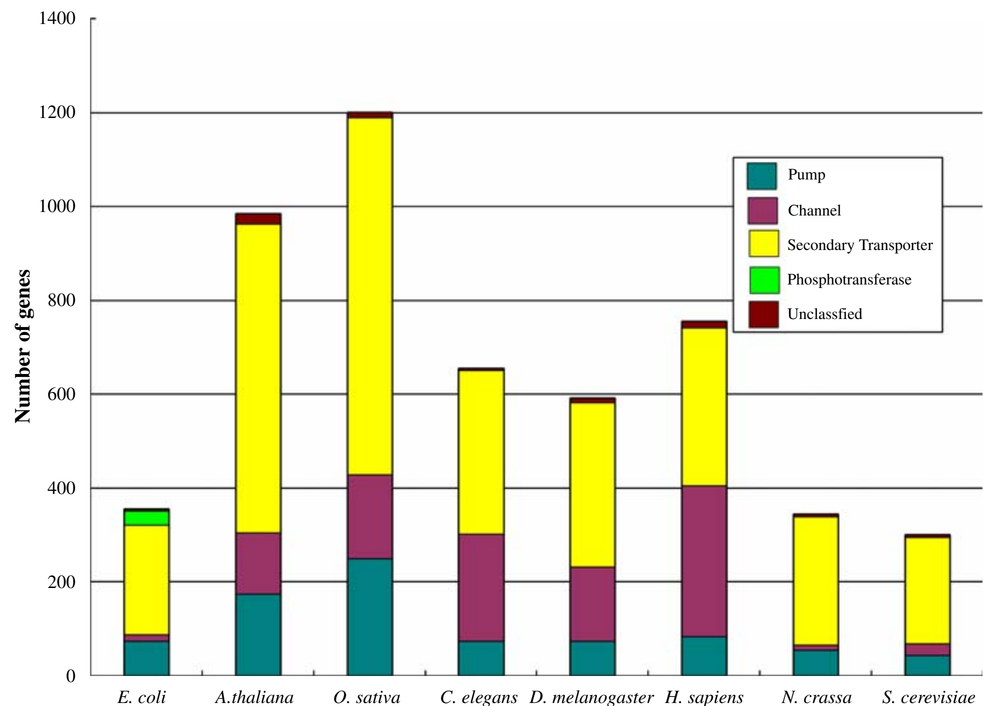

Fig. 1 *Numbers of membrane transporter proteins of each class*. Membrane transporter proteins were categorized into three classes (ATP-dependent [pump], channel, and secondary transporter) and compared among *Escherichia coli* K12-MG1655, *Arabidopsis thaliana*, *Oryza sativa*, *Caenorhabditis elegans*, *Drosophila melanogaster*, *Homo sapiens* NCBI, *Neurospora crassa* 74-OR23-IVA, and *Saccharomyces cerevisiae* S228C

pump, channel, and secondary transporter (transporter). The composition ratios of these classes of protein were also compared (Table 2, Fig. 1). The numbers of pump genes in animals (72–82) were almost the same as in bacteria (70). The numbers of secondary transporter (animals, 350; bacteria, 230) and channel (animals, 160–320; bacteria, 15) genes were increased in animals. In particular, vertebrates (humans) had more (322) channel gene species than plants (130–180). We considered that this gene diversity in the development of channel systems was caused by the acquisition of a nervous system. The electrical transmission systems in nervous systems supplying organs (e.g. muscles, kidneys) need precisely controlled ion concentrations and the ability to make immediate changes in gradients. The development of active transport systems in animals allowed the regulation of rapid movements of the body and organs. Therefore, animals presumably acquired genes for the fastest transport-system channels. Plants also had more channel gene species than bacteria, although fewer than animals. Because plants do not transmit signals for quick movement of their organs, they do not need to regulate membrane voltages as precisely as animals. Additionally, signal-transmitting systems with ligand molecules (e.g. neurotransmitters) are not specific, unlike in animals. Therefore, the numbers of voltage-gated ion channels (VICs) and ligand-dependent channels were smaller in plants than in higher animals (Table 1). On the other hand, higher plants had increased numbers of genes for pumps (170–250) and secondary active transporters (660–760). Plant cells have chloroplasts, which synthesize carbohydrates for many biological activities, including protein synthesis and functioning of the ATP-dependent pumps. Plants presumably use ATP-consuming systems more easily than animals, and the pumps transport the molecules that act as the driving forces of the secondary active transporters. Additionally, plant-specific organelles and vacuoles provide pools of ions and catabolite molecules. Co-transport molecules for secondary transport also are safely and stably stored in the vacuoles. Therefore, plants are presumably able to constantly supply co-transport molecules for secondary active transporters, independently of environmental conditions. The existence of vacuoles gives plant cells more self-sufficiency than animal cells and explains the evolution of membrane transport genes for individual cell homeostasis in plants. Therefore, pump and secondary transporter systems in plants are more divergent than in animals.

Energy (ATP, pyrophosphate)-dependent (pump) system

We compared the ATP-dependent transport genes in this diverse set of organisms (Table 1, Fig. 2, and Supplemental data-2). The main roles of the ATP-dependent (pump) proteins are (1) to transport molecules in specific directions independently of the environmental situation; and (2) to transport ions to form a concentration gradient between the areas outside and inside the membrane (active transport). Because bacteria cannot control the concentrations of ions or metabolites outside the cell, their pumps work mainly to transport molecules. In *E. coli*, most of the ATP-dependent genes (93%) encode ATP-binding cassette (ABC) proteins,

Table 1 Comparative analysis of membrane transporter gene in many organisms

Gene family	*E. coli K12*	*A. thaliana*	*O. sativa*	*C. elegans*	*D. melanogaster*	*H. sapiens*	*N. crassa 74*	*S. cerevisiae*	Material	Present in
Energy-dependent (pump)										
ABC	67	110	153	48	51	47	31	24	Various	All
ArsAB	0	0	1	0	1	1	1	1	Anion	All
F-ATPase (catalytic)	1	5	6	2	1	2	2	2	H^+, Na^+	All
H+-PPase	0	3	9	0	0	0	0	0	H^+	P, a (vacuolar)
IISP	0	3	5	0	1	3	2	9	Protein	All
MPT	0	6	14	1	18	14	8	18	Protein	Eukaryote (mitochondria)
P-ATPase	4	46	57	22	19	32	19	16	H^+, Na^+, Ca^{2+} etc.,	All
Channel										
ACC	0	0	0	0	0	7	0	0	Cation (Ca^{2+})	A (neuron)
Annexin	0	8	10	0	7	13	1	0	Ca^{2+}	Eukaryote
Bcl-2	0	0	0	0	1	12	0	0	Anion	A (mammal)
Bestrophin	0	0	0	21	4	4	0	0	Anion (Cl^-)	A, P, F, B (G^-)
CD20	0	0	0	0	0	9	0	0	Ca^{2+}	A (B-lymphocyte)
ClC	3	7	14	6	3	10	3	1	Cl^-	All
Connexin	0	0	0	0	0	18	0	0	Various	A (vertebrate)
CSC	0	2	1	0	0	0	0	0	Various	P (chloroplast)
CytB	0	19	7	0	10	13	1	9	H^+	All
E-ClC	0	4	4	0	0	4	0	0	Cl^-	A (mammal), P (distant homolog)
ENaC	0	0	0	20	25	8	0	0	Na^+	A (epithelial cell, brain)
GIC	0	19	17	9	27	20	0	0	Metal (Ca^{2+}, K^+), Glutamin	All
Hsp70	0	17	23	0	14	14	4	0	Cation	All
ICC	0	0	0	0	0	1	0	0	Cl^-	A (mammal)
ICln	0	1	2	1	0	1	0	0	Anion (Cl^-)	A, P (distant homolog)
Innexin	0	0	0	22	8	0	0	0	Various	A (invertebrate)
IRK-C	0	0	0	1	3	22	0	0	K^+	A, B
LIC	0	0	0	69	23	45	0	0	Various	A
Mid1	0	0	0	0	0	0	0	1	Cation (Ca^{2+})	Y
MIP	2	38	38	7	7	11	1	4	H_2O, CO_2, NH_3	All
MIT	2	0	0	0	0	0	2	3	Metal (Mg^{2+}, Co^{2+}, Ni^{2+})	Y, a, B
MscL	1	0	0	0	0	0	0	0	Various	B
MscS	6	8	7	0	0	0	0	0	Various	P, B
NSCC2	0	1	1	1	1	1	0	1	Cation	A, Y, F
O-ClC	0	0	0	0	1	6	0	0	Cl^-	A
PCC	0	0	0	0	5	6	0	0	Cation (Na^+, K^+ , Ca^{2+})	A
PLM	0	0	0	0	0	7	0	0	Anion	A (mammal)
RIR-CaC	0	0	0	5	3	6	0	0	Ca^{2+}	A
Tic110	0	1	2	0	0	0	0	0	Various	P
TRP-CC	0	0	0	5	7	23	0	1	Ca^{2+}	A, Y,
UT	0	0	0	0	0	2	0	0	Urea	A (vertebrate), B
VIC	1	35	18	63	31	90	2	2	K^+, Na^+ or Ca^{2+}	All

Table 1 continued

Gene family	*E. coli K12*	*A. thaliana*	*O. sativa*	*C. elegans*	*D. melanogaster*	*H. sapiens*	*N. crassa 74*	*S. cerevisiae*	Material	Present in
Phosphotransferase System (PTS)										
GPTS	6	0	0	0	0	0	0	0	Carbohydrate	B
SSPTS	23	0	0	0	0	0	0	0	Carbohydrate	B
Secondary Transporter										
AAA	0	2	3	0	0	0	0	0	ATP	Plant (chloroplast)
AAAP	0	43	77	11	15	13	4	7	Amino acid, Auxin	A, P, F, Y
AAE	1	0	0	0	0	0	0	0	Aspartate, Alanine	B
AbgT	1	0	0	0	0	0	0	0	p-Aminobenzoyl-glutamate	B
ACR3	0	0	0	0	0	0	1	1	Arsenite, Antimonite	Y, B
AE	0	7	3	4	2	10	2	1	Na^+, HCO_3^- , H^+, Cl^- , H_3BO_3 (P, Y)	A, P, Y
AEC	1	8	8	0	0	0	0	0	Auxin	P, Y, B
AGCS	1	0	0	0	0	0	0	0	Alanine, Glycine	B, a
Amt	1	6	13	6	2	4	4	3	NH_3, CO_2	All
APC	22	12	14	11	11	14	15	24	Amino acid, Polyamine	All
ArsB	2	0	0	0	5	1	0	0	Arsenite, Antimonite	All (with distant homolog)
BASS	1	5	6	0	2	5	0	1	Organic acid	All
BCCT	3	0	0	0	0	0	0	0	Betaine/Carnitine/Choline	B, a
BenE	1	0	0	0	0	0	0	0	Benzoate	B
CaCA	2	12	23	8	11	8	8	4	Ca^{2+}	All
CCC	0	1	2	6	5	9	1	1	Na^+, K^+, Cl^-	All
CDF	2	8	11	8	7	10	8	5	Zn^{2+} or Cd^{2+} etc.,	All
CHR	0	0	0	0	0	0	1	0	SO_4^{2-} , CrO_4^{2-}	F, B
CNT	3	0	0	2	2	3	1	0	Nucleoside	A, Y, B
CPA1	2	8	7	11	5	3	3	2	Na^+, H^+	All
CPA2	3	32	16	0	0	1	2	1	Na^+, K^+	All
DAACS	3	0	0	6	2	7	0	0	Dicarboxylate, Amino acid	A, B
DASS	5	4	5	4	3	5	0	3	Various	A, P, Y, B
Dcu	2	0	0	0	0	0	0	0	C4-Dicarboxylate	B (G^-)
DcuC	2	0	0	0	0	0	0	0	C4-Dicarboxylate	B (G^-)
DMT	16	121	57	15	14	18	6	9	Various	All
ENT	0	8	4	5	3	4	1	1	Nucleoside	A, P, Y
ESS	1	0	0	0	0	0	0	0	Glutamate	B
FBT	0	9	8	0	0	0	0	0	Folate, Biopterin	P, B, a
FNT	4	0	0	0	0	0	1	1	Formate, Nitrate	Y, B, a
GntP	7	0	0	0	0	0	0	0	Carbohydrate	B (E. coli, Bacillus)
GPH	6	9	8	1	1	5	2	0	Sugar	A, P, F, a, B
GUP	0	0	1	0	0	0	1	2	Glycerol	A, P, Y, F, B
HAAAP	8	1	0	0	0	0	0	0	Amino acid	B, P (distant homolog)
KDGT	1	0	0	0	0	0	0	0	2-Keto-3-Deoxygluconate	B
KUP	1	13	21	0	0	0	1	0	K^+	P, F, B
LCT	0	0	0	0	2	2	1	1	Cystine	A, P, F

Table 1 continued

Gene family	*E. coli K12*	*A. thaliana*	*O. sativa*	*C. elegans*	*D. melanogaster*	*H. sapiens*	*N. crassa 74*	*S. cerevisiae*	Material	Present in
LctP	2	0	0	0	0	0	0	0	Lactate, Glycolate	B, a
LIV-E	1	0	0	0	0	0	0	0	Amino acid (L,I,V)	B, a
LIVCS	1	0	0	0	0	0	0	0	Amino acid (L,I,V)	B
LysE	1	0	0	0	0	0	0	0	Lysine	B
MC	0	52	66	34	45	44	34	34	Various	Eukaryote
MET	0	0	0	0	0	3	0	0	Nucleoside, etc.	A
MFS	70	90	145	137	144	82	141	85	Various	All
MOP	8	56	48	0	0	2	1	3	Various (Drugs etc.,)	All
MTC	0	2	2	6	2	3	1	1	Various (Anionic substrate)	Eukaryote
NCS1	2	1	0	0	0	0	3	10	Nucleobase, Thiamine	P, F, Y, B, a
NCS2	11	12	11	5	1	4	1	0	Nucleobase,etc.	All
NhaA	1	0	0	0	0	0	0	0	Na^+,H^+	Prokaryote
NhaB	1	0	0	0	0	0	0	0	Na^+, H^+	All
NhaD	0	2	1	0	0	0	0	0	$Na^+(Li^+)$, H^+	P, B
NiCoT	0	0	0	0	0	0	1	0	Ni^{2+}, Co^{2+}	All
Nramp	1	7	10	2	1	2	2	3	Metals (Fe^{2+}, Zn^{2+} etc.,)	All
NSS	0	0	0	12	21	18	0	0	Neurotransmitters etc.,	A
OAT	0	2	2	3	8	11	0	0	Various	A, P, F, Y
OPT	0	15	34	0	0	0	4	3	Oligopeptide	P, B, a
OST	0	0	0	0	0	2	0	0	Organic compound	Eukaryote
Oxa1	1	4	5	0	1	1	0	1	Protein	All (with distant homolog)
PiT	2	1	3	5	1	2	1	1	HPO_4^{2-}, SO_4^{2-}	All
PnaS	1	0	0	1	0	2	0	0	Inorganic phosphate	A (mammal)
POT	4	50	61	3	3	4	2	1	Oligopeptide	All
RFC	0	0	0	3	3	4	0	0	Folate, Tiamine	A
RhtB	5	0	0	0	0	0	0	0	Amino acid	B, a
RND	8	2	1	24	4	7	2	1	Various	All
SSS	4	1	1	3	19	11	2	1	Various	All
SulP	1	11	15	7	9	11	4	4	SO_4^{2-}	All
Tat	1	0	1	0	0	0	0	0	Protein	B (G^-)
TDT	1	4	9	0	0	0	2	1	Tellurite, C4-Dicarboxylate	All (with distant homolog)
ThrE	1	0	0	0	0	0	0	2	Threonine, Serine	All (with distant homolog)
TRAP-T	1	0	0	0	0	0	0	0	Various	B, a
Trk	2	1	1	0	0	0	2	2	K^+	P, Y, B
ZIP	0	13	18	6	5	2	5	3	Zn^{2+}, Fe^{2+}	All
Unclassified										
Ctr1	0	0	0	0	0	0	0	1	Dipicolinic Acid	Y, B
Ctr2	0	5	7	4	3	2	2	2	Cu^{2+}	All
FeoB	1	0	0	0	0	0	0	0	Fe^{2+}	B, a
FeT	0	0	0	0	0	0	0	1	Fe^{2+}, (Co^{2+}, Cd^{2+})	Y
FP	0	0	0	0	0	1	0	0	Fe^{2+}	A (mammal)

Table 1 continued

Gene family	*E. coli K12*	*A. thaliana*	*O. sativa*	*C. elegans*	*D. melanogaster*	*H. sapiens*	*N. crassa 74*	*S. cerevisiae*	Material	Present in
LPI	0	0	0	0	0	5	0	0	Protein	A
OFeT	1	0	0	0	0	0	1	2	Fe^{2+},Fe^{3+}	Y, B
PnuC	1	0	0	0	0	0	0	0	Nicotinamide mononucleotide	B
PPI	0	0	0	0	6	6	3	0	Protein	A, F, B
PUP	1	15	4	0	0	0	0	0	Peptide, Fatty acid	P, B

Note: Present in: A = animal, P = plant, F = fungi, Y = yeast, B = bacteria, a = archea, G^- = gramm minus bacteria

and some of them are reported to encode channel proteins (Fig. 2, Table 1) (Holland et al. 2005). The ABC proteins transport various substances (e.g. ions, peptides, nucleosides, amino acids, carbohydrates, proteins) ATP-dependently in all organisms (Kolukisaoglu et al. 2002; Garcia et al. 2004). In eukaryotes, many functional units are present within one polypeptide, whereas many bacterial ABC subunits are encoded by individual genes. This results in an inverted relationship between prokaryotes (*E. coli*) (72) and primitive eukaryotes (yeast) (43). Many bacterial ABC proteins are located in the plasma membrane and serve as the main forces in energy-consuming transport for both import and export of substances. In higher eukaryotes, ABC proteins tend to export substances rather than function in import reactions.

Structural analyses revealed that ABC proteins could be classified as half size (homo- or heterodimer functional) or full size (monomer functional) in accordance with their construction. The number of genes encoding ABC proteins in animals is the same as, or slightly less than, in bacteria, most of them representing whole subunits of ABC proteins. In contrast, the number of ABC genes in plants is about twice that in animals and bacteria, primarily due to the encoding of half-size proteins (Table 1, Fig. 2, and Supplemental data-2) (Sanchez-Fernandez et al. 2001; Sugiyama et al. 2006). This increase in numbers of genes encoding half-size (e.g., WBC, ABC2) and full-size (PDR; pleiotropic drug resistance) proteins supposedly permits stage- and tissue-specific regulation of various substances (including plant-specific substances). Therefore, increases in the capacity of import and export transport systems by an increase in the number of half-size ABC proteins have allowed plants to fulfill their unique roles.

In the import and efflux of ions and the creation of ion gradients for secondary transport, P-type ATPases transport many species of ion (e.g. H^+, Na^+, K^+, Ca^{2+}) in both directions in the cell (Table 1, Fig. 2, and Supplementary data-2) (Axelsen and Palmgren 2001; Baxter et al. 2003). Control of ion concentrations outside bacterial cells is not possible. Only a few of the bacterial genes involved in systems for the uptake or efflux of ions (uptake: K^+, Mg^{2+}; efflux: Ca^{2+}, Ag^+, Zn^{2+}, Co^{2+}, Pb^{2+}, Ni^{2+}, Cd^{2+}; uptake or efflux, depending on the system: Cu^{2+}) have been characterized, and each of the enzymes encoded comprises a distinct subfamily (Banci et al. 2006).

Eukaryotes have diverged in terms of their transport substances and have also adapted genes for making the ion gradients for secondary transport. One of the most important ATPases in animals—Na^+/K^+ATPase—does not exist in plants. Na^+/K^+ATPase makes a Na^+ ion gradient across the plasma membrane and forms the basis of membrane voltage and secondary active transport in animals. On the other hand, plants use H^+ gradients for secondary transport, and more than 10 isoforms and vacuole-type H^+ATPases are involved in stage- and tissue-specific control. This difference in adapted ion gradients reflects the ion concentrations in the cells and the demand for nutrient ions. A constant supply of Na^+ ions is required to retain homeostasis of animal cells and rapid signal transmission systems (e.g. muscles, nerves). In contrast, plants use the more abundant H^+ ion to make cation gradients and have special systems for transporting H^+ ions into vacuolar pools. For transporting H^+ into vacuoles, higher plants have a vacuole-type ATPase and an H^+-translocating pyrophosphatase (H^+-PPase) (Table 1, Fig. 2, and Supplementary data-2) that are comparable to bacterial ATPase and PPase, respectively (Sivula et al. 1999; Sze et al. 2002). Therefore, plants presumably adapted bacterial H^+-ATPase systems to help in H^+ ion concentration steps, whereas animals developed completely new systems to make Na^+ ion gradients.

Ion channel systems

Ion channels are the “gates” in the membranes that open or close in response to signals such as mechanical or electrical stimulation and ligand binding. Therefore, ion channels are closely involved in determining whether or not ionic gradients are available. Multicellular organisms can control the ion concentrations in the tissues on both sides of the cell membrane, whereas monocellular organisms usually find it hard to control the ion gradient outside the cell. Thus, the use of ion channels becomes restrictive and unidirectional in these more primitive organisms. Unlike in

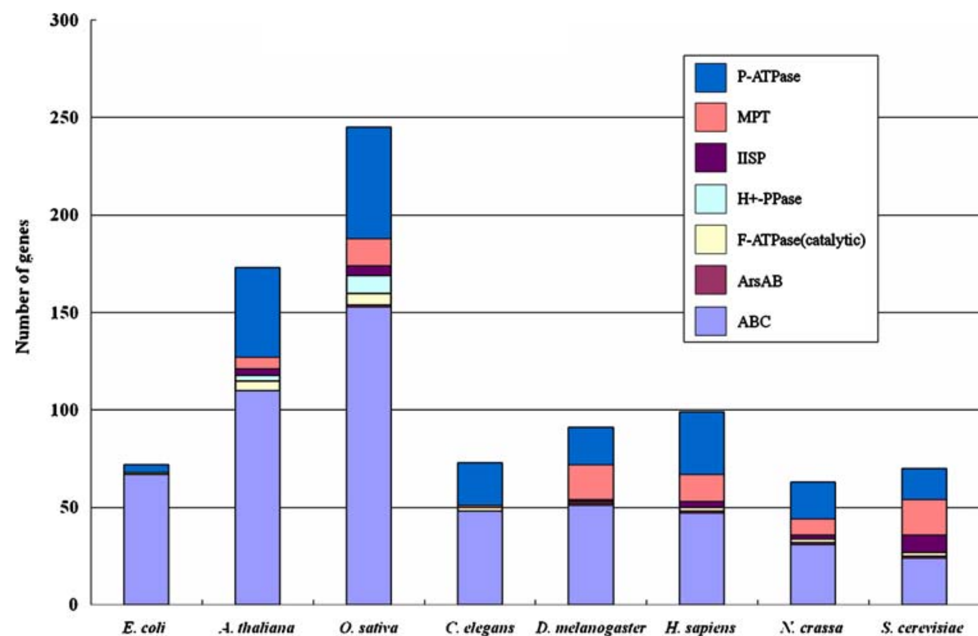

Fig. 2 *Comparison of numbers of pump genes among various organisms*. Pump gene numbers were compared among *Escherichia coli* K12-MG1655, *Arabidopsis thaliana*, *Oryza sativa*, *Caenorhabditis elegans*, *Drosophila melanogaster*, *Homo sapiens* NCBI, *Neurospora crassa* 74-OR23-IVA, and *Saccharomyces cerevisiae* S228C. ABC: ATP-binding Cassette; ArsAB: Arsenite-antimonite Efflux; F-ATPase: H^+ or Na^+-translocating F-type, V-type and A-type ATPase; H^+-Ppase: H^+-translocating Pyrophosphatase; IISP: General Secretory Pathway (Sec); MPT: Mitochondrial Protein Translocase; P-ATPase: P-type ATPase

eukaryotes, the channel system in prokaryotes is not well adapted to transport (Table 1, Fig. 3, and Supplemental data-3). Of the total number of genes encoding membrane transport proteins in prokaryotes, fewer than 5% are channel genes, and they mainly regulate osmotic homeostasis in the cell. Ions (e.g. Cl^-, K^+, metal), water, and osmolytes are imported or exported by the channels in their restricted role. In contrast, animals can make various ion gradients precisely and, in particular, can develop channel systems very well.

Compared with other systems, the channel system is the fastest at transporting molecules without consuming energy (i.e. when it is not necessary to transport against electrical gradients). Therefore, animals (especially vertebrates) have adapted these systems to nerve and muscle signal transmission. Nervous-system-specific channels such as neurotransmitter-responsible channels (connexin, ENaC, innexin, LIC, RIR-CaC, TRP-CC) are found only in animals. The numbers of channels that are membrane-voltage-dependent for Ca^{2+}, K^+, and Na^+ ion transport are dramatically increased in both vertebrates and invertebrates (Table 1, Fig. 3; Sheng et al. 2000; Du et al. 2002; Clapham 2003; Hua et al. 2003; Miyazawa et al. 2003). Because animals form Na^+ and K^+ gradients with the Na^+/K^+ ATPase pump system, there is also an increase in the numbers of species of Na^+ and K^+ ion channels in animals. Thus, the total number of channel genes in humans (320) is about twice that in plants (130–180) (Table 2, Fig. 3). This difference is supposedly related to the ecological and physiological specificity of plants. Sometimes constant acquisition of unevenly distributed resources is difficult for plants. Additionally, the ion concentrations in the vascular systems and intercellular spaces of plants, unlike those in animals, are difficult to control precisely. Therefore, some of the ion channels and membrane-voltage-dependent channels are not as divergent as in animals. Although plants lack neurotransmission systems and have fewer membrane-voltage-dependent systems that use Na^+ and K^+ ion channels, the channel genes for ion homeostasis and signal transduction (e.g. ClC, GIC) are as well developed as in animals, and the numbers of some channel genes (*CytB* and *MscS*) are in fact specifically increased in plants (Table 1, Fig. 3).

Comparison of protein structures indicates that the fundamental structures of ion channels (numbers of transmembrane domains, pore-forming helices) are common in many organisms, but local similarities in individual regions among organisms are low (especially in the N and C terminus fragments). Some of the genes (e.g. for shaker-type K^+ channel, SKOR-type K^+ channel) have low levels of similarity among the whole structures of plants and animals (Supplemental data-3).

In contrast, the major intrinsic protein (MIP) gene family, which encodes water-transport proteins, is specifically well developed in plants (Zardoya 2005). The numbers of these genes in tissues and at different stages are 3–5 times those in animals (38 vs. 7–11) (Table 1, Fig. 3) (Quigley et al. 2002; Sakurai et al. 2005). MIPs are abundant in the plasma membrane (15–20% of total membrane protein) and vacuoles (30–50% of total membrane protein) of plants; therefore, a high level of water transport is carried out at plant cell membranes. The importance of the acquisition of water has presumably resulted in the diversification and development of water channels in plants. Plants also use water pressure for regulation of movement (e.g. stomatal opening, leaf and petal

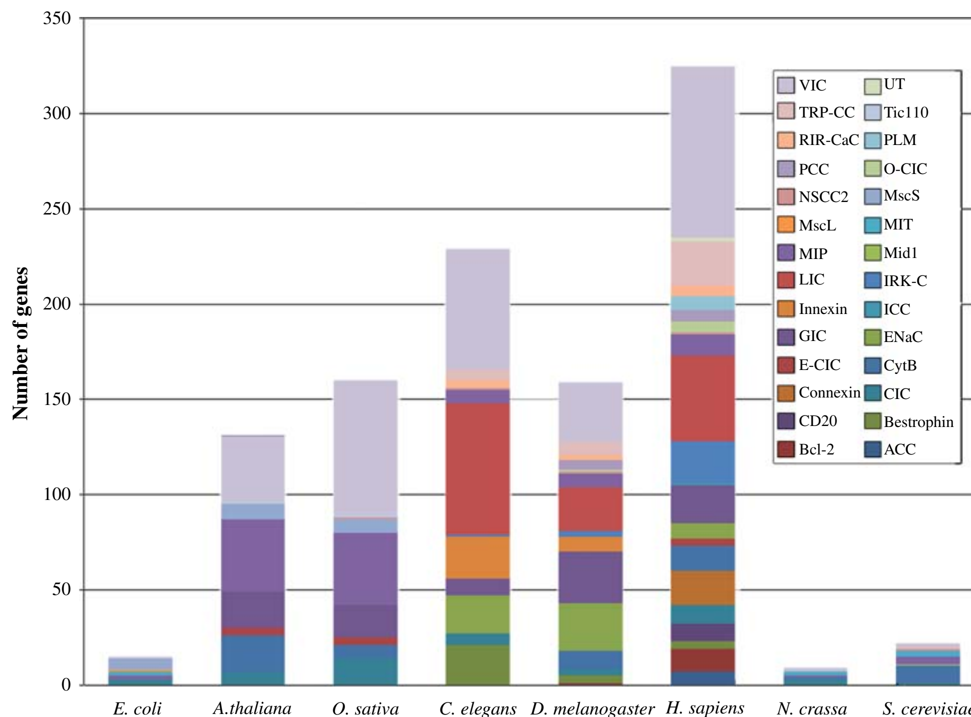

Fig. 3 *Comparison of numbers of channel genes among various organisms*. Channel gene numbers were compared among many organisms (*E. coli* K12-MG1655, *A. thaliana*, *O. sativa*, *C. elegans*, *D. melanogaster*, *H. sapiens* NCBI, *N. crassa* 74-OR23-IVA, and *S. cerevisiae* S228C). ACC: ATP-gated Cation Channel; Bcl-2: Bcl-2; Bestrophin: Anion Channel-forming Bestrophin; CD20: CD20 $Ca2^+$ Channel; ClC: Chloride Channel; Connexin: Gap Junction-forming Connexin; CytB: gp91phox Phagocyte NADPH Oxidase-associated Cytochrome b558 (CytB) H^+-channel; E-ClC: Epithelial Chloride Channel; EnaC: Epithelial Na^+ Channel; GIC: Glutamate-gated Ion Channel; Hsp70: Cation Channel-forming Heat Shock Protein-70; ICC: Intracellular Chloride Channel; Icln: Nucleotide-sensitive Anion-selective Channel; Innexin: Gap Junction-forming Innexin; IRK-C: Inward Rectifier K^+ Channel; LIC: Ligand-gated Ion Channel of Neurotransmitter Receptors; Mid1: Yeast Stretch-Activated, Cation-Selective Ca^{2+} Channel Mid1; MIP: Major Intrinsic Protein; MIT: CorA Metal Ion Transporter; MscL: Large Conductance Mechanosensitive Ion Channel; MscS: Small Conductance Mechanosensitive Ion Channel; NSCC2: Non-selective Cation Channel-2; O-ClC: Organellar Chloride Channel; PCC: Polycystin Cation Channel; PLM: Phospholemman; RIR-CaC; Ryanodine-Inositol 1,4,5-triphosphate Receptor Ca^{2+} Channel; Tic110: Chloroplast Envelope Anion Channel-forming Tic110; TRP-CC: Transient Receptor Potential Ca^{2+} Channel; UT: Urea Transporter; VIC: Voltage-gated Ion Channel

angle changes) and for transmitting signals in various homeostatic functions. Therefore, plants have presumably developed signal transduction systems that rely on water molecules instead of the neurotransmitters and membrane voltage changes used in animals.

Structural analyses indicate divergence in the level of conservation of MIP subfamilies (PIP, TIP, SIP, NIP, AQP, and GLP) (Supplemental data-3E). The plasma-membrane-intrinsic proteins (PIPs) are well conserved among organisms, but animal- (*AQP*) and plant-specific (*SIP*, *NIP*, *TIP*) genes have diverged, and closely related channel proteins have low levels of similarity within each organism (Supplementary data-3E). This also suggests a specific diversification of the water transport genes in plants. The structures of the channels in plants are simple compared with those in animals (Supplementary data-3). A decrease in the membrane spanning times and in the number of subunits of working systems can be detected in many channels (e.g. VIC [VDCC], CIC) (Supplementary data-3). Therefore, animals have developed channel systems especially for the exquisite control of ions, membrane voltages, and the signal transduction pathways of specific ligands (e.g. neurotransmitters).

Phosphotransferase system (bacteria)

Bacteria have specific membrane transport systems (phosphotransferase systems) for carbohydrates (sugar) and phosphates (Tables 1, 2) (Barabote and Saier 2005). These systems use phosphate phosphoenol pyruvate (PEP) as the energy source for phosphorylation and for transport of carbohydrates with the aid of enzyme complexes. Carbohydrates (e.g. glucose, fructose, mannitol, sorbitol) are transported against a concentration gradient, with concomitant phosphorylation. PEP is transferred via the soluble (and non-sugar-specific) enzymes EI and HPr to the enzyme complex EII. EII is made up of components A, B, and C, which, depending on the sugar specificity and bacterium involved, may be the domains of composite

Table 2 Comparison of the genome size, total and membrane transport gene numbers in many organisms

	E. coli K12	*A. thaliana*	*O. sativa*	*C. elegans*	*D. melanogaster*	*H. sapiens*	*N. crassa 74*	*S. cerevisiae*
Genome Size (Mb)	4.6	125	430	97	120	3150	40	13
Total gene number	4,290	26,000	37,000	20,621	13,489	30,000	10,082	5,804
Total Transporter Proteins	354	984	1200	654	590	754	344	300
Transporters per Mb genome	76.74	7.82	2.84	6.75	4.3	0.24	8.63	25.38
Transporters per whole gene	0.082	0.038	0.033	0.033	0.044	0.025	0.034	0.052
ATP-dependent pumps	72 (20%)	173 (18%)	249 (21%)	72 (11%)	72 (12%)	82 (11%)	53 (15%)	43 (14%)
Ion Channels	15 (4%)	131 (13%)	178 (15%)	229 (35%)	158 (27%)	322 (43%)	12 (3%)	24 (8%)
Phosphotransferase Systems (PTS)	30 (8%)	0	0	0	0	0	0	0
Secondary Transporters	233 (66%)	658 (67%)	762 (63%)	349 (53%)	351 (59%)	336 (44%)	273 (79%)	227 (75%)
Unclassified	3 (1%)	22 (2%)	11 (1%)	4 (1%)	9 (1%)	14 (2%)	6 (2%)	6 (2%)

proteins; component/domain C is a permease and is anchored to the cytoplasmic membrane. Because the amount of phosphorylation of the enzymes influences other regulatory mechanisms in the cells (e.g. catabolite repression, chemotaxis), the whole cell needs a multiple-component, complex enzymatic control system. Therefore, the larger and more complicated cells of animals and plants have not adapted this system and instead use ATP-dependent and secondary transporter systems to transport carbohydrates.

Secondary transporter (transporter) system

The secondary transporter system works via the concentration gradient of co-transporter molecules. It is efficient to use a few abundant molecules as common co-transporter molecules for various transport substrates. Therefore, in accordance with the species of ion for which there is a gradient between the inside and outside of the cell, major co-transporter molecules were selected and secondary transporter systems developed for them. There are more than 100 species of gene families in the secondary transporter systems of all organisms, and various substances are transported. Compared with those in the pump and channel systems, the genes involved in the secondary transporter system are most divergent in plants and bacteria; in animals there are about 350 of these genes (44–59% of the total) (Tables 1, 2, Fig. 4).

Organism-specific gene families in bacteria include those encoding proteins (e.g. APC, HAAAP) that transport many species of amino acid or carbohydrate (GntP, Dcu), or other substances (BCCT, BenE) (Neidle et al. 1991; Golby et al. 1998; Saier et al. 1999; Jack et al. 2000; Samsonov et al. 2002; Prakash et al. 2003). Gene families in animals include genes for specific transport proteins (NSS, MET) that carry neurotransmitters and hydrophobic compounds (Beckman and Quick 1998; Hogue et al. 1999; Yamashita et al. 2005). Those in plants include genes with dramatically increased expression, encoding proteins (AAAP, CPA2, DMT, MFS, OPT, POT) that transport such substances as amino acids, cations, and carbohydrates (Fig. 4) (Fischer et al. 1998, 2002; Pao et al. 1999; Saier et al. 1999; Jack et al. 2001; Koh et al. 2002; Wipf et al. 2002). The greater number of secondary active transporters in plants is supposedly due to the existence of vacuoles. Secondary active transporters need an ion gradient across the membranes, and vacuoles can pool many substances at high concentrations and supply co-transporter molecules instantly at any time. Therefore, the secondary transporter systems easily control transport and have diverged more in plants than in animals.

The major facilitator superfamily (MFS) is the largest secondary transporter family in whole organisms; its members transport various substances (e.g. carbohydrates, phosphates, amino acids, cations) with Na^+ or H^+ ions (Pao et al. 1999; Burckhardt and Wolff 2000; Lemoine 2000). There are many modifications of these superfamily genes among organisms. Bacteria have amino acid and drug efflux families, animals have neurotransmitter transport families, and plants have diverse carbohydrate-transport genes (Tables 1, 2, Fig. 4, and Supplementary data-4). The proteins have structures typical of secondary active transporters: 10–14 transmembrane domains, co-transporter molecules, and material-binding domains. Comparative analyses indicate that their fundamental structure—membrane-spanning, pore-forming, and substrate-binding sites—is conserved, but similarities between individual domains are generally low among organisms (Tables 1, 2, Fig. 4 and Supplementary data-4). The transporter proteins are encoded by single transcription units and work as monomers. Therefore, this transport system is easy to adapt to new purposes than are the higher pump and ion channel systems. Although the secondary transporter system can adapt to many kinds of concentration gradients, generally few ion species are used. Most of the secondary active transporters depend on two ions: Na^+ and H^+. In the whole organism, more than 80% of the genes depend on these ions as co-transporter molecules (Table 3, Fig. 5). However, in many cases each ion can be substituted: animals use Na^+ ions as co-transporter molecules and bacteria and plants use H^+ ions. This has caused animals to make a Na^+ ion gradient with Na^+/K^+ ATPase for membrane voltage and plants and bacteria to make H^+ ion gradients with

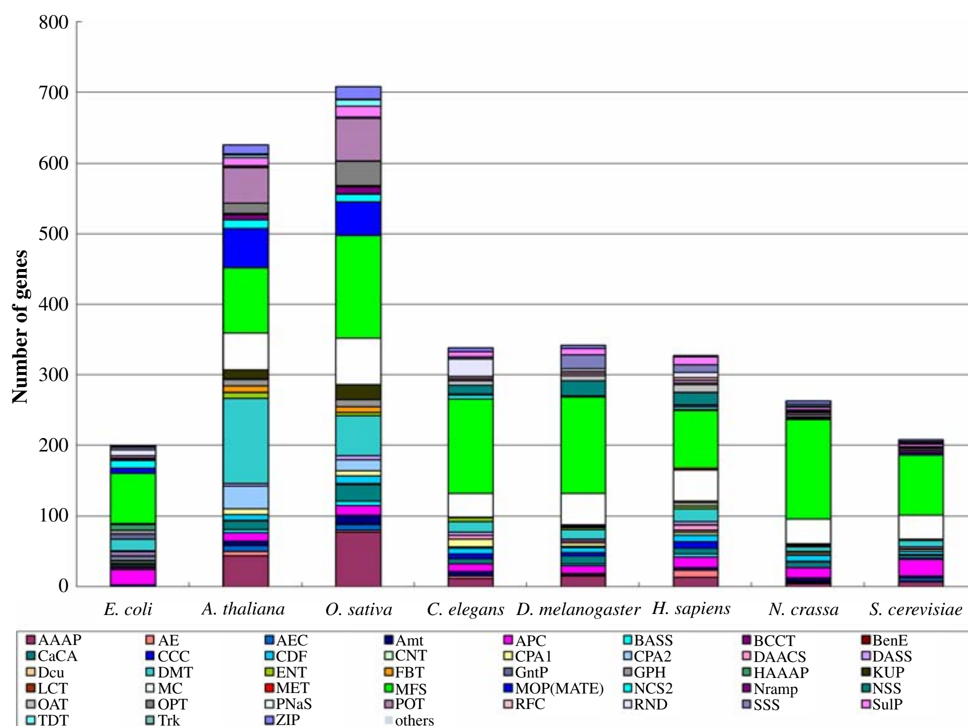

Fig. 4 *Comparison of numbers of secondary transport genes among organisms*. Secondary transporter gene numbers were compared among *Escherichia coli* K12-MG1655, *Arabidopsis thaliana*, *Oryza sativa*, *Caenorhabditis elegans*, *Drosophila melanogaster*, *Homo sapiens* NCBI, *Neurospora crassa* 74-OR23-IVA, and *Saccharomyces cerevisiae* S228C. AAAP: Amino Acid/Auxin Permease; AE: Anion Exchanger; AEC: Auxin Efflux Carrier; Amt: Ammonium or Ammonia Transporter; APC: Amino Acid-Polyamine-Organocation; BASS: Bile Acid:Na^+ Symporter; BCCT: Betaine/Carnitine/Choline Transporter; BenE: Benzoate:H^+ Symporter; CaCA: Ca^{2+}:Cation Antiporter; CCC: Cation-Chloride Cotransporter; CDF: Cation Diffusion Facilitator; CNT: Concentrative Nucleoside Transporter; CPA1: Monovalent Cation:Proton Antiporter-1; CPA2: Monovalent Cation:Proton Antiporter-2; DAACS: Dicarboxylate/Amino Acid:Cation (Na^+ or H^+) Symporter; DASS: Divalent Anion:Na^+ Symporter; DcuC: C4-dicarboxylate Uptake C; DMT: Drug/Metabolite Transporter; ENT: Equilibrative Nucleoside Transporter; FBT: Folate-Biopterin Transporter; GntP: Gluconate:H^+ Symporter; GPH: Glycoside-Pentoside-Hexuronide (GPH):Cation Symporter; HAAAP: Hydroxy/Aromatic Amino Acid Permease; KUP: K^+ Uptake Permease; LCT: Lysosomal Cystine Transporter; MC: Mitochondrial Carrier; MET: 4 TMS Multidrug Endosomal Transporter; MFS: Major Facilitator Superfamily; MOP: Multidrug/Oligosaccharidyl-lipid/Polysaccharide Flippase Superfamily; NCS2: Nucleobase:Cation Symporter-2; Nramp: Metal Ion (Mn^{2+}–iron) Transporter; NSS: Neurotransmitter:Sodium Symporter; OAT: Organo Anion Transporter; OPT: Oligopeptide Transporter; PnaS: Phosphate:Na^+ Symporter; POT: Proton-dependent Oligopeptide Transporter; RFC: Reduced Folate Carrier; RhtB: Resistance to Homoserine/Threonine; RND: Resistance-Nodulation-Cell Division; SSS: Solute:Sodium Symporter; SulP: Sulfate Permease; TDT: Tellurite-resistance/Dicarboxylate Transporter; Trk: K^+ Transporter; ZIP: Zinc (Zn^{2+})–Iron (Fe^{2+}) Permease

P-type ATPase and H^+-PPase, respectively. The transporter genes of animals and plants have specifically adapted to Na^+ and H^+ co-transporter systems (Table 3, Fig. 5). Therefore, both animals and plants have developed transporter genes adapted to ion gradients.

In animals, many molecules are transported with different co-transporter molecules. Examples are (1) molecules (e.g. neurotransmitters, organic anions, phosphates) transported with Na^+ ion (NSS, BASS, CPA2, Pnas) (Beckman and Quick 1998; Saier et al. 1999; Kramer et al. 2001; Segawa et al. 2002; Radchenko et al. 2006); (2) molecules (e.g. amino acids, nucleosides) transported with H^+ ions (e.g. AAAP, APC, DMT, LCT, MET) (Steiner et al. 1994; Jack et al. 2001; Zhai et al. 2001; Gasol et al. 2004); (3) molecules (e.g. anions, cations, sugars, nucleosides) transported with either Na^+ or H^+ ions (e.g. AE, CaCA, DAACS, GPH, CNT) (Reinders and Ward 2001; Ritzel et al. 2001; Zhu et al. 2003; Cai and Lytton 2004; Ryan et al. 2004); and (4) others (e.g. CCC, OAT, RFC) transported with other molecules (Russell 2000; Flintoff et al. 2003; Hagenbuch and Meier 2003).

In contrast, in plants, almost all substances (K^+: KUP; N (NH_4^+): Amt; phosphate: PiT, Pht [MFS], and TPT [DMT]; SO_4^{2-}: SulP; sucrose: GPH; sugar alcohol: SAT [MFS]; monosaccharide: MST [MFS]; amino acid: APC and AAAP; nucleosides: NCS1), including many cations, anions, metals, and drugs, are co-transported with H^+ ions. Only BASS (organic acid transporter) and NhaD (Na^+: H^+ antiporter = Na^+: efflux system of vacuole) use Na^+ ions as co-transporter molecules for efflux of ions from the cytoplasm, so plants do not require extensive sodium uptake (Table 3, Fig. 5) (Smith et al. 1995; Naderi and Saier 1996; Fu and Luan 1998; Nozaki et al. 1998; Pao et al. 1999; Daram et al. 1999; de Koning and Diallinas 2000; Jack et al. 2001; Khademi et al. 2004). Accumulation of H^+ ions makes solutions acidic and prevents many enzymatic and biochemical reactions, so plants pool H^+ ions in vacuoles inside the cytoplasm, thereby establishing a stable ion gradient for transport energy. Plants make H^+ gradients not only inside cells, but also outside cells by the H^+ pump, and they absorb many substances by secondary transporter systems. This H^+-adapting system presumably has led to diversification of H^+ co-transport in secondary transporter systems in plants.

Comparative analysis of membrane transporter systems in Arabidopsis and rice

We also compared the numbers of membrane transporter genes in Arabidopsis and rice. The rice genome size (430 Mb) is more than three times that of Arabidopsis (125 Mb), but the total number of membrane transporter proteins (1200) is only 1.20 times that in Arabidopsis (1000) (Table 2). The proportions of pump, channel, and secondary transporter proteins are almost the same, and the numbers of genes in many individual gene families are 1.1–1.3 times those in rice. However, Arabidopsis has more multi-efflux proteins and proteins involved in secondary active transport (CPA2, DMT, MOP, NCS2) and in channel-type signal transduction systems (CytB, GIC) (Fig. 6). Arabidopsis is a wild plant that lives in diverse soil conditions and under environmental stress, whereas rice is a cultivated crop plant grown under more stable environmental conditions. Therefore, Arabidopsis has diverged more than the rice cultivar Nipponbare to form transporter systems involved in multi-efflux and stress response signaling. On the other hand, rice has more pump and secondary transporter genes (ABC, P-type ATPase, MFS, POT) for carbohydrate and nutrient transport systems (Fig. 6). This divergence of carbohydrate transporters might have been influenced by artificial selection, during which individuals with larger seed size and numbers were chosen to be cultivated. Additionally, amino acid (AAAP), ammonia (Amt), sulfate (SulP), metal ion (ZIP), Ca^{2+} (CaCA), and K^+ transport protein gene families (VIC, KUP) are specifically diverged in rice. This may have been caused by the need for nutrient supplements for rapid growth in subtropical plants. These differences may also result from dissimilarities between monocots and dicots. Although the cell structures are the same in both plants, there are many differences in the basic structure of tissues and organs. Differences in root and vascular bundle structure may be related to differences in uptake efficiency of nutrients. The levels of uptake and transport amounts of ions such as K^+, Fe^{2+}, and other nutrients differ between monocots and dicots; this might be related to differences in the numbers of membrane transport genes.

Comparison of the membrane transport genes in Arabidopsis and rice pointed to the directions of evolution of these plants in response to the selection pressures of their differing environmental situations. The level of conservation of gene families in Arabidopsis and rice varies, depending on the category. Rice membrane transporters were 72% orthologous with those of Arabidopsis; this is higher than the 50% ortholog found in transcription factors (Xiong et al. 2005). The levels of expression of membrane transport genes are limited precisely within a small range, and these genes are critical for survival. This may be the reason why the membrane transport genes were less divergent than the transcription factors. Therefore, the specifically divergent gene families in Arabidopsis and rice are related to the indispensable and unique systems in each plant. Divergence of gene patterns might indicate differences between wild weeds and crop cultivars that have been selected for growth in specific and possibly new environments.

Table 3 Comparison of secondary transporter genes

	E. coli	*A. thaliana*	*O. sativa*	*C. elegans*	*D. melanogaster*	*H. sapiens*	*N. crassa*	*S. cerevisiae*	Present in	Material	Cotransporter	Direction
AAA	0	2	3	0	0	0	0	0	P (chloroplast)	ATP	H^+	in (same)
AAAP	0	43	77	11	15	13	4	7	A, P, F, Y	Amino acid, Auxin	H^+	in (same)
AAE	1	0	0	0	0	0	0	0	B	l-Aspartate, l-Alanine	each other	both (anti)
AbgT	1	0	0	0	0	0	0	0	B	p-Aminobenzoyl-glutamate	H^+	in (same)
ACR3	0	0	0	0	0	0	1	1	B, Y	Arsenite, Antimonite	?	out
AE	0	7	3	4	2	10	2	1	A, P, Y	Na^+, HCO_3^-, H^+, Cr, H_3BO_3	H^+, Na^+, Cl^-	both (anti)
AEC	1	8	8	0	0	0	2	4	P, Y, B	Auxin	H^+	out (anti)
AGCS	1	0	0	0	0	0	0	0	a, B	Alanine, Gycine	Na^+, H^+	in (same)
Amt	1	6	13	6	2	4	4	3	All	NH_3, CO_2	?	both
APC	22	12	14	11	11	14	15	24	All	Amino acid, Poly amine	H^+, Solute	in (same)
ArAE	3	11	9	0	0	0	0	0	P, Y, B, a	Armate acid (Malate etc.,)	?	out
ArsB	2	0	0	0	5	1	0	0	All (with distant homolog)	Arsenite, Antimonite	?	out
BASS	1	5	6	0	2	5	0	1	All	Organic acid	Na^+	in (same)
BCCT	3	0	0	0	0	0	0	0	B, a	Betaine/Carnitine/Choline	H^+	in (same)
BenE	1	0	0	0	0	0	0	0	B	Benzoate	H^+	in (same)
CaCA	2	12	23	8	11	8	8	4	All	Ca^{2+}	H^+, Na^+	both (anti)
CCC	0	1	2	6	5	9	1	1	All	Na^+, K^+, Cl^-	Na^+, K^+, Cl^-	both (same)
CDF	2	8	11	8	7	10	8	5	All	Divalent cation (Zn^{2+}, Cd^{2+})	H^+, K^+	out (antit)
CHR	0	0	0	0	0	0	1	0	F, B	SO_4^{2-} , CrO_4^{2-}	H^+	both (anti)
CNT	3	0	0	2	2	3	1	0	A, Y, B	Nucleoside	H^+, Na^+	in (same)
CPA1	2	8	7	11	5	3	3	2	All	Na^+,H^+	H^+, Na^+, Cl^-	both (anti)
CPA2	3	32	16	0	0	1	2	1	All	Na^+, K^+	H^+ (or itself)	out (anti)
DAACS	3	0	0	6	2	7	0	0	A, B	Dicarboxylate, Amino acid	H^+, Na^+	in (same)
DASS	5	4	5	4	3	5	0	3	A, P, Y, B (G^-)	Aminoacid, Sulfate, Phosphate etc.,	H^+, Na^+	in (same), both (anti)
Dcu	2	0	0	0	0	0	0	0	B (G−)	C4-Dicarboxylate	Dicarboxylate	both (anti)
DcuC	2	0	0	0	0	0	0	0	B (G−)	C4-Dicarboxylate	H^+, Dicar-boxylate	in (same = H^+) , both (anti)
DMT	16	121	57	15	14	18	6	9	All	C3 Carbohydrate, Sugar, Nucleotide etc.,	H^+, Nucleotide	out (anti)
ENT	0	8	4	5	3	4	1	1	A, P, Y	Nucleoside	?	both
ESS	1	0	0	0	0	0	0	0	B	Glutamate	Na^+	in (same)
FBT	0	9	8	0	0	0	0	0	P, a, B	Folate, Biopterin	H^+	in (same)
FNT	4	0	0	0	0	0	1	1	Y, a, B	Formate, Nitrate	H^+	in (same)

Table 3 continued

	E. coli	*A. thaliana*	*O. sativa*	*C. elegans*	*D. melanogaster*	*H. sapiens*	*N. crassa*	*S. cerevisiae*	Present in	Material	Cotransporter	Direction
GntP	7	0	0	0	0	0	0	0	B (E. coli, Bacillus)	Carbohydrate	H^+	in (same)
GPH	6	9	8	1	1	5	2	0	A, P, F, a, B	Sugar	H^+, Na^+ , Li^+	in (same)
GUP	0	0	1	0	0	0	1	2	A, P, F, Y, B	Glycerol	H^+	in (same)
HAAAP	8	1	0	0	0	0	0	0	B, P (distant homolog)	Amino acid	H^+	in (same)
KDGT	1	0	0	0	0	0	0	0	B	2-Keto-3-Deoxygluconate	H^+	in (same)
KUP	1	13	21	0	0	0	1	0	P, F, B	K^+	none	in
LCT	0	6	3	0	2	2	1	1	A, P, F	Cystine	H^+	in (same)
LctP	2	0	0	0	0	0	0	0	a, B	Lactate, Glycolate	H^+	in (same)
LIV-E	1	0	0	0	0	0	0	0	a, B	Amino acid (L, I, V)	H^+	in (same)
LIVCS	1	0	0	0	0	0	0	0	B	Amino acid (L, I, V)	H^+, Na^+	in (same)
LysE	1	0	0	0	0	0	0	0	B	Lysine	H^+, OH^-	out (OH^{-} = same; H^{+} = anti)
MC	0	52	66	34	45	44	34	34	Eukaryote	C3 Carbohydrate, Sugar, Nucleotide etc.,	Various	both (anti)
MET	0	0	0	0	0	3	0	0	A	Nucleoside, Hydrophobic compound	H^+	both (anti)
MFS	71	92	145	134	136	81	141	85	All	C3 Carbohydrate, Sugar etc.,	H^+, Na^+, Various	both (H^+, Na^+= same; others = both)
MOP (MATE)	8	56	48	0	0	2	1	3	All	Various (Drugs, Polysaccharides etc.,)	Na^+, (H^+)	out (anti)
MTC	0	2	2	6	2	3	1	1	Eukaryote	Various (Anionic substrate)	H^+	in (same)
NCS1	2	1	0	0	0	0	3	10	P, F, Y, a, B	Nucleobase, Thiamine	H^+	in (same)
NCS2	10	12	11	5	1	4	1	0	All	Nucleobase, (Ascorbate = (mouse only))	H^+, (Na^+= (Ascotbate))	in (same)
NhaA	1	0	0	0	0	0	0	0	Prokaryote	Na^+, H^+	Na^+, H^+	both (anti)
NhaB	1	0	0	0	0	0	0	0	All	Na^+, H^+	Na^+, H^+	both (anti)
NhaD	0	2	1	0	0	0	0	0	P, B	Na^+, (Li^+), H^+	Na^+, (Li^+), H^+	both (anti)
NiCoT	0	2	0	0	0	0	1	0	All	Ni^{2+}, Co^{2+}	Ni^{2+}, Co^{2+}	both (anti)
Nramp	1	7	10	2	1	2	2	3	All	Fe^{2+}, Zn^{2+}, Mn^{2+}, Co^{2+}, Ca^{2+}, Cu^{2+}, N^{i2+}, Pb^{2+}etc.,	H^+	in (same)
NSS	0	0	0	12	21	18	0	0	A	Neurotransmitters, Amino acids, Osmolytes, Cl^-etc.,	Na^+	in (same)
OAT	0	2	2	6	8	11	0	0	A, P, F, Y	Various	Anion	both (anti)
OPT	0	15	34	0	0	0	4	3	P, B, a	Oligopeptide	H^+	in (same)

Table 3 continued

	E. coli	*A. thaliana*	*O. sativa*	*C. elegans*	*D. melanogaster*	*H. sapiens*	*N. crassa*	*S. cerevisiae*	Present in	Material	Cotransporter	Direction
OST	0	0	0	0	0	2	0	0	Eukaryote	Organic compound (mostly anions)	each other	both (anti)
Oxa1	1	4	5	0	1	1	0	1	All (with distant homolog)	Protein	each other	both (anti)
PiT	2	1	3	5	1	2	1	1	All	HPO_4^{2-}, SO_4^{2-}	H^+, Na^+	in (same)
PNaS	1	0	0	1	0	2	0	0	A, B (distant homolog)	Inorganic phosphate	Na^+	in (same)
POT	4	50	61	3	3	4	2	1	All	Oligopeptide	H^+	both (same)
RFC	0	0	0	3	3	4	0	0	A	Folate, Tiamine	H^+, OH^-, anion	both (anti)
RhtB	5	0	0	0	0	0	0	0	a, B	Amino acid	H^+	out (anti)
RND	8	2	1	24	4	7	2	1	All	Heavy metals, Drugs, Lipids etc.,	H^+	out (anti)
SSS	4	1	1	3	19	11	2	1	All	Sugar, Amino acid, Organo cation, Anion	Na^+, (H^+ can replace, but reduces affinity)	in (same)
SulP	1	11	15	7	9	11	4	4	All	SO_4^{2-}	H^+	in (same)
Tat	1	0	1	0	0	0	0	0	B (G^-), P (distant homolog)	Protein	none	out
TDT	1	4	9	0	0	0	2	1	All (with distant homolog)	Tellurite, C4-Dicarboxylate	H^+	in (same)
ThrE	1	0	0	0	0	0	0	2	All (with distant homolog)	Threonine, Serine	H^+	both (anti).
TRAP-T	1	0	0	0	0	0	0	0	a, B	Various	H^+	in (same)
Trk	2	1	1	0	0	0	2	2	P, Y, B	K^+	H^+	in (same)
ZIP	0	13	18	6	5	2	5	3	All	Zn^{2+}, Fe^{2+}	none	in

Note:

Present in: A = animal, P = plant, F = fungi, Y = yeast, B = bacteria, a = archea, G^- = gramm minus bacteria;

Direction: “in” , “out”, and “both” indicate transport direction of materials through cell membrane, and “same” means material and cotranspoter move to the same dirction, “anti” means the different directions

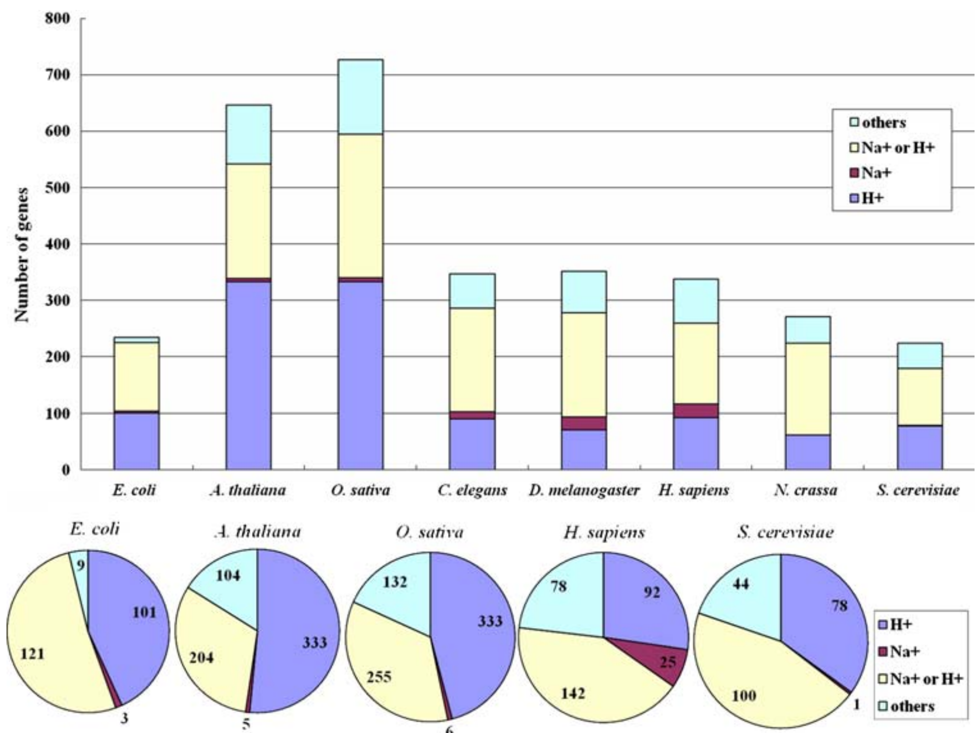

Fig. 5 *Comparison of co-transport molecules of secondary active transporters among various organisms*. Numbers of co-transport molecules of secondary active transporters were compared among *Escherichia coli* K12-MG1655, *Arabidopsis thaliana, Oryza sativa, Caenorhabditis elegans, Drosophila melanogaster, Homo sapiens* NCBI, *Neurospora crassa* 74-OR23-IVA, and *Saccharomyces cerevisiae* S228C

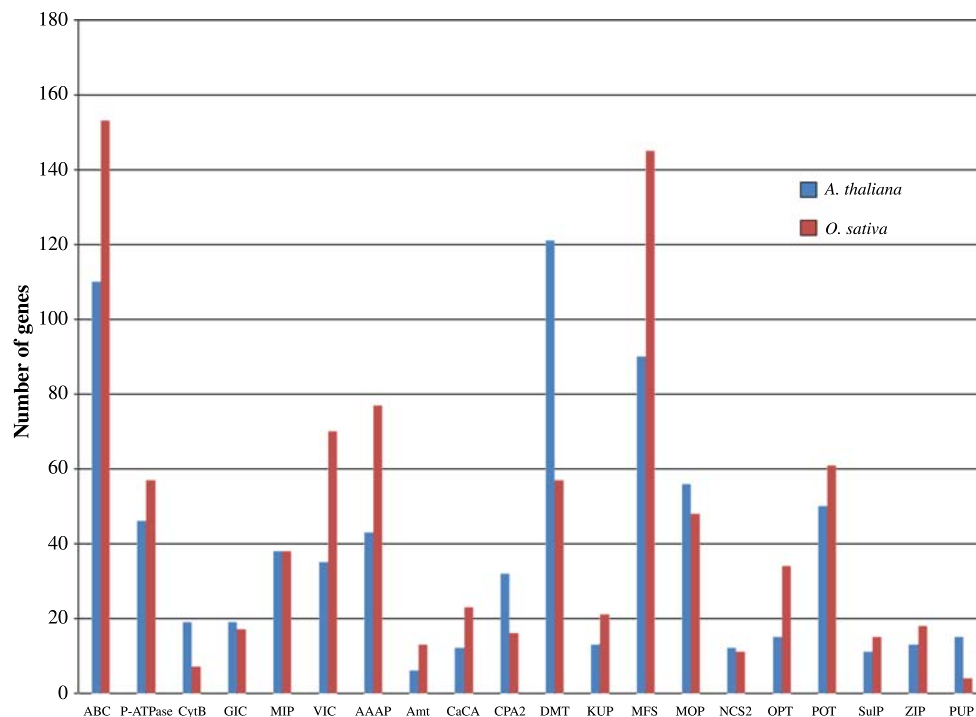

Fig. 6 *Comparison of membrane transport genes in Arabidopsis and rice*. Numbers of the membrane transporter gene families are compared in Arabidopsis and rice. ABC: ATP-binding Cassette; P-ATPase: P-type ATPase; CytB: gp91phox Phagocyte NADPH Oxidase-associated Cytochrome b558 (CytB) H^+-channel; GIC: Glutamate-gated Ion Channel; MIP: Major Intrinsic Protein; VIC: Voltage-gated Ion Channel; AAAP: Amino Acid/Auxin Permease; Amt: Ammonium or Ammonia Transporter; CaCA: Ca^{2+}:Cation Antiporter; CPA2: Monovalent Cation:Proton Antiporter-2; DMT: Drug/Metabolite Transporter; KUP: K^+ Uptake Permease; MFS: Major Facilitator Superfamily; MOP: Multidrug/Oligosaccharidyl-lipid/Polysaccharide Flippase Superfamily; NCS2: Nucleobase: Cation Symporter-2; OPT: Oligopeptide Transporter; POT: Proton-dependent Oligopeptide Transporter; SulP: Sulfate Permease; ZIP: Zinc (Zn^{2+})–Iron (Fe^{2+}) Permease; PUP: Peptide Uptake or Activated Fatty Acid Export Permease

Comparison of the overall gene compositions of bacteria (*E. coli*), animals (*H. sapiens*), and plants

In the eight diverse organisms that we compared, the number of membrane transporter genes (300–1200) varied less than that of genes in other categories. The minimum number of genes indispensable to retaining cell membrane transport homeostasis thus seems to be 300–350, as found in bacteria, yeasts, and fungi. Many of the additional newly diverged genes of higher animals and plants are channel transporter genes and secondary active transporters that facilitate adaptation to fluctuating concentration gradients present in their environments. Moreover, many newly acquired transporters are highly specifically stage- and tissue-regulated and transport special substrates such as neurotransmitters (ACC) in neural cells or carbohydrates (POT) in leaf tissue.

Comparison of the overall gene compositions of bacteria (*E. coli*), animals (*H. sapiens*), and plants (*A. thaliana* and *O. sativa*) reveals the strategies for osmotic pressure adjustment and the features of the substance-transport systems in each organism (Fig. 7). Since a monad cannot control the ion environment of its external world and has no need to communicate with other cells, the role of its transporter proteins is restricted to the control of material transport into and out of the cell. Therefore, transport depends mainly on an energy-consuming system (pump) and an internal ion-gradient-dependent system (secondary transporter). Because the bacterium has a cell wall, osmotic pressure is opposed by cell wall pressure and there is no need to form a molecular concentration gradient to prevent excessive accumulation within the cell. Additionally, bacteria are small (1–5 μm, <1/10 of the size of an animal cell) and do not have many of the organelles and membrane structures possessed by higher organisms. Therefore, transport of substances is simple and it is easy to control their concentrations in the cell. The genes encoding the ATP-dependent pump (ABC), phosphotransferase (PTS), small conductance mechanosensitive ion channel (MscS), and secondary active transporters (e.g. APC, DMT, MFS, NCS2, RND) with H^+ or Na^+ as co-transporter molecules have diverged in bacteria (Fig. 7).

Fig. 7 *Summaries of comparison of membrane transport genes in bacteria, animals, and plants*. Bacteria-specific genes = blue; animal-specific genes = red; plant-specific genes = green; bacteria- and animal-specific genes = purple; bacteria- and plant-specific genes = brown; genes with divergent numbers in organisms = yellow. ABC: ATP-binding Cassette; ArsAB: Arsenite-Antimonite Efflux; F-ATPase: H^+or Na^+-translocating F-type, V-type and A-type ATPase; H^+-Ppase: H^+-translocating Pyrophosphatase; IISP: General Secretory Pathway (Sec); MPT: Mitochondrial Protein Translocase; P-ATPase: P-type ATPase; ACC: ATP-gated Cation Channel; Annexin: Annexin; Bcl-2: Bcl-2; Bestrophin: Anion Channel-forming Bestrophin; CD20: CD20 Ca2$^+$ Channel; ClC: Chloride Channel; Connexin: Gap Junction-forming Connexin; CSC: Chloroplast Outer Envelope Solute Channel; CytB: gp91phox Phagocyte NADPH Oxidase-associated Cytochrome b558 (CytB) H^+-channel; E-ClC: Epithelial Chloride Channel; EnaC: Epithelial Na^+ Channel; GIC: Glutamate-gated Ion Channel; Hsp70: Cation Channel-forming Heat Shock Protein-70; ICC: Intracellular Chloride Channel; Ic ln: Nucleotide-sensitive Anion-selective Channel; Innexin: Gap Junction-forming Innexin; IRK-C: Inward Rectifier K^+ Channel; LIC: Ligand-gated Ion Channel of Neurotransmitter Receptors; Mid1: Yeast Stretch-Activated, Cation-Selective Ca^{2+} Channel Mid1; MIP: Major Intrinsic Protein; MIT: CorA Metal Ion Transporter; MscL: Large Conductance Mechanosensitive Ion Channel; MscS: Small Conductance Mechanosensitive Ion Channel; NSCC2: Non-selective Cation Channel-2; O-ClC: Organellar Chloride Channel; PCC: Polycystin Cation Channel; PLM: Phospholemman; RIR-CaC; Ryanodine-Inositol 1,4,5-triphosphate Receptor Ca^{2+} Channel; Tic110: Chloroplast Envelope Anion Channel-forming Tic110; TRP-CC: Transient Receptor Potential Ca^{2+} Channel; UT: Urea Transporter; VIC: Voltage-gated Ion Channel; AAA: ATP:ADP Antiporter; AAAP: Amino Acid/Auxin Permease; AAE: Aspartate:Alanine Exchanger; AbgT: *p*-Aminobenzoyl-glutamate Transporter; ACR3: Arsenical Resistance-3; AE: Anion Exchanger; AEC: Auxin Efflux Carrier; AGCS: Alanine or Glycine:Cation Symporter; Amt: Ammonium or Ammonia Transporter; APC: Amino Acid-Polyamine-Organocation; ArsB: Arsenite-Antimonite (ArsB) Efflux; BASS: Bile Acid:Na^+ Symporter; BCCT: Betaine/Carnitine/Choline Transporter; BenE: Benzoate:H^+ Symporter; CaCA: Ca^{2+}:Cation Antiporter; CCC: Cation-Chloride Cotransporter; CDF: Cation Diffusion Facilitator; CHR: Chromate Ion Transporter; CNT: Concentrative Nucleoside Transporter; CPA1: Monovalent Cation:Proton Antiporter-1; CPA2: Monovalent Cation:Proton Antiporter-2; DAACS: Dicarboxylate/Amino Acid:Cation (Na^+ or H^+) Symporter; DASS: Divalent Anion:Na^+ Symporter; Dcu: C4-Dicarboxylate Uptake; DcuC: C4-dicarboxylate Uptake C; DMT: Drug/Metabolite Transporter; ENT: Equilibrative Nucleoside Transporter; ESS: Glutamate:Na^+ Symporter; FBT: Folate-Biopterin Transporter; FNT: Formate-Nitrite Transporter; GntP: Gluconate:H^+ Symporter; GPH: Glycoside-Pentoside-Hexuronide (GPH):Cation Symporter; GUP: Glycerol Uptake; HAAAP: Hydroxy/Aromatic Amino Acid Permease; KDGT: 2-Keto-3-Deoxygluconate Transporter; KUP: K^+ Uptake Permease; LCT: Lysosomal Cystine Transporter; LctP: Lactate Permease; LIV-E: Branched Chain Amino Acid Exporter; LIVCS: Branched Chain Amino Acid:Cation Symporter; LysE: L-Lysine Exporter; MC: Mitochondrial Carrier; MET: 4 TMS Multidrug Endosomal Transporter; MFS: Major Facilitator Superfamily; MOP: Multidrug/Oligosaccharidyl-lipid/Polysaccharide Flippase Superfamily; MTC: Mitochondrial Tricarboxylate Carrier; NCS1: Nucleobase:Cation Symporter-1; NCS2: Nucleobase:Cation Symporter-2; NhaA: Na^+:H^+ Antiporter A; NhaB: Na^+:H^+ Antiporter B; NhaD: Na^+:H^+ Antiporter D; NiCoT: Ni^{2+}–Co^{2+} Transporter; Nramp: Metal Ion (Mn^{2+}–iron) Transporter; NSS: Neurotransmitter:Sodium Symporter; OAT: Organo Anion Transporter; OPT: Oligopeptide Transporter; OST: Organic Solute Transporter; Oxa1: Cytochrome Oxidase Biogenesis; PiT: Inorganic Phosphate Transporter; PnaS: Phosphate:Na^+ Symporter; POT: Proton-dependent Oligopeptide Transporter; RFC: Reduced Folate Carrier; RhtB: Resistance to Homoserine/Threonine; RND: Resistance-Nodulation-Cell Division; SSS: Solute:Sodium Symporter; SulP: Sulfate Permease; Tat: Twin Arginine Targeting; TDT: Tellurite-resistance/Dicarboxylate Transporter; ThrE: Threonine/Serine Exporter; TRAP-T: Tripartite ATP-independent Periplasmic Transporter; Trk: K^+ Transporter; ZIP: Zinc (Zn^{2+})–Iron (Fe^{2+}) Permease; GPTS: general carbohydrate phosphotransferase; SSPTS: sugar specific phosphotransferase; Ctr1: Dipicolinic Acid Transporter; Ctr2: Copper Transporter; FeoB: Ferrous Iron Uptake; FeT: Low Affinity Fe^{2+} Transporter; FP: Ferroportin; LPI: Lysosomal Protein Import; OfeT: Iron/Lead Transporter; PnuC: nicotinamide mononucleotide(NMN) uptake permease; PPI: Peroxisomal Protein Importer; PUP: Peptide Uptake or Activated Fatty Acid Export Permease ►

Increases in the size and complexity of cells with the emergence of organelles and an internal membrane structure arose in the process of evolution from prokaryote to eukaryote. The eukaryote's inclusion of the prokaryote's transport genes in the mitochondrion or chloroplast allowed the transport of homeostatic substrate to organelles, as well as other materials and the products of specific biosynthesis (tricarboxylic acid cycle, electron-transport chain, and photosynthesis). Some of the genes are indistinguishable between eukaryotic transcript and organelle transporter, but there are 50–100 typical organelle transporters (e.g. MC, MCX, Tat) in eukaryotes, and they make a large group (7–12% of all transporters). Furthermore, with the development of multicellular eukaryotes there came a need for organisms to develop a system of transmission between cells. Animals and plants developed specific systems to solve the problem. Animals lost the cell wall structure and gained cellular mobility and flexibility, which also promoted integration of the whole body and specialization of tissues and cells. The loss of the cell wall also led to the need to control osmotic pressure against the plasma membrane. Animal cells regulate osmotic pressure by raising the Na^+ concentration outside the plasma membrane by active transport with Na^+/K^+ ATPase. This Na^+ gradient is used not only for homeostasis of the osmotic pressure of the membrane, but also as an electrical potential to control the voltage-dependent channel system and for co-transport of symport or antiport molecules of secondary transportation. Animals then developed the channel system (e.g. Bcl-2, connexin, E-ClC, EnaC, ICC, Ic ln, LIC, PCC, PLM, RIR-CaC, VIC) for rapid transmission in nerve and muscle tissues, together with an Na^+ gradient dependent on the secondary transport system (e.g. NSS, PnaS, SSS). This Na^+ ion gradient adaptation system is a feature of the newly added transporters and has evolved with animal specificities, i.e. the need for rapid movement and adaptation to environmental stimulation.

In contrast to animals, plants have retained the cell wall instead of acquiring mobility. Because their cell structures and sizes became complicated and large, plants also evolved a means of controlling osmotic pressure; this means differed from the cell-wall-dependent system of bacteria. Because the plant cell controls osmotic pressure by the turgor pressure of the cell wall, it does not require a sodium or other ion gradient and lacks an Na^+/K^+ ATPase for control of osmotic pressure against the plasma membrane. On the other hand, an increase in permeability to water is necessary for smooth transmission of water pressure in the large and complicated structures of the cell. Therefore, water channels are abundant in both the plasma membrane and the vacuoles (20–50% of membrane proteins), and the diversity of water channels (MIP) is also four to five times higher than that in bacteria and animals. Compared with circulation systems in animals, whole-plant circulation systems are simple and less controlled. Plant cells also need to be more self-complementary in their homeostasis. Additionally, plants have less choice in their environmental conditions (e.g. soil, weather, stress) and must have more transport systems for cell homeostasis than animals. This is why plant cells include vacuoles to pool many substances and bioactive compounds.

Because plants obtain energy through photosynthesis, there are more ATP-dependent (pump) transporters (ABC, P-type ATPase) in their cells than in animal cells. Unlike the case in animals, voltage-dependent systems and Na^+-ion-dependent secondary transport systems have not developed in plants; systems that rely on H^+ rather than Na^+ have developed as part of plant secondary transporter systems. Because the increase in H^+ brings acidification, plants maintain the neutrality of the cytoplasm by accumulating H^+ in vacuoles, which are membrane-bound organelles separated from the other cytoplasmic contents. By having an internal ion pool, plant cells can control the ion gradient easily and safely. Therefore, secondary transport genes (e.g. *CPA2*, *DMT*, *MFS*, *MOP*, *POT*) that use H^+ as a co-transporter molecule have diverged far in plants (Fig. 7). The inclusion of pooling and energy-synthesizing organelles in the cell may be why plant cells (10–100 μm) are larger than animal cells (10–30 μm).

Conclusions

Comparative analysis of membrane transporters among these eight diverse organisms indicates the type of cell homeostasis, as evidenced by the pattern of gene conservation and diversification. Evolutionary changes in gene families, in general, indicate the dynamics of alterations in biological systems and gene networks. Therefore, analysis of large categories of gene families may reveal many basic concepts of biological systems. In practice, analyses of the membrane transporter mechanism are useful in revealing changes in the absorption of molecules by, or their efflux from, cells and tissues. This information also is useful for examining changes in soil adaptability, nutritional demand, and stress tolerance in plants. It may also help to improve the harvest of crop cultivars or extend areas habitable by plant species. Gene networks are intricately related, and analysis of the whole genetic structure is needed to gain a full understanding of biological phenomena and systems of gene regulation. We are continuing to analyze whole categories of genes in an effort to develop an overview of total gene networks.

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