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ORIGINAL ARTICLE

Comparative Genomic Analysis of Integral Membrane Transport Proteins in Ciliates

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ABSTRACT

Integral membrane transport proteins homologous to those found in the Transporter Classification Database (TCDB; www.tcdb.org) were identified and bioinformatically characterized by transporter class, family, and substrate specificity in three ciliates, *Paramecium tetraurelia* (Para), *Tetrahymena thermophila* (Tetra), and *Ichthyophthirius multifiliis* (Ich). In these three organisms, 1,326 of 39,600 proteins (3.4%), 1,017 of 24,800 proteins (4.2%), and 504 out of 8,100 proteins (6.2%) integral membrane transport proteins were identified, respectively. Thus, an inverse relationship was observed between the % transporters identified and the number of total proteins per genome reported. This surprising observation provides insight into the evolutionary process, giving rise to genome reduction following whole genome duplication (as in the case of Para) or during pathogenic association with a host organism (Ich). Of these transport proteins in Para and Tetra, about 41% were channels (more than any other type of organism studied), 31% were secondary carriers (fewer than most eukaryotes) and 26% were primary active transporters, mostly ATP-hydrolysis driven (more than most other eukaryotes). In Ich, the number of channels was selectively reduced by 66%, relative to Para and Tetra. Para has four times more inorganic anion transporters than Tetra, and Ich has nonselectively lost most of these. Tetra and Ich preferentially transport sugars and monocarboxylates while Para prefers di- and tricarboxylates. These observations serve to characterize the transport proteins of these related ciliates, providing insight into their nutrition and metabolism.

CILIATES are unicellular eukaryotes named for the hair-like cilia that cover their bodies. They are found in many aquatic environments ranging from coastal waters to hydrothermal vents. They play important ecological roles in their environments by preying on smaller microorganisms such as bacteria, algae, yeast, and other ciliates, providing food for invertebrates, fish larvae and protozoans, thus allowing nutrient transfer to larger organisms (Dopheide et al. 2009). The nearly 5,000 known ciliate species comprise a major portion of the planet's plankton (Caron et al. 2012). These organisms have adopted a range of life styles including heterotrophic, photosynthetic, aerobic, anaerobic, free living, symbiotic, and parasitic (Coyne et al. 2011; Ricard et al. 2008).

Ciliates, such as *Tetrahymena thermophila* (Tetra) and *Paramecium tetraurelia* (Para) in particular, are important model organisms that have been used to make numerous important discoveries in disciplines ranging from basic cellular and molecular processes to complex genomics (Bracht et al. 2013; Coyne et al. 2012). Although unicellular, they share many of the same genes as multicellular organisms, and they have many large and complex organelles that can be targeted for study (Beisson et al. 2010a,b).

All ciliates have two nuclei, a large somatic nucleus, the macronucleus, wherein the genes are expressed, and a small silent germline nucleus, the micronucleus, which is used exclusively in reproduction (Prescott 1994). Their macronuclei can be restructured, fragmented, scrambled, rejoined, degraded, rebuilt and amplified in various ways, mediated in part by transposons that are controlled epigenetically (Nowacki et al. 2008, 2010; Swart et al. 2013; Yao et al. 2003). Lastly, Both Tetra and Para are easy to culture and have rapid rates of reproduction (Cassidy-Hanley 2012).

Ichthyophthirius multifiliis (Ich) is a parasitic ciliate that is closely related to Tetra (Alvarez-Pellitero 2008). Ich's parasitic lifestyle has resulted in a greatly reduced genome as compared to Tetra. Ich parasitizes a wide range of fish, causing White Spot Disease (Dickerson and Clark 1998). It invades the epidermis of fish and eventually grows to such a large size that it can be seen by the naked eye as a white spot on the surface of the infected fish. Infection of the gills can result in the death of the fish by asphyxiation (Coyne et al. 2011). Ich does not need an intermediate host to reproduce, but it is not known if it can reproduce sexually. Ich outbreaks have resulted in extensive economic loss in both aquaculture and the ornamental fish industry (Ling et al. 2012). Prevention of these outbreaks becomes increasingly important as we look to fish farming to feed our burgeoning human population (Picon-Camacho et al. 2012).

Transport proteins, comprising 5–10% of an organism's genome, are essential for cell homeostasis and survival (Paulsen et al. 2001). They provide vital cellular processes such as uptake of nutrients, export of wastes, and signal transduction (Lam et al. 2011). A comparative genome analysis of the transporters found within Tetra, Para, and Ich should help elucidate key aspects of their lifestyles and physiology (Barabote et al. 2007; Lorca et al. 2007; Youm and Saier 2012).

Para is not as closely related to the other two ciliates and has undergone at least two (possibly three) whole genome duplication events (Aury et al. 2006; Prescott 1994; Sogin and Silberman 1998), probably one more duplication than observed for Tetra or Ich (Eisen et al. 2006). Tetra and Para shared their last common ancestor after the first genome duplication event. Although Tetra and Ich are much more closely related, their stark differences in lifestyle have produced two very different genomes. By seeing what transporters were lost, retained or expanded, we can gain important insight into the metabolic requirements, means of pathogenicity (in Ich), and adaptability to different environments.

In this paper, we analyze the genomes of Para, Tetra, and Ich for all integral membrane transport proteins that correspond to currently recognized transporters included within the Transporter Classification Database (TCDB; www.tcdb.org; Saier et al. 2006, 2009, 2014; Youm and Saier 2012). These systems fall into several classes including (1) channels/pores, (2) secondary carriers, (3) primary active transporters, (4) group translocators, (5) transmembrane electron flow carriers, (8) auxiliary transport proteins, and (9) transporters of unknown mechanism of action. The transport proteins identified in this study have been analyzed by class, family topology and substrate. With this information in hand, we then carried out detailed comparative studies.

MATERIALS AND METHODS

The ciliate genomes analyzed were the most complete and up to date for each organism at the time these studies were initiated. The FASTA formatted protein sequences of *T. thermophila* strain SB210, *P. tetraurelia* strain d4-2 and *I. multifiliis* strain d were used (Aury et al. 2006; Coyne et al. 2011; Eisen et al. 2006). Each sequence in the proteomes was used as a query and blasted against TCDB using the program, G-BLAST (Reddy and Saier 2012).

G-BLAST is a tool to screen all proteins encoded within a genome against all protein entries in TCDB. The program retrieves information for both the query and top hit sequences, TC#, protein size in number of amino acyl residues, number of transmembrane segments (TMSs), *e*-value for the query and hit proteins, regions of sequence similarity and regions of TMS overlap. The low complexity filter was not used as it is normally of value only for larger datasets including proteins with multiple repeat elements. In addition, each open reading frame was scanned with the HMMTOP 2.0 program to predict the number of putative TMSs. The Web-based Hydropathy, Amphipathicity, and Topology (WHAT) program (Tusnady and Simon 2001; Zhai and Saier 2001) was used with a window size of 19 residues and an angle of 100° (as is appropriate for alpha helices) to display the hydropathy plot for individual proteins in order to resolve the differences in the numbers of TMSs between the proteins retrieved and their TCDB homologs. The plot generated by WHAT allows the user to judge if a program such as HMMTOP has missed a TMS or has predicted a TMS inappropriately. A cut-off value of 0.001 was used with the G-BLAST program to eliminate false positives and proteins with unreliable degrees of sequence divergence.

Proteins with no predicted TMSs were eliminated so that only integral membrane proteins, primarily multispanning membrane proteins, were retrieved. Proteins with only an N-terminal signal sequence are numerous because these proteins include almost all secreted proteins that are exported via the Sec translocase. The topological prediction programs often miss these TMSs, recording the proteins to have zero TMSs. Consequently, the numbers of 0 or 1 TMS proteins retrieved were not reliable and were therefore not always recorded.

The G-BLAST algorithm, set up with the parameters described above, is able to recognize all transporter protein homologs that are sufficiently similar in sequence to any sequence in TCDB to give a score of less than (smaller than) 0.001. Every such sequence retrieved was confirmed. It should be noted that transport proteins of functions that have not yet been identified may also have been excluded from our study, but their identification will only be possible when such proteins are identified. Thus, we feel confident that, given our current knowledge of transport proteins, we have identified all integral membrane transport proteins encoded in the three genomes to the extent possible.

The limitations of our methods are illustrated with the identification of F and V-type ATPases in paragraph two of the Discussion section. Although TCDB contains numerous sequences from ciliates, facilitating the identification of the large majority of transport proteins in the three organisms studied, these methods have their limitations.

The results reported in this paper represent a comprehensive evaluation of the transporters encoded in the ciliates, limited only by the considerations noted above.

Candidate proteins were subsequently examined in greater detail to estimate their probable substrate specificities. On the basis of the numbers and locations of TMSs as well as degrees of sequence similarity with entries of known function in TCDB, transport proteins were classified into families and subfamilies of homologous transporters according to the classification system presented in TCDB (Saier et al. 2006, 2009, 2014). Regions of sequence similarity were examined to insure that homology was in the transmembrane regions and not merely in hydrophilic domains (Youm and Saier 2012).

The substrate specificities of particular homologs identified in the sequenced genomes were often predicted based on homology to functionally characterized proteins. Assignment to a family or subfamily within the TC System often allows prediction of substrate type with confidence (Busch and Saier 2002; Felce and Saier 2004; Harvat et al. 2005; Saier 2000; Zhang et al. 2003).

RESULTS

Paramecium tetraurelia transporters

Overview

According to the Transporter Classification (TC) System, transporters are classified into five well-defined categories (classes 1–5) and two poorly defined categories (classes 8 and 9) as mentioned above (see TCDB; www.tcdb.org; Busch and Saier 2002; Saier 2000; Saier et al. 2006, 2009). *Paramecium tetraurelia* strain d4-2 (Para) has been reported to have a macronuclear genome that is 70.07 million base pairs in size and encodes 39,580 proteins. Of these proteins, 1,326 were predicted to be transporters, 3.4% of all proteins encoded. Table S1 and Fig. 1A present an overall summary of the classes and subclasses of transporters found in Para. Only integral membrane transport proteins, mostly those that provide the transmembrane pathway for solute translocation, are reported.

Of all the major classes of transport proteins included in TCDB, Para encodes representatives of classes 1, 2, 3, and 9. 557 (39%) of these proteins are channels; 496 (34.7%) are secondary carriers; 349 (24.5%) are primary active transport proteins, and 26 (1.8%) are of unknown mechanism of action. It therefore, appears that a wide diversity of transport protein classes are important to Para.

539 channel-type proteins are alpha-type channels (Subclass 1.A). A single member of the beta-barrel porins (Subclass 1.B) (belonging to the Autotransporter 1 family; TCID 1.B.12.3.1) was identified, and no channel-forming toxins (Subclass 1.C) were found. Seventeen members of the vesicle fusion pore (Subclass 1.F) were retrieved.

All secondary carriers belong to subclass 2.A (uniporters, symporters, and antiporters). The vast majority of

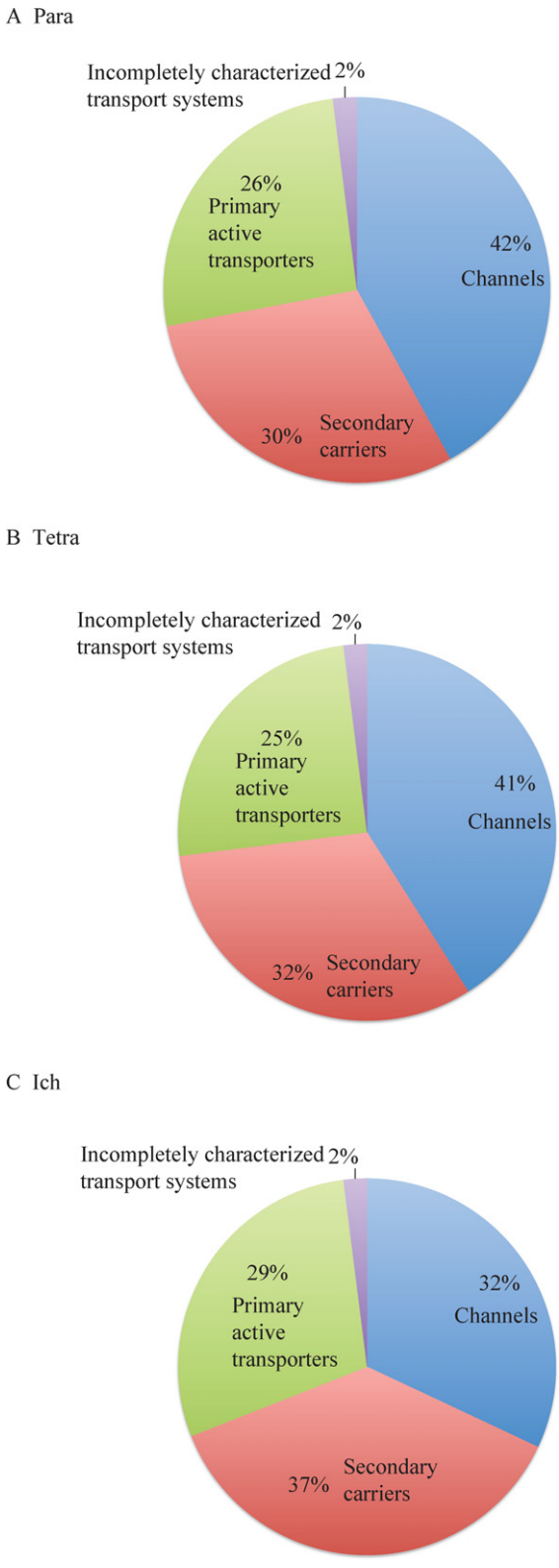

Figure 1 A–C. Percentages of the major classes of recognized transport proteins found in *Paramecium tetraurelia* strain d4-2 (A), *Tetrahymena thermophila* strain SB210 (B) and *Ichthyophthirius multifiliis* strain d serotype (C).

primary active carriers belong to subclass 3.A (P-P-bond-hydrolysis-driven transporters). The remaining 15 of the 349 putative primary active carriers belong to subclass 3.D (oxidoreduction-driven transporters). Twenty-nine proteins belong to class 9.A (recognized transporters of unknown biochemical mechanism).

Substrate type

More than half of the transporters found in Para are devoted to the transport of inorganic molecules. Table 1 presents an overview of these transporters based on substrate specificity. They can be nonselective or exhibit selectivity toward cations or anions. Most of the nonselective transporters (nine proteins) are channels; another four are recognized transporters of unknown biochemical mechanism, and the remaining two are primary active transporters. Five hundred and ninety-five of all putative transporters found in Para are devoted to the transport of inorganic cations, the vast majority being channels (444 proteins). Ninety-seven cation transporters are primary active transporters, 48 are secondary carriers, and the remaining 6 are poorly defined systems. In addition to cation transporters, there are 77 inorganic anion transporters; channels account for 50 of these proteins, the rest being secondary carriers.

Nearly 7% of all transporters in Para take up carbon sources. Carbon compounds are subdivided into (1) sugars and polyols, (2) monocarboxylates, (3) di-/tricarboxylates, (4) noncarboxylate organoanions, and (5) aromatic compounds. Secondary carriers make up the vast majority of transporters that act on carbon sources. The breakdown is 40 for sugars and polyols, 6 for monocarboxylates, 26 for di- and tricarboxylates, 5 for organoanions, and 6 for aromatic compounds. A single channel seems to catalyze transport of monocarboxylates. Lastly, 6 and 4 primary active carriers transport sugars and polyols and organoanions, respectively.

Table 1. Distribution of transporters in *Paramecium tetraurelia* (P), *Tetrahymena thermophila* (T) and *Ichthyophthirius multifiliis* (I) based on substrate specificity

	Channels			Primary active transporters			Secondary carriers			Poorly defined system			Total number of systems		
Substrate	P	T	I	P	T	I	P	T	I	P	T	I	P	T	I
Inorganic Molecules													687	503	242
Nonselective	9	0	0	2	0	0	0	0	0	4	0	0	15	0	0
Cations	444	385	143	97	51	44	48	40	39	6	7	4	595	483	230
Anions	50	11	10	0	0	0	27	9	2	0	0	0	77	20	12
Carbon sources													**94**	**106**	**47**
Sugar and polyols	0	0	0	6	0	0	40	63	28	0	0	0	46	63	28
Monocarboxylates	1	0	0	0	0	0	6	20	5	0	0	0	7	20	5
Di- and tri carboxylates	0	0	0	0	0	2	26	6	8	0	0	0	26	6	10
Organoanions	0	0	0	4	12	2	5	0	0	0	0	0	9	12	2
Aromatic compounds	0	0	0	0	0	0	6	5	2	0	0	0	6	5	2
Amino acids and their derivatives													**110**	**114**	**57**
Amino acids and conjugates	17	5	7	6	19	0	42	45	27	0	0	0	65	69	34
Amines, amides, polyamines, and organocations	18	1	4	1	0	0	20	40	18	0	0	0	39	41	22
Peptides	0	0	0	6	4	1	0	0	0	0	0	0	6	4	1
Vitamins, cofactors, and cofactor precursors													**68**	**30**	**22**
Vitamins and vitamin or cofactor precursors	0	0	0	10	2	0	52	24	18	6	4	2	68	30	20
Enzyme and redox cofactors	0	0	0	0	0	2	0	0	0	0	0	0	0	0	2
Substrate cofactors	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Siderophores; siderophores-Fe complexes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Drugs, dyes, sterols, and toxics										0			**26**	**22**	**19**
Multiple drugs	0	0	0	11	14	10	11	5	4	0	0	0	22	19	14
Specific drugs	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0
Pigments	0	0	0	4	1	2	0	0	0	0	0	0	4	1	2
Other hydrophobic substances	0	0	0	0	1	3	0	0	0	0	0	0	0	1	3
Bases and derivatives	0	0	0	18	25	2	45	33	30	4	4	1	**67**	**62**	**33**
Macromolecules													**177**	**113**	**66**
Carbohydrates	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Proteins	1	2	0	61	31	10	3	1	1	3	2	0	68	36	11
Lipids	0	0	0	95	75	51	11	2	3	3	0	1	109	77	55
Nucleic acids	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Unknown	17	13	0	28	22	15	52	26	3	0	5	0	**97**	**66**	**18**
Total	557	417	164	349	257	144	394	320	188	26	22	8	**1326**	**1016**	**504**

The bolded numbers represent subcategory totals (e.g., total number of systems with carbon source substrates).

The substrate category amino acids and their derivatives is broken down into three subcategories: (1) amino acids and conjugates, (2) amines, amides, polyamines, and organocations, and (3) peptides. Amino acids and their conjugates are transported primarily by secondary carriers (42 proteins), but 17 are channels and 6 are primary active transporters. Twenty secondary carriers transport amines, amides, polyamines and organocations, while 18 channels and one primary active transporter are specific for these compounds. All six peptide transporters are primary active transporters.

Vitamins, cofactors, and cofactor precursors comprise a category that includes both enzyme and redox factors as well as siderophores and siderophore-Fe^{3+} complexes. In Para, no system is specific for siderophores. Secondary carriers (52 systems) make up a large majority of these vitamin/cofactor transporters. Ten primary active transporters and six poorly defined systems act on these compounds.

The next category, drugs, dyes, sterols, and toxins, consists of the four subcategories: multiple drugs, specific drugs, pigments, and other hydrophobic substances. Both primary active transporters and secondary carriers contribute equally to the transport of multiple drugs (11 each). In addition, primary active carriers are involved in the transport of pigments (four proteins). Transporters for specific drugs or other hydrophobic substances were not identified.

The next important substrate category includes macromolecules with 177 proteins acting on these substances. Macromolecules are broken down into four subcategories: carbohydrates, proteins, lipids, and nucleic acids. Not a single transporter that transports carbohydrates or nucleic acids was found in Para, but 61 primary active transport proteins act on proteins. Only a single channel, three secondary carriers, and three poorly characterized systems apparently function to transport proteins. Ninety-five primary active transport proteins appear to act on lipids, but 11 secondary carriers and three poorly defined systems may also transport lipids.

Transporters that act on nucleobases and their derivatives (mostly nucleosides and nucleotides) fall into several different transport protein classes. Forty-five secondary carriers, 18 primary active transporters and four poorly defined systems probably transport these compounds. 97 of the putative transporters in Para do not have a known substrate. Of these transporters, 52 are secondary carriers, 28 are primary active transporters, and 17 are channels.

Superfamilies

An overview of all the superfamilies is presented in Table 2, showing that the Voltage-gated Ion Channel (VIC) superfamily has an enormous representation in Para with 425 proteins. In second place, the MFS superfamily has 145 proteins. Closely following the MFS, 133 P-ATPases and 105 ABC exporters were identified. The Mitochondrial Carrier (MC) family and the Ca^{2+}-dependent Cl^- channel (Ca-ClC) family also have substantial representation with 82 and 44 proteins, respectively.

Topologies

Para transport proteins were examined according to predicted topology (Fig. 2A–D). Generally, there are substantially more proteins with even numbered TMSs than odd numbered TMSs as is usually the case (Saier 2003). Six and 12 TMSs proteins dominate all others with 285 and 154 representatives, respectively. Interestingly, most of the six TMS proteins are channel proteins (249) and most of the 12 TMS proteins are secondary carriers (123). Primary active transporters include many proteins with 4 TMSs (35) and 10 TMSs (43).

Tetrahymena thermophila transporters

Overview

Tetrahymena thermophila strain SB210 (Tetra) has a genome that is 103 million base pairs in size and encodes an estimated 24,770 proteins. Table S2 and Fig. 1B present an overall summary of the classes and subclasses of transporters found in Tetra. Of these proteins, 1,017 (4.2%) are recognized transport proteins.

Tetra encodes representatives of classes 1, 2, 3, 5, and 9. 417 (40%) of these proteins are simple channels; 346 (33.2%) are secondary carriers; 257 (24.6%) are primary active transport proteins; a single transmembrane electron carrier (0.1%) was found, and 22 (2.1%) are of unknown mechanism of action.

As with Para, a large majority of channel type transporters in Tetra are alpha-type channels (Subclass 1.A) with 408 representatives. In addition to alpha-type channels, Tetra has two homologs of beta-barrel porins (Subclass 1.B) and two putative pore-forming toxins (Subclass 1.C). Lastly, five proteins belong to the vesicle fusion pore family (Subclass 1.F).

All electrochemical potential-driven transporters in Tetra belong to subclass 2.A (uniporters, symporters and antiporters). 252 of the 257 identified primary active transport proteins function as pyrophosphate-bond hydrolysis-driven transporters (Subclass 3.A). A single probable member of subclass 3.B (decarboxylation-driven transporters) was identified. Such a system has never been characterized in a eukaryote, so its functional assignment must be considered tentative. The remaining proteins are oxidoreduction-driven transporters (Subclass 3.D). Unlike Para and Ich, Tetra has a single transporter that is homologous to a transmembrane 1-electron transfer carrier (Subclass 5.B). Finally, 22 transport proteins are of unknown biochemical mechanism (Subclass 9.A).

Substrate types

Table 1 presents the breakdown of transporters found in Tetra based on class and substrate specificity. Inorganic molecules comprise by far the largest category of substrates transported with 503 members, almost half of all transporters found. These transporters generally exhibit specificity toward cations or anions; there are few if any nonselective transporters. Anion transporters are divided almost equally between channels and secondary carriers with 11 and 9 proteins, respectively. The majority of

Table 2. Distribution of transporters encoded within the genomes of *Paramecium tetraurelia*, *Tetrahymena thermophila* and *Ichthyopthirius multifiliis* according to TC Family

TC subclass	Superfamily or Family (Abbreviation)	Substrate	No. of proteins in		
			Para	Tetra	Ich
	The Voltage-gated Ion Channel (VIC) Superfamily (1.A.1-1.A.5 + 1.A.10): Total Numbers		425	374	140
1.A.1	The Voltage-gated Ion Channel (VIC) Family	Cation	392	342	105
1.A.3	The Ryanodine-Inositol 1,4,5-triphosphate Receptor Ca^{2+} Channel (RIR-CaC) Family	Cation; Ca^{2+}	25	22	32
1.A.4	The Transient Receptor Potential Ca^{2+} Channel (TRP-CC) Family	Cation; Ca^{2+}	0	1	1
1.A.5	The Polycystin Cation Channel (PCC) Family	Cation	8	7	2
1.A.10	The Glutamate-gated Ion Channel (GIC) Family of Neurotransmitter Receptors	Ion	0	2	0
1.A.11	The Ammonia Channel Transporter (AMT) Family	Ammonia	14	1	4
1.A.14	The Testis-Enhanced Gene Transfer (TEGT) Family	Ca^{2+}; Unknown	17	9	0
1.A.17	The Calcium-Dependent Chloride Channel (Ca-ClC) Family	Cl^-	44	11	7
1.A.38	The Golgi pH Regulator (GPHR) Family	Anion	1	1	3
1.A.54	The Presenilin ER Ca^{2+} Leak Channel (Presenilin) Family	Ca^{2+}	4	10	1
1.A.75	The Mechanical Nociceptor, Piezo (Piezo) Family	Cation	5	3	1
1.C.57	The Clostridial Cytotoxin (CCT) Family	Unknown	7	2	2
1.F.1	The Synaptosomal Vesicle Fusion Pore (SVF-Pore) Family	Neurotransmitter	17	5	7
	The Major Facilitator Superfamily (MFS) (2.A.1-2.A.2 + 2.A.71) total numbers		145	126	72
2.A.1	The Major Facilitator Superfamily (MFS)	Solute	132	122	64
2.A.2	The Glycoside-Pentoside-Hexuronide (GPH):Cation Symporter Family	Glycoside-Pentoside-Hexuronide	3	3	3
2.A.71	The Folate-Biopterin Transporter (FBT) Family	Folate-Biopterin	10	1	4
	Cation Diffusion Facilitator (CDF) Superfamily (2.A.4 + 2.A.19) Total Numbers		14	11	8
2.A.4	The Cation Diffusion Facilitator (CDF) Family	Cation	6	6	5
2.A.19	The Ca^{2+}: Cation Antiporter (CaCA) Family	Ca^{2+} and Cation	8	5	3
2.A.5	The Zinc (Zn^{2+})-Iron (Fe^{2+}) Permease (ZIP) Family	Zinc (Zn^{2+})-Iron (Fe^{2+})	5	7	8
2.A.6	The Resistance-Nodulation-Cell Division (RND) Superfamily	Drug/Complex Carbohydrate	8	1	1
2.A.7	The Drug/Metabolite Transporter (DMT) Superfamily	Drug/Metabolite	14	34	4
	The Amino Acid-Polyamine-Organocation (APC) Superfamily (2.A.18 + 2.A.22) Total Numbers		26	27	7
2.A.18	The Amino Acid/Auxin Permease (AAAP) Family	Amino Acid/Auxin	22	24	5
2.A.22	2.A.22 The Neurotransmitter:Sodium Symporter (NSS) Family	Neurotransmitter	4	3	2
2.A.29	Mitochondrial Carrier (MC) Superfamily	Many different substrates	82	40	38
	The Cation:Proton Antiporter Superfamily (CPA) (2.A.36 + 2.A.98)Total Numbers		22	17	16
2.A.36	The Monovalent Cation:Proton Antiporter-1 (CPA1) Family	Cation:Proton	22	16	16
2.A.98	The Putative Sulfate Exporter (PSE) Family	Sulfate	0	1	0
2.A.43	The Lysosomal Cystine Transporter (LCT) Family	Cystine	3	2	2
2.A.50	The Glycerol Uptake (GUP) Family	Glycerol	3	2	2
2.A.51	The Chromate Ion Transporter (CHR) Family	Chromate Ion	5	0	2
2.A.54	The Mitochondrial Tricarboxylate Carrier (MTC) Family	Tricarboxylate	2	1	1
2.A.55	The Metal Ion (Mn^{2+}-iron) Transporter (Nramp) Family	Metal Ion	0	3	3
2.A.57	The Equilibrative Nucleoside Transporter (ENT) Family	Nucleoside	2	12	4

(continued)

Table 2 (continued)

TC subclass	Superfamily or Family (Abbreviation)	Substrate	No. of proteins in Para	Tetra	Ich
2.A.66	The Multidrug/Oligosaccharidyl-lipid/Polysaccharide (MOP) Flippase Superfamily	Multidrug/Oligosaccharidyl-lipid/Polysaccharide	15	18	6
2.A.89	The Vacuolar Iron Transporter (VIT) Family	Iron	2	0	2
2.A.92	The Choline Transporter-like (CTL) Family	Choline	19	7	8
2.A.102	The Putative 4-Toluene Sulfonate Uptake Permease (TSUP) Family	4-Toluene Sulfonate	5	5	2
3.A.1	The ATP-binding Cassette (ABC) Superfamily	Many different substrates	105	136	40
3.A.2	The H^+- or Na^+-translocating F-type, V-type and A-type ATPase (F-ATPase) Superfamily	H^+- or Na^+	22	9	14
3.A.3	The P-type ATPase (P-ATPase) Superfamily	Ion	133	77	65
3.A.5	The General Secretory Pathway (SEC) Family	Protein	23	10	6
3.A.8	The Mitochondrial Protein Translocase (MPT) Family	Protein	6	7	3
3.A.10	The H^+, Na^+-translocating Pyrophosphatase (M+-PPase) Family	H^+	13	3	7
3.A.20	The Peroxisomal Protein Importer (PPI) Family	Protein	26	8	0

ion-selective transporters are channels (385 proteins), but 51 and 40 cation-specific primary active transporters and secondary carriers were identified, respectively. Lastly, seven cation-selective transporters function by unknown mechanisms (Class 9A).

Transport of carbon sources in Tetra is primarily accomplished by secondary carriers. These include 63 for sugar and polyols, 20 for monocarboxylates, 6 for di/tricarboxylates, and 5 for aromatic compounds. However, 12 primary active carriers transport organoanions.

Channels play a minor role in the transport of amino acids and their derivatives. Forty secondary carriers act on amines, amides, polyamines, and organocations as substrates, while only a single channel serves this function. The same is true for amino acids and their conjugates; 45 secondary carriers, 19 primary active transporters, and only 5 channels function in this capacity. Primary active transporters have the distinction of being the only class that transports peptides (four proteins).

Vitamins, cofactors, and their precursors are the substrates of only 30 recognized transporters in Tetra. Secondary carriers are most important for these substrates with 24 such proteins. Four more are of unknown mechanism, and the remaining two are primary active transporters.

Drugs, pigments, and other hydrophobic substances are transported primarily by primary active transporters with 14 multidrug transporters, one pigment transporter, and a single "other" hydrophobic substance transporter. Secondary carriers play a minor role in this category with five being multidrug carriers and only one being drug-specific. Channels play no role in the transport of these substrates.

Macromolecular transporters account for more than 9% of all transporters found in Tetra. Similar to Para, not a single carbohydrate exporter was found. Almost all (106 of 113) macromolecular transporters are primary active transporters; 31 transport proteins and 75 transport lipids. Of the remaining protein transporters, two are channels, two are transporters of unknown biochemical mechanism, and one is a secondary carrier. The remaining two lipid transporters are secondary carriers.

Transport of nucleobases and their derivatives is accomplished nearly equally by primary active transporters and secondary carriers with 25 and 33, respectively. The remaining four transporters of this specificity are of an unknown mechanism.

Sixty-six transporters have unpredictable substrate specificities. 13 of them are channels, 22 are primary active transporters, 26 are secondary carriers, and 5 are known transporters with unknown biochemical mechanism.

Superfamilies

Table 2 presents an overview of the superfamilies and families found in Tetra. The VIC superfamily is the largest with 374 members. The ABC and MFS superfamilies make up a distant second and third with 136 and 126 proteins, respectively. P-ATPases have a substantial representation with 77 transporters along with MCs with 40 transporters.

Topologies

Figures 3A–D and 4 present an overview of the topologies for all transporters in Tetra and for each of the major transporter classes. 4, 6, 8, 10, and 12 TMSs proteins dominate transporters with odd numbers of putative TMSs. Programs that predict topology are often in error by one or two TMSs; possibly proteins with odd numbered TMSs are overrepresented. Channels mostly have 4 or 6 TMS; secondary carriers usually have 10, 11, or 12 TMSs, and primary active transport proteins have more variable numbers of TMSs (Saier 2003).

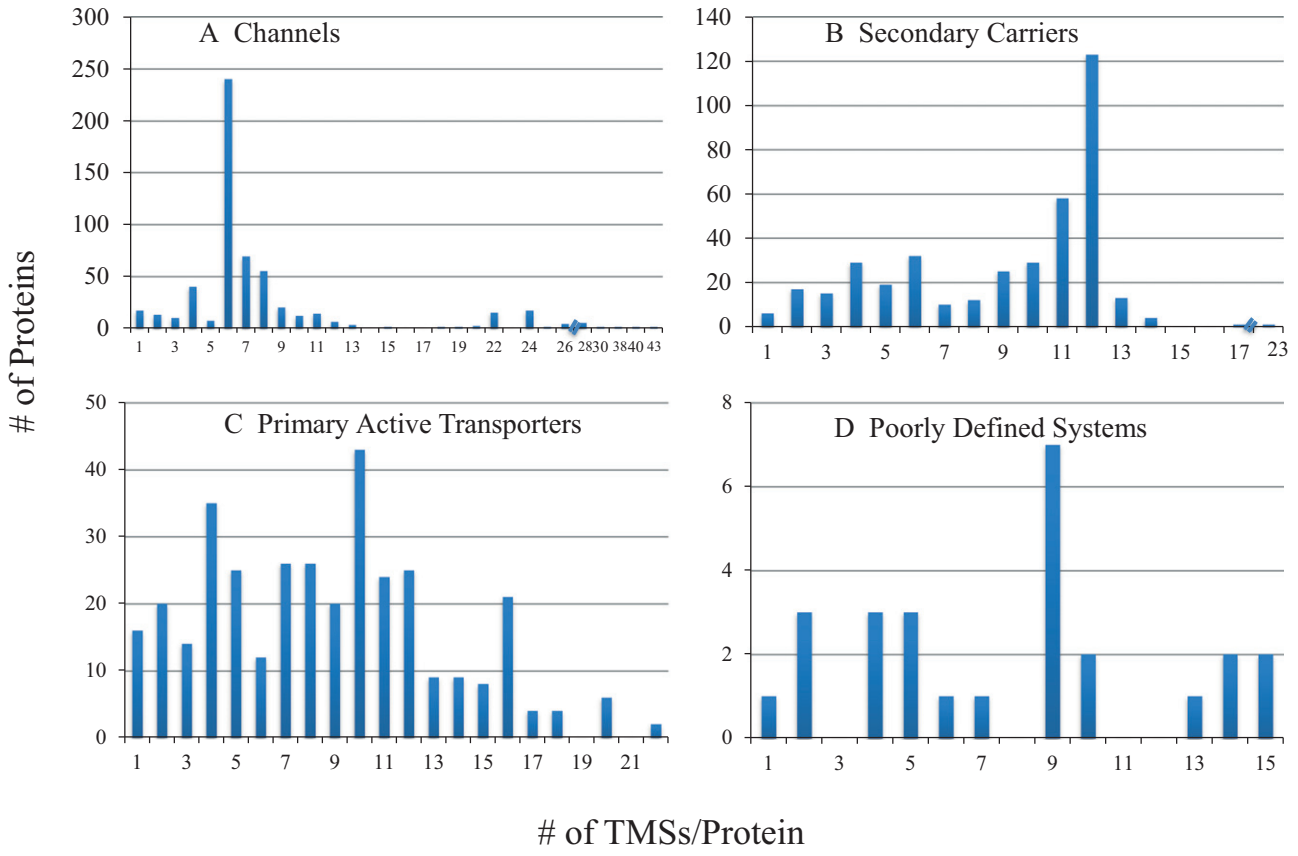

Figure 2 **A–D.** Distribution of predicted topological types among the identified putative transporter proteins in *Paramecium* based on transporter class; Channels (A), secondary carriers (B), primary active transporters (C) and poorly defined systems (D). The number of putative TMSs (*x*-axis) is plotted vs. the numbers of proteins of that topology (*y*-axis).

Ichthyophthirius multifiliis transporters

Overview

Ichthyophthirius multifiliis of strain d serotype (Ich) has a genome that is reported to be 48.7 million base pairs in size and encodes 8,056 putative proteins. Examination of the sequences of these proteins revealed that many of them are fragments, and consequently, the actual number of proteins is less certain than for the other two ciliates studied. The number of transport proteins found in each class and each family is also less certain. Table S3 and Fig. 1C present an overall summary of the classes and subclasses of transporters found in Ich. Of these proteins, 504 are believed to be transport proteins based on the results of a G-BLAST search with a cut-off value of 0.001. Thus, about 6.3% of the predicted proteins encoded within the genome of Ich are homologous to parts of recognized transport proteins.

Ich encodes representatives of TC classes 1, 2, 3, and 9. 164 (32.5%) of these proteins are simple channels; 188 (37.3%) are secondary carriers; 144 (28.6%) are primary active transport proteins, and 8 (1.6%) are of unknown mechanism of action. These percentages differ from those of Para and Tetra, showing that channel proteins were preferentially lost in Ich. Perhaps Ich, being a parasite, is less dependent on signal transduction and tactile behavior.

Of the channel-type proteins, almost all found in Ich are alpha-type channels (Subclass 1.A). No outer membrane porins (Subclass 1.B) and pore-forming toxins (Subclass 1.C) were identified. However, seven proteins belong to the Vesicle Fusion Pore family (Subclass 1.F). The fact that all three ciliates have these proteins is a clear indication that ciliates release neurotransmitters by an exocytosis mechanism similar to that documented in animals.

Secondary carriers (uniporters, symporters, antiporters; Subclass 2.A) comprise the largest class in Ich, with members belonging to several well represented families (see below). The majority of primary active transporters are pyrophosphate bond hydrolysis-driven transporters (Subclass 3.A). No decarboxylation-driven transporters (Subclass 3.B) were found, but there are 6 oxidoreduction-driven H^+ transporters (Subclass 3.D). Lastly, there are eight transporters that belonged to subclass 9.A, known transporters of unknown biochemical mechanism.

Substrate types

Close to half of all transporters found in Ich are involved in the transport of inorganic molecules. Channels account for

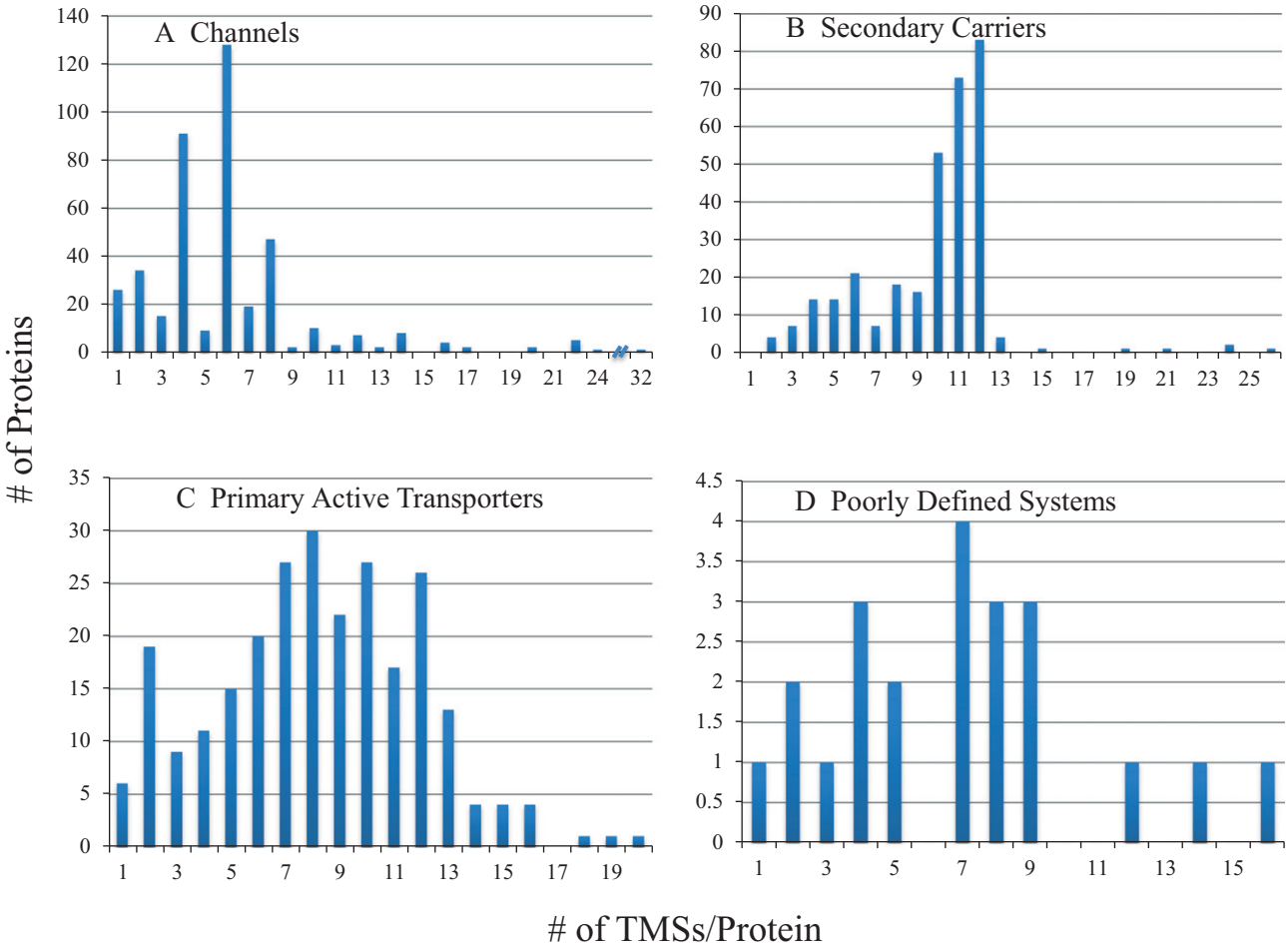

Figure 3 **A–D.** Distribution of predicted topological types among the identified putative transporter proteins in *Tetrahymena* based on transporter class; Channels (A), secondary carriers (B), primary active transporters (C) and poorly defined systems (D). The number of putative TMSs (*x*-axis) is plotted vs. the numbers of proteins of that topology (*y*-axis).

143 of the 230 cation transporters and 10 of the 12 anion transporters. Forty-four primary active inorganic cation transporters and 39 secondary carriers were identified. A few anion-selective secondary carriers were also detected. Lastly, four of the putative cation-selective transporters are of unknown mechanisms of action. No nonselective or transmembrane electron flow carriers were identified in Ich.

Table 1 presents different classes of Ich transporters with the numbers of such transporters as well as the types of substrates transported. Secondary carriers are the largest class for many of these substrates. Categories 2 (carbon sources), 3 (amino acids and their derivatives), 4 (vitamins, cofactors, and cofactor precursors), and 7 (nucleobases and derivatives) are predominantly transported by secondary carriers. With respect to carbon sources, two primary active transporters are involved in the transport of di/tricarboxylates, and two transport organoanions. The rest of the transporters are secondary carriers with the breakdown: 28 specific for sugars and polyols, 5 for monocarboxylates, 8 for di/tricarboxylates, and 2 for aromatic compounds.

Amino acids and their derivatives are transported predominantly by secondary carriers. Of the amino acid transporters in Ich, 7 are channels and the remaining 27 are secondary carrier. A similar pattern is found in the substrate subcategory of amines, amides, polyamines, and organocations where 4 of these proteins form channels and the remaining 18 are secondary carriers. The only peptide transporter present in Ich functions by primary active transport.

Vitamins, cofactors and cofactor precursors are substrates of 22 of the transporters. 20 of these are secondary carriers, and two are of unknown biochemical mechanism of action. Finally, nucleobases and their derivatives are transported mostly by 30 secondary carriers. Just two transporters specific for these compounds are primary active carriers, and a single transporter of unknown mechanism probably transports these substances.

Surprisingly for a pathogen, drugs, dyes, sterols, and toxins are substrates of a relatively small number of transporters in Ich, 19 proteins. The majority of these are multiple drug transporters of which 10 are primary active transport-

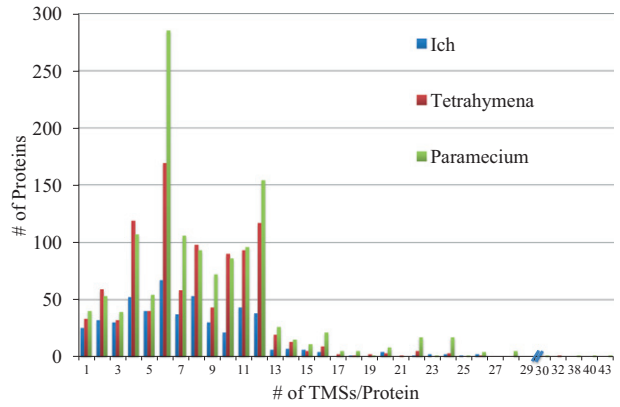

Figure 4 Distribution of predicted topological types among the identified putative transporter proteins in *Paramecium*, *Tetrahymena*, and *Ichthyophthirius*. The number of putative TMSs (*x*-axis) is plotted vs. the numbers of proteins of that topology (*y*-axis).

ers and four are secondary carriers. Pigments and other hydrophobic substances are transported only by primary active transporters.

Macromolecular transporters, which act on substrate proteins and lipids, make up a sizable portion of the transporters found in Ich. The vast majority of them are primary active transporters, which account for 61 of these 66 putative transport proteins. As for the other two ciliates studied, we did not identify even a single complex carbohydrate exporter in Ich. There are 11 protein exporters; 10 are primary active carriers and one is a secondary carrier. Of the 55 transport proteins that act on lipids, primary active carriers play a major role (51 lipid transport proteins), with only three secondary carriers and a single putative lipid transporter of unknown biochemical mechanism.

Substrates could not be predicted for 18 putative transporters. Fifteen of these are primary active transporters while three are secondary carriers.

Superfamilies

As stated before, Table 2 presents a summary of all the superfamilies and families found in Ich. The VIC superfamily dwarfs all other families with 140 members. The MFS and the P-ATPase superfamily come in second and third with 72 and 64 members, respectively. The ABC and MC

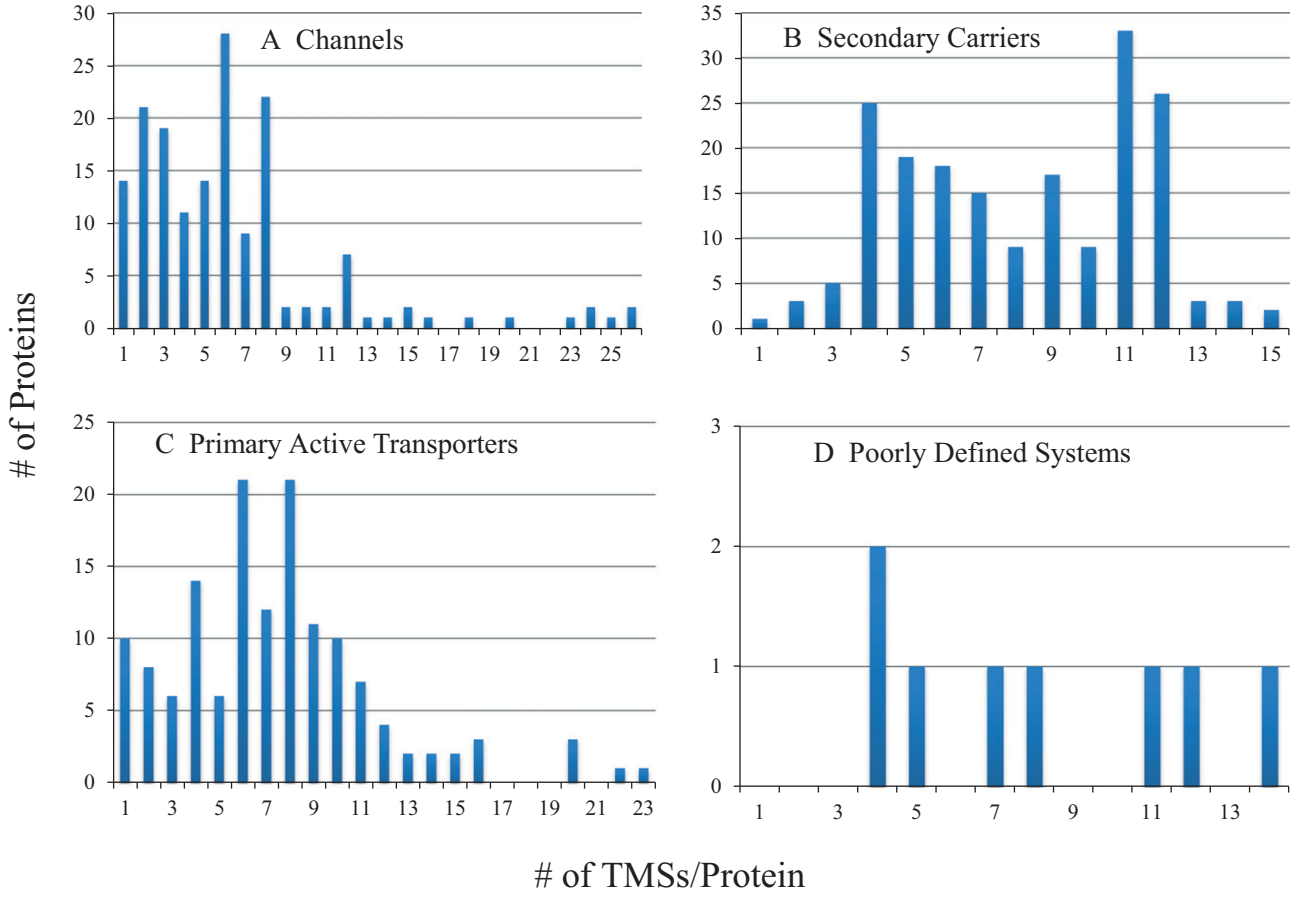

Figure 5 **A–D**. Distribution of predicted topological types among the identified putative transporter proteins in *Ichthyophthirius* based on transporter class; Channels (A), secondary carriers (B), primary active transporters (C) and poorly defined systems (D). The number of putative TMSs (*x*-axis) is plotted vs. the numbers of proteins of that topology (*y*-axis).

families make similar contributions with 40 and 38 family members, respectively. Lastly, Ich also has 16 proteins in the CPA1 family and 6 proteins that are homologs of the c-subunit of V-type ATPases.

Topologies

Figure 5A–D present histograms with an overview of the different topologies found in Ich according to transporter class. Channel proteins with even numbers of TMSs predominate over those with odd numbers. Secondary carriers show a similar trend, but the proteins tend to have more TMSs than found in channels. Primary active transporters have a wide range of topologies, but transporters with 4, 6, and 8 TMSs are most numerous.

Distribution of transporters according to TC family

Table 2 summarizes the distribution of transporters in the three ciliates analyzed according to TC family. Every family for which even a single member was represented is included in Table 2. The highlights will be presented here. As noted above, the VIC superfamily is well represented in ciliates with 425 in Para, 374 in Tetra and 140 in Ich. Most of these belong to the VIC family (1.A.1), but the RIR-CC family (1.A.3) and the PCC family (1.A.5) are also represented. In general, Para has more than Tetra, while Ich has the fewest as expected based on the total number of transporters encoded in these three genomes.

Para has 14 Amt family NH_3 channels while Tetra has only one, and Ich may have up to four. TEGT channels are relatively poorly characterized but may be Ca^{2+} channels (Bullynok et al. 2012). Para has 17 while Tetra has half this number and Ich has none.

Ca^{2+}-regulated Cl^- channels (1.A.17) show an unusual distribution with 44 in Para, 11 in Tetra and 7 in Ich. The large numbers in Para may reflect the whole genome duplications reported only for this organism (Aury et al. 2006). On the other hand, Tetra has over twice the number of presenilin Ca^{2+} leak channels (1.A.54) as Para, while Ich has only one. Possibly greater representation of presenilins in Tetra compensates for the deficiency of TEGT channels in this organism compared to Para. It is also interesting that ciliates have mechanosensitive piezo nocioreceptor (Pitts et al. 2012) cation channels, characterized only in animals (Kim et al. 2012).

MFS carriers occur in ciliates in large numbers with Para > Tetra > Ich. However of the other major superfamilies of carriers, the RND superfamily is over represented in Para, the DMT superfamily is over represented in Tetra, and the APC and amino acid/auxin permease (AAAP) families are equally represented in these two organisms. Surprisingly, Para has four times the number of SSS solute:Na^+ symporter (SSS; 2.A.21) as Tetra while neurotransmitter:Na^+ symporters (NSS; 2.A.22) are hardly represented.

Mitochondrial carriers (2.A.29) are twice as numerous in Para as in Tetra or Ich, and interestingly, there has been

Table 3. Representation of transporters belonging to known families within the MFS listed according to TC number with their substrate ranges and modes of active transport indicated

TC number	Family name	Known substrate range	Para	Tetra	Ich
2.A.1.1	The Sugar Porter (SP) Family	Sugar and sugar derivative (uniport; symport); urate (antiport)	18	31	14
2.A.1.2	The Drug:H^+ Antiporter-1 (12 Spanner) (DHA1) Family	Drug, polyamine, neurotransmitter, sugar, nucleobase/side, siderophore, lipid (antiport); vitamin (symport)	7	9	3
2.A.1.4	The Organophosphate: Pi Antiporter (OPA) Family	Carbohydrate phosphate (antiport)	6	5	3
2.A.1.9	The Phosphate: H^+ Symporter (PHS) Family	Phosphate	4	0	0
2.A.1.11	The Oxalate:Formate Antiporter (OFA) Family	Oxalate/formate (antiport)	6	16	3
2.A.1.13	The Monocarboxylate Porter (MCP) Family	Monocarboxylate	0	3	0
2.A.1.15	The Aromatic Acid:H^+ Symporter (AAHS) Family	Aromatic acid, vitamin (symport)	5	0	0
2.A.1.19	The Organic Cation Transporter (OCT) Family	Organic cation	19	28	19
2.A.1.21	The Drug:H+ Antiporter-3 (12 Spanner) (DHA3) Family	Drug, siderophore (antiport)	0	0	7
2.A.1.22	The Vesicular Neurotransmitter Transporter (VNT) Family	Neurotransmitter	0	0	1
2.A.1.25	The Peptide-Acetyl-Coenzyme A Transporter (PAT) Family	Peptide, glycopeptide, acyl-CoA (symport)	10	3	1
2.A.1.28	The Feline Leukemia Virus Subgroup C Receptor (FLVCR) Family	Unknown	22	13	5
2.A.1.29	The Unknown Major Facilitator-3 (UMF3) Family	Unknown	1	0	0
2.A.1.43	The Putative Magnetosome Permease (PMP) Family	Tetracyline	1	1	1
2.A.1.49	The Endosomal Spinster (Spinster) Family	Unknown	3	1	1
2.A.1.53	The Proteobacterial Intraphagosomal Amino Acid Transporter (Pht) Family	Amino acid	29	11	11
2.A.1.58	The N-Acetylglucosamine Transporter (NAG-T) Family	*N*-acetylglucosamine	1	1	1
2.A.2	The Glycoside-Pentoside-Hexuronide (GPH):Cation Symporter Family	Glycoside-Pentoside-Hexuronide	3	3	4
2.A.71	The Folate-Biopterin Transporter (FBT) Family	Folate-Biopterin	10	1	4

Table 4. Representation of transporters belonging to known families within the MC Superfamily listed according to TC number with their substrate in *Paramecium tetraurelia*, *Tetrahymena thermophila* and *Ichthyophthirius multifiliis*

TC number	Known substrate	Para	Tetra	Ich
2.A.29.1	ATP/ADP	16	11	6
2.A.29.2	Dicarboxylate/tricarboxylate	16	3	6
2.A.29.3	Uncoupling Proteins (H^+)	2	0	1
2.A.29.4	Phosphate	2	2	1
2.A.29.5	Iron	3	3	1
2.A.29.6	Peroxisomal	0	0	1
2.A.29.7	Citrate	3	1	1
2.A.29.8	Carnitine/Acyl carnitine	13	4	4
2.A.29.9	Amino acid	2	0	1
2.A.29.10	Folate	6	3	3
2.A.29.11	ADP-glucose	0	0	1
2.A.29.12	Coenzyme A	1	2	2
2.A.29.13	Succinate/fumarate	2	1	1
2.A.29.14	Aspartate/glutamate	3	4	1
2.A.29.15	Oxaloacetate/malonate/ sulfate/thiosulfate	1	0	0
2.A.29.16	Nucleoside	0	0	1
2.A.29.17	ADP/ATP	0	1	0
2.A.29.18	S-adenosylmethionine	5	3	2
2.A.29.20	Adenine nucleotide	1	0	1
2.A.29.23	ATP-Mg^{2+}/inorganic phosphate	4	2	3
2.A.29.24	Protons and chloride	2	0	0
2.A.29.27	NAD^+	0	0	1

virtually no reduction in the number of Ich MC carriers relative to Tetra. The increased numbers in Para presumably reflect the late genome duplication for this organism that did not occur in Tetra (Aury et al. 2006). However, this situation is the reverse for the equilibrative nucleoside transporter (ENT; 2.A.57) family where there are six times as many family members in Tetra as in Para. The MOP (2.A.66) and ABC (3.A.1) superfamilies also show higher representation in Tetra than in Para.

Of the membrane-bound components of the F- or V-type ATPases in ciliates, only c-subunits were detected in each organism. This could be because c-subunits can also function as ductin channels involved in the release of neurotransmitters (Bloc et al. 2000; Jin et al. 2012; Tomina and Takahata 2012). In addition, all three ciliates have the a-subunit of the V_O domain of V-type ATPases. No other membrane-bound subunit was detected. One explanation could be that ciliate homologs of the other subunits have diverged in sequence so that they were not detected by the G-BLAST search using the cut-off value of 0.001.

The numbers of P-type ATPases (3.A.3), the general secretory pathway proteins (Sec; 3.A.5) and H^+-transporting pyrophosphatases (3.A.10) are nearly double in Para relative to Tetra or Ich. There may be two multicomponent Sec systems in Para rather than one, possibly resulting from the most recent genome duplication event (Aury et al. 2006).

Some of the largest superfamilies were examined to see if a pattern could be observed with respect to the distribution of carriers within the constituent families. Table 3 presents the data for the MFS (Reddy et al. 2012). Sugar porters (SP; 2.A.1.1) of the MFS are well represented, with Tetra having more (31) than Para (18) or Ich (14). The Drug:H^+ antiporter-1 family (DHA1; 2.A.1.2) is also well represented, but surprisingly, the DHA2 family is not represented, and the DHA3 family is represented only in Ich, which has seven members. This last fact may be related to its pathogenicity. Of the MFS anion transporters, all three organisms have OPA family members for the uptake of sugar phosphates, but an inorganic phosphate uptake system (PHS family; 2.A.1.9) is found only in Para. Oxalate:formate antiporters (OFA; 2.A.1.11) are present in all three organisms, but Tetra has more than twice the representation as Para, and Para has twice the representation as Ich. Only Tetra has monocarboxylate porters of the MFS (MCP; 2.A.1.13), but only Para has aromatic acid:H^+ symporters (AAHS; 2.A.1.15). The peptide/acetyl-CoA (PAT) family (2.A.1.25) and especially the Feline Leukemia Virus C Receptor (FLVCR) family (2.A.1.28; transport function unknown) are present with the order of representation being Para > Tetra > Ich. Surprisingly, the Pht family (2.A.1.53), previously identified and characterized as amino

Table 5. Integral membrane ABC export proteins in *Paramecium tetraurelia*, *Tetrahymena thermophila* and *Ichthyophthirius* multifiliis arranged by family with family designations and possible substrate

ABC family TC number	Family designation	Known substrate(s)	Para	Tetra	Ich
3.A.1.106	The Lipid Exporter (LipidE) Family	Lipid	0	2	0
3.A.1.201	The Multidrug Resistance Exporter (MDR) Family (ABCB)	Drug	9	15	3
3.A.1.203	The Peroxysomal Fatty Acyl-CoA Transporter (P-FAT) Family (ABCD)	Peroxysomal long chain fatty acyl	5	2	2
3.A.1.204	The Eye Pigment Precursor Transporter (EPP) Family (ABCG)	Drug	13	34	5
3.A.1.205	The Pleiotropic Drug Resistance (PDR) Family (ABCG)	Drug	0	1	0
3.A.1.207	The Eukaryotic ABC3 (E-ABC3) Family	Unknown	6	4	2
3.A.1.208	The Drug Conjugate Transporter (DCT) Family (ABCC)	Drug conjugate	43	50	14
3.A.1.209	The MHC Peptide Transporter (TAP) Family (ABCB)	Peptide	1	4	0
3.A.1.210	The Heavy Metal Transporter (HMT) Family (ABCB)	Heavy metal	1	2	1
3.A.1.211	The Cholesterol/Phospholipid/Retinal (CPR) Flippase Family (ABCA)	Cholesterol/Phospholipid	25	22	12
3.A.1.212	The Mitochondrial Peptide Exporter (MPE) Family (ABCB)	Peptide	2	0	1

Table 6. Integral membrane P-type ATPase proteins in *Paramecium tetraurelia*, *Tetrahymena thermophila* and *Ichthyophthirius multifiliis* arranged by family with family substrate(s) indicated

TC number	Known substrate range	Para	Tetra	Ich
3.A.3.1	Na^+, K^+	9	20	5
3.A.3.2	Ca^{2+}	34	9	13
3.A.3.5	Cu^{2+}	2	2	0
3.A.3.8	Lipid/phospholipid	65	27	35
3.A.3.10	Ca^{2+}	1	1	1
3.A.3.14	Mn^{2+}	5	0	2
3.A.3.15	Mn^{2+}	2	4	0
3.A.3.16	Mn^{2+}	4	6	4
3.A.3.19	Unknown	0	0	1
3.A.3.20	Unknown	11	8	4

acid porters only in a restricted group of bacteria, is well represented with 29 members in Para and 11 each in Tetra and Ich. All other MFS families are either absent or represented in low numbers.

The APC superfamily (Wong et al. 2012) shows very restricted occurrence in ciliates. The largest of these families in nature, the APC family, is not even represented as is true of several other families specific for amino acids. However, the AAAP family (2.A.18), a constituent of the APC superfamily, is equally represented in Para and Tetra with 22–24 members while Ich has 5. The NSS family (2.A.22) has lower representation as noted above, but members are found in all three organisms. No other member of an APC family was identified.

Mitochondrial carriers (Gutierrez-Aguilar and Baines 2013) show a more uniform distribution in the three ciliates (Table 4). Thus, the ATP/ADP exchangers are most numerous followed by the di/tricarboxylic acid exchangers, the carnitine:acylcarnitine antiporters and the folate transporters in that order. However, 22 MC superfamily families were identified in these ciliates, most families being represented by just one or a few members in any one organism.

As might be expected, ABC uptake and bacterial export systems are essentially lacking in ciliates. However the eukaryotic Drug Conjugate Transporter family is by far the best represented, with 43, 50 and 14 members for Para, Tetra, and Ich, respectively (Table 5; Al-Shawi 2011; Cuthbertson et al. 2010; Zolnerciks et al. 2011). The cholesterol/phospholipid flippase family is in second place followed by the Eye Pigment Precursor family that is of unknown function in ciliates.

P-type ATPase families show an unusual distribution in ciliates (Table 6). Lipid flippases (3.A.3.8) are by far more numerous than members of any other P-ATPase family. While Para has 65 such systems, Tetra has 27 and Ich has 35. Ca^{2+}-ATPases were found in smaller numbers, but similar relative proportions (34; 9; 13), but Na^+, K^+-ATPases show much greater representation in Tetra than either Para or Ich. Five families of unknown substrate specificity are also found in the ciliates (Thever and Saier 2009). Some of these (families 3.A.3.14 – 16) may function in manganese detoxification (Chesi et al. 2012).

Characterized transporters in *P. tetraurelia* and *T. thermophila*

Relatively few transporters have been characterized from ciliates, and almost all of those that have been characterized are derived from Para and Tetra. The functionally characterized systems that we have identified in literature searches are tabulated in Table 7 for Para and Table 8 for Tetra. These tables also list some proteins that proved to be distantly related to any previously entered proteins in TCDB as they increase the scope of the database. References for the functionally characterized proteins are provided in the table.

In Para, two potassium channel proteins of the voltage-gated ion channel family (1.A.1) have been characterized. These two proteins are both cyclic nucleotide regulated channels belonging to the same VIC subfamily (1.A.1.5) (Jegla and Salkoff 1995; Ling et al. 1998). Eight proteins were identified that belong to the Ryanodine-Inositol 1,4,5-triphosphate Receptor Ca^{2+} Channel (RIR-CaC) Family (Table 7). Calcium-release channels belong to this family (Docampo et al. 2014; Ladenburger and Plattner 2011; Ladenburger et al. 2009; Plattner et al. 2012). Three of these proteins are annotated as inositol 1,4,5-triphosphate like receptors (Docampo et al. 2014). One member appears to occur in the contractile vacuole where it functions in calcium homeostasis and osmoregulation by promoting the expulsion of water and cations (Ladenburger et al. 2006). Interestingly, all eight of these channels more closely resemble the inositol-triphosphate receptors of animals than the ryanodine receptors of animals (see TCDB). Although, not characterized, we identified calcium-activated chloride channel (CaClC) homologs (1.A.17) in two distinct subfamilies. Also identified but not characterized are single members of the mechanosensitive Piezo Family (1.A.75) and the mitochondrial Mg^{2+}/Ca^{2+} Uniporter (MCU) Family (1.A.77).

Two porin proteins, most likely in two different organelles, one in mitochondria and one in peroxisomes were identified. Of these, only the mitochondrial protein (VDAC family) has been characterized (Ludwig et al. 1989). This porin exhibits the properties of a voltage-gated general diffusion porin with cation-selectivity and a pore diameter of 1.3 nm. Two large putative functionally uncharacterized toxins, homologous to bacterial Vegetative Insecticidal Protein (Vip3) Family (1.C.105), were identified and entered into TCDB.

A Para magnesium transporter in the CaCA family (2.A.19) has been characterized both structurally and functionally (Haynes et al. 2002; Preston and Kung 1994). As is typical of members of this family, this protein exhibits 10 putative TMSs in a 5 + 5 TMSs arrangement and behaves like a channel. The mutant form is called "eccentric" and exhibits backwards-swimming behavior (Preston and Kung 1994).

Several ATP driven transporters were also identified (see Table 7). One is an ABC transporter (3.A.1.207.1); a second is a V-type ATPase with 13 subunits, and the third and fourth are two calcium P-type ATPases, one being the plasma membrane-type (PMCA) (Wassmer et al. 2006, 2009) and the other being the endoplasmic reticulum type (SERCA).

Table 7. Transport proteins from *Paramecium tetraurelia* in TCDB

TCID	Uniprot accession no.	No. of AA	TMSs	Description from TCDB
1.A.1.5.21	Q6JV75	543	7	K^+ channel protein, PAK2.1 of 543 aas. Contains a cyclic nucleotide-binding domain (Jegla and Salkoff 1995; Ling et al. 1998)
1.A.1.5.22	O96386	772	6	K^+ channel protein, PAK11-MAC of 772 aas. Contains a cyclic nucleotide-binding domain (Jegla and Salkoff 1995; Ling et al. 1998)
1.A.3.2.4	A0CX44	3,036	4	The Inositol 1,4,5- triphosphate (InsP3)-like receptor (3,036 aas) (Docampo et al. 2014; Ladenburger et al. 2009)
1.A.3.2.7	Q3SDX7	2,888	4	Contractile vacuole complex calcium-release channel (CRC)II; IP3Rn (Ladenburger et al. 2006). Functions in osmoregulation by promoting expulsion of water and some ions including Ca^{2+}. Also functions in calcium homeostasis (Docampo et al. 2014; Ladenburger et al. 2006)
1.A.3.2.8	G7ZUA6	2,021	4	Putative IP3R calcium-release channel VI-3 of 2,021 aas (Docampo et al. 2014)
1.A.3.2.9	G7ZU97	2,972	6	CRCI-1a; IP3R. Functions similarly to TC# 1.A.3.2.7 (Docampo et al. 2014)
1.A.3.2.10	G7ZUA0	2,598	4	Calcium-release channel III, CRCIII1a of 2,598 aas. Associated with recycling vesicles engaged in phagosome formation (Ladenburger and Plattner 2011)
1.A.3.2.11	G7ZUA3	3,127	6	Calcium-release channel IV3b, CRCIV3b, of 3,127 aas. Display structural and functional properties of ryanodine receptors (Ladenburger et al. 2009). Localized to the alveolar sacs of the cortical subplasmalemmal Ca^{2+}-stores (Plattner et al. 2012). Involved in exocytosis in response to ryanodine receptor agonists (Docampo et al. 2014)
1.A.3.2.12	G7ZUA4	2,589	4	Calcium-release channel V-4b, CRCV4b of 2,589 aas. Occurs in parasomal (alveolar) sacs (clathrin coated pits) (Docampo et al. 2014)
1.A.3.2.13	G7ZUA5	2,774	4	Calcium-release channel VI-2b, CRCVI2b. Localized to the contractile vacuole (Docampo et al. 2014)
1.A.17.2.2	A0CAP8	1,610	10	Ca-ClC Family homolog
1.A.17.3.1	A0CIB0	1,371	7	Ciliate CaClC homolog
1.A.75.1.5	A0EF36	2,544	41	Hypothetical protein, HP (2,544 aas; > 40 TMSs)
1.A.77.1.8	A0E7U6	352	2	Ciliate MCU homolog 362 aas; 2 TMSs
1.B.8.3.2	Q3SE03	305	0	Mitochondrial porin of 305 aas (Porin3_VDAC superfamily). Exhibits the properties of a voltage-dependent general diffusion porin with cation-selectivity and a pore diameter of 1.3 nm (Ludwig et al. 1989)
1.B.69.1.7	A0BTG7	202	4	Uncharacterized protein of 202 aas and 4 TMSs
1.C.105.2.3	A0EEH7	973	0	Uncharacterized protein of 973 aas. This protein is also homologous to the nucleoporin, 1.I.1.1.1; Q02455 and the TypeIV protein secretion system, 3.A.7.12.1; O25262
1.C.105.2.5	A0DML3	1,418	0	Uncharacterized protein of 1,418 aas
2.A.19.9.1	Q8I0U1	550	11	Mg^{2+} transporter (Mg^{2+}-specific channel-like exchanger) of 550 aas (Haynes et al. 2002; Preston and Kung 1994). Has 10 putative TMSs in a 5 + 5 TMS arrangement and exhibits properties of a channel (Haynes et al. 2002). The mutant form is called 'eccentric' and exhibits backwards-swimming behavior (Preston and Kung 1994)
2.A.66.3.4	A0D5K0	469	14	Hypothetical protein; RFT1 homolog
2.A.105.1.5	A0CVJ5	117	3	MPC (117 aas; 3 TMSs)
3.A.1.207.1	A0ECD9	1,209	10	The hypothetical protein, HP (1,209 aas; 10TMSs:1+6+3; 2-4 are homologous to 8–10; the FtsX domain) (*P. tetraurelia* has at least five paralogues)
3.A.2.2.8	Multicomponent			V-type ATPase with 13 subunits (Wassmer et al. 2009)
3.A.3.2.37	O76974	1,036	10	SERCA P-type ATPase of 1,036 aas
3.A.3.2.38	Q3SEE6	1,146	9	Plasma membrane Ca^{2+} ATPase (PMCA) of 1,146 aas (Plattner 2014)
9.A.6.1.2	A0DG78	879	14	Mcd4 homolog

Table 8 lists the functionally characterized and unique proteins found in Tetra. Most of these have not been functionally characterized, and only those that have not already been described for Para will be discussed here. One interesting finding is that Tetra appears to possess at least three distinct MACPF domain-containing proteins in two different subfamilies (1.C.39.6 and 7). A probable multidrug-resistance MATE efflux pump (2.A.66) and an ABC MDR transporter were identified as were several other ABC transporters, probably involved in iron/sulfur transport and long chain fatty-acid transport. Finally, several P-type ATPases belonging to different subfamilies were also identified (Thever and Saier 2009).

DISCUSSION

Overview of ciliate transporters

Our laboratory is concerned with transport protein function, evolution and ecology. In order to understand these

Table 8. Transport proteins from *Tetrahymena thermophila* in TCDB

TCID	Uniprot accession no.	No. of AA	TMSs	Description
1.A.3.2.3	Q23K98	2,872	6	The cation channel family protein, IsnP3-like protein (2,872 aas)
1.A.43.2.1	Q23G77	307	6	CrcB-like protein of 307 aas and 6 TMSs in an apparent 3 + 3 arrangement
1.A.77.1.15	I7M0N6	362	2	Mitochondrial calcium uniporter, MCU, of 362 aas
1.B.8.3.1	Q22Z08	309	0	Putative mitochondrial porin of 309 aas (Porin3_VDAC superfamily)
1.C.39.6.2	Q23MJ4	342	1	MACPF domain-containing protein (342 aas)
1.C.39.6.3	Q23I78	681	1	Duplicated MACPF protein (681 aas) The first half resembles 1.C.39.6.2 more than the second half
1.C.39.7.1	Q23QV5	518	1	MAC/Perforin domain protein
1.C.105.2.4	Q24GL4	642	4	Uncharacterized protein of 642 aas
2.A.18.10.4	Q24DJ2	519	11	AAAP homolog
2.A.19.9.2	I7M0U0	625	10	Probable Mg^{2+}-specific channel-like exchanger of 625 aas
2.A.66.1.17	Q22BG1	605	11	MATE efflux pump, MatE
3.A.1.201.19	Q22BT5	663	4	Mitochondrial iron/sulfur complex transporter, AbcB13 of 663 aas
3.A.1.201.20	I7LXH0	1,334	9	Putative multidrug resistance transporter of 1,334 aas, AbcB15
3.A.1.203.10	I7MJ28, I7LWG7	719, 694	6, 6	Long chain fatty-acid transporter consisting of a heterodimer of AbcD1 (719 aas) and AbcD2 (694 aas)
3.A.1.207.2	Q22NS1	1,234	10	Putative permeases; Duf214 protein [1,234 aas; 10TMSs: 1+6+3; 2–4 are homologous to 8–10 (the FtsX domain)]
3.A.1.210.10	I7MMI0	542	1	Mitochondrial ABC iron/sulfur complex transporter, AbcB12 of 542 aas
3.A.2.1.4	Multisubunit			The F-type ATPase of the ciliate, *Tetrahymena thermophila* (strain SB210). The eight established or putative ATPase subunits are provided. These are distantly related to subunits of other studied F-type ATPases (Balabaskaran Nina et al. 2010). Other associated proteins/subunits are presented by Balabaskaran Nina et al. (2010)
3.A.3.10.11	Q23QW3	1,328	7	This protein was previously designated the functionally uncharacterized P-type ATPase 16 (FUPA16) (Thever and Saier 2009). Probable manganese exporter by similarity
3.A.3.10.12	Q23QV7	1,982	10	P-type ATPase of 1,982 aas
3.A.3.10.15	I7MI73	1,807	16	This protein was previously designated the functionally uncharacterized P-type ATPase 19 (FUPA19 of 1,807 aas) (Thever and Saier 2009). The unusually large size and number of TMSs is unique to this protein. Whether this is a consequence of an artifact of sequencing is not known. It may be a Mn^{2+}-ATPase (by similarity)
3.A.3.10.16	Q22V52	1,072	9	This protein was previously designated the functionally uncharacterized P-type ATPase 20 (FUPA20) (Thever and Saier 2009). It may be a Mn2+-exporting ATPase (by similarity)
9.A.54.3.1	Q22WA5	612	9	LMBR1-like conserved protein

crucial aspects of transport proteins, we have created a classification system maintained in the TCDB, adopted by the International Union of Biochemistry and Molecular Biology as the only internationally acclaimed system of transport protein classification. This system has been extensively described in publications from our laboratory (Busch and Saier 2002; Saier 2000; Saier et al. 2006, 2009, 2014). It allows one to screen fully sequenced genomes (or proteomes) to identify recognizable transport proteins encoded within the genome of any organism, using software developed specifically for this purpose (Reddy and Saier 2012).

Application of the G-BLAST program to the three ciliates described in this paper revealed an interesting relationship, namely that the percentages of integral membrane transport proteins encoded within the genomes of these organisms are inversely proportional to their genome sizes. Thus, the genome encoding the largest number of proteins belongs to Para with 39,600 proteins, but only 1,326 recognized integral membrane transport proteins (3.3%) were identified. Tetra, encoding 24,800 proteins, codes for 1,017 recognized integral membrane transporters (4.1%). Ich, encoding 8,100 proteins, has 6.2% of its proteins as integral membrane transport proteins. From this observation, it seems clear that genome content expansion, as in the case of Para, resulted in the preferential loss of transporters over evolutionary time, while genome reduction, as in the case of Ich, resulted in preferential retention of transport proteins. It should be noted that the percentages of transporters encoded within the genomes of these ciliates are underestimates, first, because we are only recording integral membrane constituents of these systems (not the energizing proteins such as ATPases, associated regulatory proteins and other auxiliary proteins that may be required or facilitate transport), and second, because ciliates represent a distinct phylogenetic group of organisms with great protein sequence divergence as compared to other organisms, probably leading to some transport protein homologs that could not be recognized by G-BLAST using the cut-off selected for these studies, 0.001 (Reddy and Saier 2012). For example, outer membrane mitochondrial porins of the VDAC family (1.B.8)

were not identified by G-BLAST, but using PSI-BLAST with iterations, these proteins could be identified and were entered into TCDB. Subsequent TC BLAST searches revealed that use of Tetra VDAC as the query sequence retrieved VDAC family members in plants, fungi and slime molds with values greater than (poorer than) 0.001, while the Para VDAC proved to be too distantly related to any of the VDAC family members in TCDB except those derived from other ciliates to be retrieved. In addition, we did not detect certain subunits of large transport protein complexes such as the F- and V-type ATPases even though these subunits must be present. In fact, ciliate F- and V-type ATPase components have been reported to be present but highly sequence divergent (Balabaskaran Nina et al. 2010; Wassmer et al. 2009). In agreement with this conclusion, we could identify integral membrane c-subunits but not a- and b- subunits. In fact, all organisms need F-type ATPases to interconvert chemical (ATP) and chemiosmotic (*pmf*) energy, and almost all eukaryotes need V-type ATPases to acidify intracellular organelles.

Channel proteins

In previous studies, animals had been found to possess more channels than almost any other type of eukaryote (Ren and Paulsen 2005). Thus, mammals have about 37%, *Caenorhabditis elegans* has 36% and *Drosophila melanogaster* has 26% while all other eukaryotes including plants, fungi and most unicellular eukaryotes as well as prokaryotes have far fewer channels. Our studies revealed that ciliates have even higher percentages of channel proteins than do mammals, 41% for Tetra and 42% for Para, and as Para and to a lesser extent Tetra have more genes than do humans and mice, these unicellular eukaryotes have far more channels. Most of these are cation-specific channels of the VIC family, in agreement with an earlier report suggesting that ciliates have more K^+ channels than animals (Haynes et al. 2003). These channels play important roles in ciliates in generating action potentials that control exocytosis, motility, tactic, and tactile behavior (Kung and Saimi 1982; Mohamed et al. 2002; Oami and Takahashi 2003; Schwab et al. 2008; Yano et al. 2013). They may also serve other functions currently unrecognized.

Individual families were first compared between mammals (humans are cited below) and ciliates (Para and Tetra). Although these two ciliates show marked differences, our comparisons with mammals represent a general overview, assuming that these organisms are representative of their respective kingdoms. The ciliates have far more VIC family (1.A.1) and RIR-CaC (1.A.3) channels than do humans, but they have virtually no TRP-CC channels (1.A.4; 21 for humans, 0 or 1 for the ciliates) or GIC channels (1.A.10; 19 for humans, 0 or 2 for the ciliates) (Docampo et al. 2014; Plattner 2014). These three organisms have similar numbers of PCC channels (1.A.5; 11 for humans, 7–8 for ciliates). All of these channel families belong to the VIC superfamily (Chang et al. 2004; Lam et al. 2011). These results demonstrate a tremendous dependency on VIC channels as well as ryanodine- and inositol-triphosphate receptor calcium channels (Lencesova and Krizanova 2012; Van Petegem 2012), but a nearly complete loss of TRP calcium channels (Abramowitz and Birnbaumer 2009; Woodard et al. 2007) relative to mammals. As mammalian TRP-CC family members mediate sensitivity to numerous stimuli including pain, noxious compounds, temperature, pH, anesthetics, toxins, mechanical stimuli, osmotic pressure, etc., it can be concluded that ciliates either lack sensitivity to many of these agents and conditions, or they use different types of channels to detect them. While Ca^{2+}-activated and Ca^{2+}-calmodulin-dependent Na^+ and K^+ conductances (Clark 1995; Ishida et al. 1991; Preston et al. 1990; Saitow et al. 1997) as well as K^+-induced Ca^{2+} conductances (Oami and Takahashi 2003) and Mg^{2+} currents (Preston and Kung 1994) have been documented, in few cases, have the channel proteins responsible for these activities been identified. Thermal adaptation in ciliates has also been documented (Krenek et al. 2012) although again, the molecular constituents involved remain to be elucidated. The studies reported here provide the first clues as to the channel types that are likely to be responsible.

Some channel families are over represented in Para compared to Tetra. These include the ammonium channels of the AMT family (1.A.11) with 14 members in Para and only one in Tetra and the CaCIC chloride channels (1.A.17) with 44 members in Para but only 11 members in Tetra. By contrast, Tetra has two and a half times more presenilin Ca^{2+} channels than Para. All ciliates have proteins of the Synaptosomal Vesicle Fusion Pore family (1.F.1), but Para has about three times as many of these proteins as does Tetra or Ich. This observation together with the selective occurrence of NSS (2.A.22) suggests that the activities of at least some of the channel systems described above are likely to be sensitive to neurotransmitter regulation as is true in animals.

Secondary carriers

Most eukaryotes have large numbers of secondary carriers and fewer ATP-hydrolysis-driven primary active transporters. For different eukaryotes, including animals, plants and fungi, percentages of secondary active transporters range between 44% and 85% while primary ATP driven pumps range between 11% and 22% (Ren and Paulsen 2005). By contrast, Para and Tetra possess fewer secondary carriers (30–32%) but more primary active transport proteins (~25%). These observations suggest that ciliates depend more on primary sources of energy such as ATP to drive transport than secondary sources of energy such as proton and sodium electrochemical gradients (*pmf* and *smf*). This observation could reflect the primary sources of energy generated by ciliates as observed for many other organisms (Paulsen et al. 2000), but it could also be a consequence of the use of ion gradients for purposes of generating action potentials that control tactile and tactic behaviors. Perhaps utilization of ion gradients for action potential generation results in decreases and fluctuations

in the magnitudes of these gradients, preventing their use for purposes of nutrient acquisition.

Analysis of secondary carriers by family revealed that in many cases, the three ciliates have overall representation as expected based on total number of genes. However, in some families, major differences were observed between Para and Tetra. The MFS, the largest superfamily of secondary carriers found in nature, is also the dominant superfamily found in ciliates with numbers essentially proportional to genome content. Among the dominant representatives of the MFS family (2.A.1) within the MFS superfamily, we find large numbers of transporters for sugars, drugs, organic cation/anions, peptides, and heme as well as proteins of unknown specificities. In addition, smaller numbers of these porters are specific for sugar phosphates, inorganic phosphate and sphingosine 1-phosphate. For one family of the MFS, the FBT family (2.A.71), specific for folate, and biopterin analogues, Para has 10 members while Tetra has only 1.

Most divalent cation transporters are found in the two non-pathogenic ciliates in similar numbers. Surprisingly lipid export proteins of the RND superfamily are overrepresented in Para, while DMT superfamily nucleotide sugar exchange porters in the golgi and endoplasmic reticulum are overrepresented in Tetra. However, the APC superfamily is equally represented in these two organisms. Within the APC superfamily, some surprising results were obtained. For example, for most organisms, the nearly ubiquitous APC family (2.A.3) is more prevalent than other families in this superfamily, but this family is not represented in any one of the three ciliates studied. Instead, the AAAP family (2.A.18) is dominant with 22 and 24 members in Para and Tetra, respectively, and the Neurotransmitter:Sodium Symporter family (NSS; 2.A.22) is equally represented in these two ciliates. Members of the other nine families within the APC superfamily (Wong et al. 2012) were not found in any of the three ciliates studied. We conclude that ciliates use different sets of amino acid transporters than most previously studied organisms, a fact that may have evolutionary and metabolic significance. The differences observed presumably reflect the differing substrate specificities of carriers included within these three families. For example, APC family members transport polyamines and organocations as well as amino acids, while AAAP and NSS family members are more specific for amino acids. This fact tentatively suggests that ciliates do not utilize polyamines and organocations as sources of nutrition.

The MC family (2.A.29) has twice the representation in Para as in Tetra, but with essentially no diminution in Ich relative to Tetra. Members of the MC superfamily provide metabolic communication between the cytoplasm and the mitochondrial matrix (Ferramosca and Zara 2013; Palmieri et al. 2011). It can be concluded that Para took advantage of the increased numbers of MCs following its last genome duplication, perhaps to benefit the organism by increasing the gene dosage (Aury et al. 2006). The loss of these carriers would have disadvantaged Ich during its genome size reduction, as Para, not Tetra or Ich, preferentially utilizes di- and tricarboxylates over monocarboxylates, because the di- and tricarboxylates depend largely upon mitochondria for metabolism. These considerations account for the retention of all of these carriers relative to Tetra and Ich.

The CPA superfamily includes two families responsible for the exchange of monovalent cations (Na^+ and K^+) for protons (H^+), CPA1 (2.A.36), and CPA2 (2.A.37). All three ciliates have about 20 CPA1 family members, but the CPA2 family is lacking, even though members of both families catalyze similar reactions. However, CPA1 family members are predominantly Na^+/H^+ exchangers while CPA2 family members are predominantly K^+/H^+ exchangers, and while CPA1 family members are found ubiquitously, CPA2 members are not found in all types of organisms. For example, while CPA2 members are prevalent in bacteria, plants, fungi, and slime molds, they are not found in animals, certain single celled eukaryotes and most archaea. It seems likely that ciliates need sodium:proton antiporters more than potassium:proton antiporters, and that ciliates are among the organismal types that lack CPA2 family members. In this regard and several others, such as the profusion of VIC family members, we note parallels between ciliates and animals that do not occur with other well studied eukaryotic phyla.

The equilibrative nucleoside transporter family (ENT; 2.A.57) has only two members in Para but twelve members in Tetra and four in Ich. Substantial representation is observed for the MOP superfamily with similar numbers in the two non-pathogenic ciliates (15 and 18 for Para and Tetra, respectively) with a threefold decrease for Ich. All of these MOP superfamily members appear to function in toxic compound export, although many multidrug-resistance exporters also export natural products (Kung and Saimi 1982; Mohamed et al. 2002; Oami and Takahashi 2003; Saier and Paulsen 2001; Saier et al. 1998; Schwab et al. 2008; Yano et al. 2013, see TCDB). These differences between the three ciliates studied presumably reflect their different natural environments and metabolic needs.

Primary active transporters

The two dominant families of primary active transporters in ciliates are the ABC and P-type ATPase families (3.A.1 and 3.A.3, respectively; Table 6). Tetra has 136 ABC transporters while Para has 105 and Ich has 40. By contrast, Para has 133 P-type ATPases while Tetra has 77 and Ich has 65. Thus, Tetra has more ABC permeases than Para, but Para has almost twice as many P-type ATPases. Furthermore, while Ich has lost more than two-thirds of its ABC transporters, it has retained almost all of the P-type ATPases found in Tetra. The situation observed for P-type ATPases is similar to that for MCs, and the same arguments may apply.

Of the ABC porters, all belong to families 201–212, members of which are derived predominantly from eukaryotes. Not even one ABC family member in ciliates belongs to one of the many prokaryotic exporter families, even though these can be found in other eukaryotes. This

observation suggests that there has been very little horizontal transfer of genetic material between ciliates and bacteria, an observation that contrasts with cellular slime molds and fungi where horizontal transfer from bacteria has been appreciable (Andersson 2011). An explanation for the presence of bacterial genes in slime molds and fungi has traditionally been that these eukaryotes live together with bacteria and use them as sources of nutrition. However, ciliates are also known to contain internal bacteria and use them as food sources (Kisand and Noges 2004; Muylaert et al. 2002; Sime-Ngando et al. 1999).

A large fraction of the ABC porters in ciliates appear to be drug export proteins (families 201, 204 and 208; Table 6). However, family 203 porters are peroxysomal Acyl-CoA transporters, while porters of families 209 and 212 may be peptide transporters. These last mentioned families, however, are underrepresented compared to the drug exporters. It is noteworthy that there are large numbers of proteins belonging to family 211, all of which are likely to be lipid/sterol exporters. Many drug exporters have also been shown to act on lipids and other natural products (Quazi and Molday 2011). Family 207, consisting of ABC3 porters of unknown specificity (Wang et al. 2009), is well represented in ciliates although absent in multicellular eukaryotes. This is an example where the parallels between ciliates and animals break down.

P-type ATPases found in ciliates include Na^+, K^+-ATPases (3.A.3.1), Ca^{2+}-ATPases (3.A.3.2), copper-ATPases (3.A.3.5), and lipid-ATPases (3.A.3.8). Families 3.A.3.10-20 are of unknown function, but those within families 14, 15, and 16 may transport manganese (Chesi et al. 2012; Thever and Saier 2009). H^+-, Mg^+-, and heavy metal-ATPases are not present in ciliates although they are present in large numbers in plants, fungi and many single celled eukaryotes. They are also absent in animals, providing another parallel between ciliates and animals. As ciliates (e.g., Para) have been reported to be resistant to certain heavy metals, Zn^{2+}, Cd^{2+}, and Ni^{2+} (Rehman et al. 2009), it seems reasonable to suggest that ciliates are suitable agents of bioremediation in polluted wastewaters (Gertler et al. 2010).

Of course, ciliates possess F-type and V-type ATPases (3.A.2), but as noted above, we were not able to identify all proteins known to be integral membrane components of these systems. It is nevertheless interesting to note that Para has more than twice as many c-subunit homologs as does Tetra. The same observation was made for constituents of the general secretory pathway (Sec; 3.A.5); see Table 2. However, the mitochondrial protein translocon (MPT; 3.A.8) has equal numbers in Para and Tetra and half this number in Ich. Interestingly, Para has three to four times more H^+-translocating pyrophosphatases (PPases: 3.A.10) and peroxisomal protein importer constituents (PPI; 3.A.20) than Tetra, perhaps a consequence of the late genome duplication event that occurred in Para but not Tetra.

CONCLUDING REMARKS

In conclusion, generalizing from the best-characterized ciliates for which whole genome data are available, ciliates have an extraordinary number of channel proteins, more than any other type of organism studied, as far as we are aware. They resemble animals more than other types of eukaryotes in terms of their complements of transporters including P-type ATPases, ABC exporters, and Na^+:H^+ antiporters. They devote a large fraction of their transport repertoire to inorganic cation homeostasis and signaling but have fewer secondary carriers and more primary active transporters than most other eukaryotes. Except for MDR pumps, primary active ABC transporters are used almost exclusively for macromolecular (protein and lipid) transport although polysaccharide exporters were not detected. In fact, ciliates secrete proteins and carbohydrates, and use a variety of polysaccharides for nutrition (Arregui et al. 2007; Profousova et al. 2011; Wereszka and Michalowski 2012). The paucity of polysaccharide export proteins could be explained if ciliates use exocytosis for polysaccharide export (Cheng and Hufnagel 1992; Gutierrez and Orias 1992; Turkewitz 2004). The studies reported here provide a wealth of novel information relevant to the ecology, evolution, physiology, and pathology of model ciliates and probably to the characteristics of many other ciliates of industrial and bioremediative importance (Dubber and Gray 2011; Gertler et al. 2010; Harikrishnan et al. 2010).

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1. TC classification and functional predictions of putative transport proteins found in *Paramecium*.
Table S2. TC classification and functional prediction of putative transport proteins from *Tetrahymena*.
Table S3. TC classification and functional predictions of putative transport proteins from *Ichthyophthirius*.