TRAP transporters: an ancient family of extracytoplasmic solute-receptor-dependent secondary active transporters

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Tripartite ATP-independent periplasmic transporters (TRAP-T) represent a novel type of secondary active transporter that functions in conjunction with an extracytoplasmic solute-binding receptor. The best characterized TRAP-T family member is from *Rhodobacter capsulatus* and is specific for C₄-dicarboxylates [Forward, J. A., Behrendt, M. C., Wyborn, N. R., Cross, R. & Kelly, D. J. (1997). *J Bacteriol* 179, 5482–5493]. It consists of three essential proteins, DctP, a periplasmic C₄-dicarboxylate-binding receptor, and two integral membrane proteins, DctM and DctQ, which probably span the membrane 12 and 4 times, respectively. Homologues of DctM, DctP and DctQ were identified in all major bacterial subdivisions as well as in archaea. An orphan DctP homologue in the Gram-positive bacterium *Bacillus subtilis* may serve as a receptor for a two-component transcriptional regulatory system rather than as a constituent of a TRAP-T system. Phylogenetic data suggest that all present day TRAP-T systems probably evolved from a single ancestral transporter with minimal shuffling of constituents between systems. Homologous TRAP-T constituents exhibit decreasing degrees of sequence identity in the order DctM > DctP > DctQ. DctM appears to belong to a large superfamily of transporters, the ion transporter (IT) superfamily, one member of which can function by either protonmotive force- or ATP-dependent energization. It is proposed that IT superfamily members exhibit the unusual capacity to function in conjunction with auxiliary proteins that modify the transport process by providing (i) high-affinity solute reception, (ii) altered energy coupling and (iii) additional yet to be defined functions.

Keywords: ion transport, dicarboxylates, TRAP-T family, phylogeny, molecular evolution

INTRODUCTION

All organisms use transport systems to facilitate the vectorial movement of nutrients and other solutes across biological membranes. Based on molecular architecture and the energy used to drive transport, five types of transport systems have been distinguished (Fig. 1; Nikaido & Saier, 1992): (i) facilitators (Fig. 1a) mediate

facilitated diffusion but are incapable of substrate accumulation; (ii) secondary active transporters (Fig. 1b) couple substrate translocation to the downhill influx of H⁺ or Na⁺ (Maloney, 1994; Maloney & Wilson, 1996); (iii) primary active transporters (Fig. 1d) are energized by the utilization of a primary source of energy such as ATP, and one type of these systems, the ATP-binding cassette (ABC) uptake permeases of prokaryotes, employ high-affinity extracytoplasmic solute-binding proteins (Ames, 1986; Boos & Lucht, 1996; Higgins, 1992); (iv) group-translocators of the phosphoenolpyruvate (PEP):sugar phosphotransferase system (PTS) (Fig. 1e) couple sugar translocation to PEP-dependent sugar phosphorylation (Postma *et al.*, 1993); (v) tripartite ATP-independent periplasmic transporters

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Abbreviations: gb, GenBank; IT, ion transporter; p.m.f., protonmotive force; sp, SWISS-PROT; TC, transport classification; TMS, transmembrane spanning α -helix; TRAP-T, tripartite ATP-independent periplasmic transporters.

Fig. 1. Models of recognized classes of transporters. (a) Facilitator, (b) secondary transporter, (c) TRAP transporter, (d) binding-protein-dependent ABC-type transporter, (e) phosphotransferase system. BP, binding protein; filled circles, solute to be translocated. Modified from Nikaido & Saier (1992).

(TRAP-T; Fig. 1c), only recently discovered, employ a periplasmic binding protein as do ABC uptake permeases, but they use the protonmotive force (p.m.f.) as a source of energy (Forward *et al.*, 1997).

Many secondary active transport proteins exhibit a common transmembrane topology of $6 + 6 = 12$ transmembrane spanning α -helices (TMSs; Nikaido & Saier, 1992). Recently, a new classification system, the transport classification (TC) system, has been proposed (Saier, 1998) which integrates function and phylogeny for classifying the immense diversity of transport systems found in nature (see our transport classification web site: <http://www-biology.ucsd.edu/~msaier/transport/titlepage.html>). Carrier-type facilitators (TC no. 2) and ATP-driven active transporters (TC no. 3),

are among the twelve primary categories of transport systems recognized in the TC system.

As noted above, ATP-driven uptake systems of the ABC superfamily (Ames, 1986; Higgins *et al.*, 1990; Saier, 1998) function in conjunction with periplasmic solute-binding receptors that release the solute to the integral membrane translocator (Boos & Lucht, 1996; Higgins *et al.*, 1990; Hyde *et al.*, 1990; Fig. 1d), and equivalent receptors are essential constituents of TRAP-T systems. These systems have been shown to be specific for C_4 -dicarboxylates in *Rhodobacter capsulatus* (Forward *et al.*, 1997; Shaw *et al.*, 1991; Shaw & Kelly, 1991) or glutamate in *Rhodobacter sphaeroides* (Jacobs *et al.*, 1996). The former system is encoded within the *dctPQM* operon (Forward *et al.*, 1997; Hamblin *et al.*, 1990) where DctP is the periplasmic C_4 -dicarboxylate-binding protein (M_r 36 128), DctQ is a small integral membrane protein (M_r 24 763) with 4 putative TMSs and DctM is a large integral membrane protein (M_r 46 827) with 12 putative TMSs. Insertional mutagenesis of any one of the three *dct* genes results in complete loss of p.m.f.-dependent transport activity (Forward *et al.*, 1997). Glutamate uptake in *Rhodobacter sphaeroides*, which is dependent on a glutamate-binding protein, is also driven by the p.m.f. (Jacobs *et al.*, 1996).

In the present study we identified all DctP, DctQ and DctM homologues in the current databases and conducted phylogenetic analyses on representative TRAP-T protein constituents. We thus define the three families of functionally related proteins that presumably comprise TRAP-T systems. Phylogenetic analyses lead to the conclusion that such systems are ancient, and that all currently identified systems probably arose from a single primordial system with little or no shuffling of constituents between them. In *Bacillus subtilis*, an organism with a completely sequenced genome, a DctP homologue may function independently of DctQ and DctM homologues as a transcriptional sensor. TRAP-T DctP proteins are proposed to be homologous to extracytoplasmic solute-binding receptors of ABC uptake systems. We provide evidence that DctM is a member of a large superfamily of ion transporters and suggest that members of this superfamily have the unusual capacity to associate with auxiliary proteins in order to modify their transport characteristics. Some of the data on which our conclusions are based are included on our phylogenetic web site (<http://www-biology.ucsd.edu/~msaier/phylo/titlepage.html>) rather than in the text of this paper. This site will be referred to as the Phylo web site in the text.

COMPUTER METHODS

Protein and translated nucleotide databases were screened using the FASTA (Pearson & Lipman, 1988), BLAST (Altschul *et al.*, 1990) and PSI-BLAST (Altschul *et al.*, 1997) programs. The statistical significance of sequence similarities between putative members of the various families was established using the RDF2 (Pearson & Lipman, 1988) and GAP (Devereaux *et al.*, 1984) programs with 500 random shuffles. Binary comparison scores are expressed in standard deviations (SD; Dayhoff *et al.*,

Table 1. Members of the DctP family

Abbreviation	Name or description in database	Organism	Size (no. of residues)	Database and accession no.
DctP Rca	C ₄ -dicarboxylate-binding periplasmic protein	<i>Rhodobacter capsulatus</i>	333	spP37735
DctP Rsp	C ₄ -dicarboxylate-binding periplasmic protein	<i>Rhodobacter sphaeroides</i>	337	gbAF005842
SmoM Rsp	Periplasmic mannitol-binding protein	<i>Rhodobacter sphaeroides</i>	365	gbAF018073
Y146 Hin	Hypothetical protein HI0146 precursor (strain Rd KW20)	<i>Haemophilus influenzae</i>	329	spP44542
Y052 Hin	Hypothetical protein HI0052 precursor	<i>Haemophilus influenzae</i>	328	spP71336
YiaO Hin	Hypothetical protein HI1028 precursor	<i>Haemophilus influenzae</i>	328	spP44992
YiaO Eco	Hypothetical 36·0 kDa protein precursor in <i>avtA-selB</i> intergenic region	<i>Escherichia coli</i>	328	spP37676
Y4mM Rsp	Hypothetical 57·1 kDa protein Y4 mM	<i>Rhizobium</i> sp. strain NGR234	541	spP55572
Orf1 Ssp	Putative C ₄ -dicarboxylate binding protein (Orf-ID sll1314)	<i>Synechocystis</i> sp. strain PCC6803	369	gbD90907
Orf2 Ssp	Hypothetical protein (Orf-ID slr1962)	<i>Synechocystis</i> sp. strain PCC6803	370	gbD90912
Orf279 Bpe	Similar to C ₄ -dicarboxylate-binding periplasmic protein of Rca	<i>Bordetella pertussis</i>	280	Not available*
Orf Bsu	Similar to C ₄ -dicarboxylate-binding periplasmic protein of Rca	<i>Bacillus subtilis</i>	350	gbAB001488
DctP Ral	Hypothetical protein; similar to YiaO	<i>Ralstonia</i> sp. strain TFD41	209	gbAF045553
DctP Wsu	Putative C ₄ -dicarboxylate periplasmic binding protein precursor	<i>Wolinella succinogenes</i>	329	gbAJ132740
DctP Tma	Putative periplasmic substrate-binding protein precursor	<i>Thermotoga maritima</i>	336	gbAE001714
DctP ₁ Pae	Hypothetical protein	<i>Pseudomonas aeruginosa</i>	411	Not available†
DctP ₂ Pae	Hypothetical protein	<i>Pseudomonas aeruginosa</i>	331	Not available†
DctP ₃ Pae	Hypothetical protein	<i>Pseudomonas aeruginosa</i>	346	Not available†
DctP ₄ Pae	Hypothetical protein	<i>Pseudomonas aeruginosa</i>	332	Not available†
Orf ₁ Afu‡	Hypothetical protein (AF0635)	<i>Archaeoglobus fulgidus</i>	330	gbAE001060
Orf ₂ Afu‡	Hypothetical protein (AF0467)	<i>Archaeoglobus fulgidus</i>	355	gbAE001072
Orf ₃ Afu‡	Hypothetical protein (AF0998)	<i>Archaeoglobus fulgidus</i>	347	gbAE001036

* Forward *et al.* (1997).

† Kindly provided by PathoGenesis Inc. (Seattle, USA) prior to publication.

‡ The three Orfs of *Archaeoglobus fulgidus* lack significant sequence similarity to the bacterial DctP homologues, but they (1) map adjacent to the three genes encoding the putative Dct(Q)M homologues, and (2) exhibit significant sequence and motif similarity with the glutamine- and glutamate-binding proteins of *Escherichia coli*.

1983). A value of 9 SD (probability that the observed degree of sequence similarity occurred by chance is $< 10^{-19}$) is deemed sufficient to establish homology when this value is obtained for two aligned sequences of at least 60 residues in comparable portions of the proteins (Saier, 1994).

Multiple sequence alignments were constructed and phylogenetic analyses were performed using the TREE program (Feng & Doolittle, 1990) and the CLUSTAL X program (Thompson *et al.*, 1997). In order to correctly align the DctP, DctQ and DctM family proteins using the TREE program, N- and C-terminal extensions of non-homology found in some or all of the homologues were artificially removed. Mean hydropathy and mean similarity analyses were conducted for all protein families analysed based on the resultant multiple alignments of the protein members of these families. Well-conserved portions of the alignments generated with the TREE program, and the complete alignments generated with the

CLUSTAL X program, are presented on our Phylo web site (see Introduction). Multiple alignments and bootstrapped phylogenetic trees (Felsenstein, 1985) of the DctP, DctM and DctQ family proteins obtained using the CLUSTAL X program can also be found on this web site. Differences between the trees generated with the TREE and the CLUSTAL X programs primarily reflect the different assumptions upon which these programs are based (Young *et al.*, 1999). Bootstrapping, applied to the trees derived by use of the latter program, do not evaluate these assumptions and therefore do not provide a true measure of confidence as discussed previously (Young *et al.*, 1999).

Mean hydropathy, similarity and amphipathicity plots were generated using a sliding window of 21 residues (Le *et al.*, 1999). The hydropathy values described by Kyte & Doolittle (1982) were used in the first of these three analyses. Motif searches were conducted using the MEME and MAST programs

(Bailey & Gribskov, 1998; Grundy *et al.*, 1997) and signature sequences were defined according to Bairoch *et al.* (1997). All signature sequences were screened against the SWISS-PROT and TREMBL databases. Protein abbreviations used in this study as well as accession numbers which allow easy access to the sequences and primary references are provided in Tables 1–3.

RESULTS

As noted above, TRAP-T of *Rhodobacter capsulatus* consists of three essential non-homologous proteins, DctP, DctQ and DctM. Homologues of each of these proteins were identified using the search methods described under Computer Methods and analysed as described below.

The DctP family

Table 1 lists established proteins of the DctP family. Twelve of the twenty-two proteins (those above the dashed line in Table 1) were used for the reported analyses. All family members analysed, with the exception of the *Bacillus subtilis* Orf, were from Gram-negative bacteria. In several organisms, multiple DctP homologues were found, and in these cases, the paralogues were found to be highly divergent. Three paralogues are encoded within the complete genome of *Haemophilus influenzae*, and two paralogues were found in *Synechocystis* sp. strain PCC6803 and in *Rhodobacter sphaeroides*. Interestingly, the two DctP paralogues in *Rhodobacter sphaeroides* exhibit different substrate-binding specificities with DctP Rsp shown to bind C₄-dicarboxylates (M. D. Omrani & D. J. Kelly, unpublished data) and SmoM Rsp proposed to function as a mannitol-binding protein (GenBank accession number AAC45772). An unsequenced glutamate-binding protein has been proposed to be a constituent of a TRAP-T system in this same organism (Jacobs *et al.*, 1996). Members of the DctP family are generally of similar sizes, ranging from 280 to 350 amino acid residues with the exception of Y4 mM from *Rhizobium* sp. strain NGR234, which is much larger, having 541 residues. This protein proved to be a fused DctP–DctQ homologue, and consequently the two domains were artificially spliced apart for the reported analyses.

Fig. 2 presents mean hydropathy (a) and mean similarity (b) plots for the 12 analysed N-terminally truncated members of the DctP family. The mean hydropathy plot revealed that DctP family members are hydrophilic with two distinct regions showing partial hydrophobic character (from alignment positions 80–110 and from positions 180–200). The latter region and the adjacent hydrophilic region (positions 200–220) show the highest percentage similarity. The poorly conserved N-terminal leader sequences of these proteins were removed to facilitate correct alignment of the proteins as indicated in the Computer Methods section.

Fig. 2(c) presents a phylogenetic tree of DctP family members derived using the TREE program of Feng &

Fig. 2. Mean hydropathy plot (a), mean similarity plot (b) and phylogenetic tree (c) for the DctP family. The black bars in (a) indicate the portions of the multiple alignment that are presented on our Phylo web site. These plots were based on a multiple alignment of truncated sequences lacking the N-terminal, poorly conserved hydrophobic signal sequence. The TREE program was used to generate the alignment as well as the tree. The configurations of this and other phylogenetic trees reported in this paper were confirmed using the CLUSTAL X program with bootstrapping, and the results are presented on our Phylo web site (see Computer Methods). The abbreviations used in (c) are explained in Table 1.

Doolittle (1990). The tight clustering and short branch lengths connecting some members from dissimilar bacterial species reflect their high degrees of sequence similarity and indicate that these proteins are

Table 2. Members of the DctQ family

Abbreviation	Name or description in database	Organism	Size (no. of residues)	Database and accession no.
DctQ Rca	Small integral membrane transport protein	<i>Rhodobacter capsulatus</i>	227	gbX63974
Orf Rsp	Similar to DctQ of <i>Rhodobacter capsulatus</i>	<i>Rhodobacter sphaeroides</i>	228	gbAF005842
Y147 Hin	Hypothetical protein HI0147	<i>Haemophilus influenzae</i>	633	spP44543
Y051 Hin	Hypothetical protein HI0051	<i>Haemophilus influenzae</i>	165	spP44484
YiaM Hin	Hypothetical protein HI1030	<i>Haemophilus influenzae</i>	161	spP44994
YiaM Eco	Hypothetical 17.5 kDa protein in <i>avtA-selB</i> intergenic region	<i>Escherichia coli</i>	157	spP37674
Y4mM Rsp	Hypothetical 57.1 kDa protein Y4 mM	<i>Rhizobium</i> sp. strain NGR234	541	spP55572
Orf Ssp	Hypothetical protein (Orf-ID sll1102)	<i>Synechocystis</i> sp. strain PCC6803	179	gbD90913
DctQ Ral	Hypothetical protein, similar to YiaM	<i>Ralstonia</i> sp. strain TFD41	185	gbAF045553
DctQ ₁ Pae	Hypothetical protein	<i>Pseudomonas aeruginosa</i>	210	Not available*
DctQ ₂ Pae	Hypothetical protein	<i>Pseudomonas aeruginosa</i>	217	Not available*
DctQ ₃ Pae	Hypothetical protein	<i>Pseudomonas aeruginosa</i>	164	Not available*

* Kindly provided by PathoGenesis Inc. (Seattle, USA) prior to publication.

orthologues, having arisen by speciation. Interestingly, the three DctP paralogues of *Haemophilus influenzae*, Y146, YiaO and Y052, do not cluster, suggesting that they diverged from each other early in evolutionary history. The most divergent members of the family are Orf279 of *Bordetella pertussis* and Orf1 of *Synechocystis* sp. strain PCC6803.

A phylogenetic tree for the DctP family was also derived using the CLUSTAL X program, and bootstrapping was applied in order to evaluate the reliability at each node. This tree (and the corresponding bootstrapped trees for the DctQ and DctM family proteins) are presented on our Phylo web site (see Introduction for address). As expected, the results confirmed the reliability of nodes more distant from the centre of the unrooted tree but revealed a lesser degree of reliability for branch points near the centre.

Short but well-conserved and gap-free portions of the complete multiple alignment upon which the plots and tree shown in Fig. 2 were based are presented on our Phylo web site. From one of these alignments, a potential signature sequence for the DctP family was derived:

[SANF]-[RKNSQ]X2-[LIV]-x(2)-[LIVWPF]-[ADES]-[DHE]-[LIVMF]-x-G-[LQM]-[KRD]-[LIVM]-R-[LIVTA]

(x = any residue; alternative residues at any position are presented in square brackets)

This signature sequence was screened against the SWISS-PROT and TREMBL databases and was found to retrieve only members of the DctP family. By these criteria, it is thus a bona fide signature sequence and can

be used to identify sequences of new members of the DctP family as they become available.

The DctQ family

Table 2 lists proteins that make up the DctQ family. Eight protein members (Table 2, above the dashed line) were included in our analyses. Three DctQ paralogues were found in *Haemophilus influenzae*. No DctM or DctQ homologue could be found in *Bacillus subtilis* although a DctP homologue is present in this organism (see Discussion). The sizes of DctQ family members range from 161 to 228 amino acid residues with the exceptions of Y147 of *Haemophilus influenzae* and Y4 mM of *Rhizobium* sp. strain NGR234, which are larger due to fusion with other TRAP-T family constituents (DctM and DctP homologues, respectively). The C- and N-terminal regions of these proteins, respectively, were truncated in order to eliminate the non-DctQ homologous regions.

Fig. 3 presents mean hydropathy (a) and mean similarity (b) plots for the eight analysed members of the DctQ family. The mean hydropathy plot reveals four putative TMSs with greatest sequence similarity between residue positions 80 and 100. The portion of the multiple alignment indicated by the bar in Fig. 3(a) is presented on our Phylo web site.

Fig. 3(c) presents the DctQ family phylogenetic tree. Implications of clustering and branch length have been discussed above for the DctP family. The *Rhodobacter* orthologues, DctQ Rca and Orf Rsp, associate in a tight cluster as seen in the DctP family. The three DctQ

Fig. 3. Mean hydropathy plot (a), mean similarity plot (b) and phylogenetic tree (c) for the DctQ family. The black bar in (a) indicates the portion of the multiple alignment that is displayed on our Phylo web site. The abbreviations used in (c) are explained in Table 2. All three figures were derived from the multiple alignment generated with the TREE program as outlined in the legend to Fig. 2.

paralogues of *Haemophilus influenzae* display pronounced sequence divergence, but the putative YiaM orthologues of *Escherichia coli* and *Haemophilus influenzae* form a distinct cluster.

The most conserved portion of the complete multiple alignment is a gap-free 35 residue segment which shows

three fully conserved amino acyl residues and several positions with only conservative substitutions (see our Phylo web site). The following signature sequence for the DctQ family was derived from this portion of the multiple alignment:

[LIVC]-F-x-[WLY]-[LIVMF]-x(2)-[LIVFG]-G-[AI]-
[LIVAS]-[LIVY]-x(6)-H-[LIVM]-x-[LIV]

It proved to be specific to proteins of the DctQ family.

The DctM family

Table 3 lists proteins of the DctM family, eleven of which (those above the dashed line), all from Gram-negative bacteria, were included in the reported analyses. With the exceptions of DctM homologues from *Rhodobacter sphaeroides*, *Rhodobacter capsulatus* and *Salmonella typhimurium*, the protein sequences were obtained from completely sequenced genomes. The sizes of bacterial DctM family members were found to range from 389 to 445 residues with the exceptions of DctM of *Treponema pallidum* (632 residues), Y147 of *Haemophilus influenzae* (633 residues) and the three paralogues of *Archaeoglobus fulgidus* (643, 679 and 684 residues). These proteins have sizes equivalent to the approximate sum of DctQ and DctM, and in the case of the *Haemophilus influenzae* protein, the fusion of the DctM and DctQ homologues could be demonstrated. All of these larger homologues exhibit the same apparent topology with 16 putative TMSs. We therefore suggest that this type of fusion also occurred in the *T. pallidum* and *Archaeoglobus fulgidus* homologues but that extensive sequence divergence precluded recognition of the DctQ moiety.

Fig. 4 presents mean hydropathy (a) and mean similarity (b) plots for the 11 analysed members of the DctM family. The mean hydropathy plot reveals nine regions of hydrophobicity which span 20 residues or more. Three of these regions (residue positions 110–150, 250–300 and 350–400) probably encompass two TMSs each. Thus, the plot is consistent with the presence of 12 TMSs. The mean similarity plot reveals that almost without exception, each peak of high similarity aligns with a corresponding peak of hydrophobicity. Most of these hydrophobic peaks are about 80% similar.

Fig. 4(c) presents the DctM family phylogenetic tree. As observed for the DctP and DctQ family trees, DctM orthologues of *Rhodobacter*, DctM Rca and Orf Rsp form a tight cluster, although the DctM paralogues of *Haemophilus influenzae* display pronounced divergence. The YiaN orthologues of *Escherichia coli* and *Haemophilus influenzae* cluster together as do the DctP-like YiaO homologues and the DctQ-like YiaM homologues.

A portion of the complete multiple alignment upon which the plots and tree shown in Fig. 4 were based is presented on our Phylo web site. This gap-free alignment shows 3 fully conserved amino acyl residues in 34

Table 3. Members of the DctM family

Abbreviation	Name or description in database	Organism	Size (no. of residues)	Database and accession no.
DctM Rca	Large integral membrane transport protein	<i>Rhodobacter capsulatus</i>	440	gbX63974
Orf Rsp	Similar to DctM in Rca	<i>Rhodobacter sphaeroides</i>	443	gbAF005842
Y147 Hin	Hypothetical protein HI0147	<i>Haemophilus influenzae</i>	633	spP44543
Y050 Hin	Hypothetical protein HI0050	<i>Haemophilus influenzae</i>	401	spP44483
YiaN Hin	Hypothetical protein HI1029	<i>Haemophilus influenzae</i>	425	spP44993
YiaN Eco	Hypothetical 45.5 kDa protein in <i>autA</i> - <i>selB</i> intergenic region	<i>Escherichia coli</i>	424	spP37675
Y4mL Rsp	Hypothetical 44.6 kDa protein Y4ML	<i>Rhizobium</i> sp. strain NGR234	419	spP55571
Orf Ssp	Hypothetical protein (Orf-ID sll1103)	<i>Synechocystis</i> sp. strain PCC6803	445	gbD90913
OrfC Bpe	OrfC gene product	<i>Bordetella pertussis</i>	389	gbX64876
YgiK Sty	Hypothetical 46.1 kDa protein in <i>plsC</i> 3' region	<i>Salmonella typhimurium</i>	435	spP40800
DctM Tpa	Putative dicarboxylate transporter (TP0958)	<i>Treponema pallidum</i>	632	gbAE001264
DctM Ral	Hypothetical protein, similar to YiaN	<i>Ralstonia</i> sp. strain TFD41	389	gbAF045553
DctM ₁ Ape	Hypothetical protein, similar to YiaN	<i>Aeropyrum pernix</i>	731	gbAP000063
DctM ₂ Ape	Hypothetical protein, similar to YiaN	<i>Aeropyrum pernix</i>	560	gbAP000063
DctM Tma	Hypothetical protein, similar to YiaN	<i>Thermotoga maritima</i>	427	gbAE001714
DctM ₁ Pae	Hypothetical protein	<i>Pseudomonas aeruginosa</i>	427	Not available*
DctM ₂ Pae	Hypothetical protein	<i>Pseudomonas aeruginosa</i>	427	Not available*
DctM ₃ Pae	Hypothetical protein	<i>Pseudomonas aeruginosa</i>	426	Not available*
DctM ₁ Afu	Hypothetical protein (AF0636)	<i>Archaeoglobus fulgidus</i>	679	gbAE001060
DctM ₂ Afu	Hypothetical protein (AF0466)	<i>Archaeoglobus fulgidus</i>	684	gbAE001072
DctM ₃ Afu	Hypothetical protein (AF0989)	<i>Archaeoglobus fulgidus</i>	643	gbAE001036

* Kindly provided by PathoGenesis Inc. (Seattle, USA) prior to publication.

residue positions with several positions exhibiting only conservative substitutions. The signature sequence for the DctM family is:

[LIVM]-[LIVMT]-A-[LIV]-P-[FL]-[FY]-[LIVMA]-x(2)-[GS]-x(2)-[LM]-x(2)-[GST]-x(2)-[GAST]-x(2)-[LIVM]

Additional members of the TRAP-T families in bacteria and archaea

After completion of the sequence analyses reported, new sequences that proved to be homologous to the protein constituents of the three TRAP-T families were deposited in the databases. Some of these proteins are tabulated in Tables 1–3 below the dashed lines. Bacterial homologues identified include a complete DctPQM system from *Ralstonia* sp. strain TFD41 (Nakatsu *et al.*, 1998), three complete paralogous DctPQM systems from *Pseudomonas aeruginosa* and one additional DctP paralogue from *Pseudomonas aeruginosa*. The comparison scores obtained with TRAP-T constituents of *Rhodobacter capsulatus* establish or strongly suggest homology (Table 4). The high comparison scores of the *Pseudomonas aeruginosa* proteins with those of *Rhodo-*

bacter capsulatus suggest that they may also be dicarboxylate transporters (see Table 4). Hydropathy plots of the constituents of the complete systems from *Ralstonia* sp. strain TFD41 and *Pseudomonas aeruginosa* revealed that these proteins are similar in apparent topology to those described above.

By screening the incompletely sequenced genomes deposited in the NCBI databases, we identified homologues of complete TRAP-T systems in a number of additional bacteria. Thus, in the following organisms, DctM, DctP and DctQ homologues were detected with BLAST probability scores smaller than: e^{-25} for DctM, e^{-20} for DctP and 0.07 for DctQ when the *Rhodobacter capsulatus* proteins were used as the query sequences. Complete systems identified (with decreasing scores for DctM homologues in the order presented) were from the following bacteria: *Vibrio cholerae*, *Shewanella putrefaciens*, *Bordetella pertussis*, *Enterococcus faecalis*, *Actinobacillus actinomycetemcomitans* and *Caulobacter crescentus*. Many additional bacteria exhibit one or two homologues of the TRAP-T system, for example *Thiosphaera pantotropha* (now *Paracoccus pantotrophus*) (DctM) (Berks *et al.*, 1995), and *Thermotoga*

Fig. 4. Mean hydropathy plot (a), mean similarity plot (b) and phylogenetic tree (c) for the DctM family. The black bar in (a) indicates the portion of the multiple alignment that is displayed on our Phylo web site. The abbreviations used in (c) are explained in Table 3. All three figures were derived from the multiple alignment generated with the TREE program as outlined in the legend to Fig. 2.

maritima (DctPM). The fact that *Enterococcus faecalis*, a Gram-positive bacterium, exhibits all three Dct homologues allows us to conclude that TRAP-T systems are probably found in all major bacterial subdivisions.

Three DctM paralogues are encoded within the completely sequenced genome of the hyperthermophilic, sulfate-reducing archaeon *Archaeoglobus fulgidus*

(Klenk *et al.*, 1997) and two more are found in a second archaeon, *Aeropyrum pernix* (Table 3). The *Archaeoglobus fulgidus* proteins proved to be more similar to the *Ralstonia* DctM protein than to the other bacterial homologues listed in Table 3. The comparison scores of these proteins with the *Ralstonia* DctM protein are presented in Table 4. Because these three proteins exhibit comparison scores with each other in excess of 20 SD, and two of them give comparison scores with the *Ralstonia* protein of 10 SD or greater, they can all be considered to be homologues. Fig. 5 shows a portion of the complete multiple alignment of the three *Archaeoglobus fulgidus* paralogues together with three selected bacterial members of the DctM family. Six positions are fully conserved, and only conservative substitutions are observed at many other positions. Consequently, there is little question that these proteins are homologues.

A mean hydropathy plot of the three *Archaeoglobus fulgidus* proteins is shown in Fig. 6(a). Sixteen putative TMSs are predicted, the same number that is observed for the bacterial DctQ plus DctM homologues (Figs 3a and 4a). PSI-BLAST searches revealed that all three *Archaeoglobus fulgidus* proteins exhibit an N-terminal region of 120–140 residues that is absent in all but one of the bacterial DctM protein homologues. The one exception is the Y147 protein of *Haemophilus influenzae*, which exhibits a fused DctQ–DctM structure with 16 putative TMSs. Attempts to establish significant sequence or motif similarity between the N-terminal sequences of the *Archaeoglobus fulgidus* proteins and the DctQ family homologues from bacteria were not successful. However, four observations suggested to us that they are equivalent: (1) they exhibit similar sizes; (2) they show similar topologies; (3) they show the same domain association as is present in the Y147 protein of *Haemophilus influenzae*; and (4) the bacterial DctQ homologues are far more divergent than the bacterial DctM homologues (see next section and Figs 3b and 4b). We therefore suggest that all three *Archaeoglobus fulgidus* homologues include N-terminal DctQ equivalents even though we cannot prove this postulate.

Adjacent to each of the genes encoding one of the three paralogous Dct(Q)M proteins in *Archaeoglobus fulgidus*, we found a gene encoding a DctP-like protein (see Table 1). All of the latter three genes were homologous to each other. While homology between Orf₁ and Orf₂ was pronounced (percentage identity of 38%), Orf₃ proved to be more distant from the two other paralogues (percentage identity of 21.5% and 23.4%, respectively). This is in agreement with the observation that DctM₃ is also more distant from DctM₁ and DctM₂. All three putative DctP paralogues exhibit a hydrophilic nature except for hydrophobic N-termini as expected for extracytoplasmic solute-binding receptors (Fig. 6b). PSI-BLAST searches revealed motif similarity of these three archaeal proteins to the periplasmic glutamine-binding protein of *Escherichia coli*. Thus, three complete TRAP-T systems appear to be present in *Archaeoglobus fulgidus*.

Table 4. Comparison scores of newly identified TRAP-T constituents

	Comparison scores (SD) with <i>Rhodobacter capsulatus</i>		
	DctM	DctP	DctQ
DctMPQ of <i>Ralstonia</i> sp. strain TFD41	13	12	4
DctM ₁ P ₁ Q ₁ of <i>Pseudomonas aeruginosa</i>	62	45	14
DctM ₂ P ₂ Q ₂ of <i>Pseudomonas aeruginosa</i>	57	51	11
DctM ₃ P ₃ Q ₃ of <i>Pseudomonas aeruginosa</i>	45	17	1
DctP ₄ of <i>Pseudomonas aeruginosa</i>	–	39	–

	Comparison score (SD) with	
	DctM of <i>Ralstonia</i> sp. strain TFD41	DctM ₁ of <i>Archaeoglobus fulgidus</i>
DctM ₁ of <i>Archaeoglobus fulgidus</i>	11	–
DctM ₂ of <i>Archaeoglobus fulgidus</i>	10	42
DctM ₃ of <i>Archaeoglobus fulgidus</i>	7.5	24

DctM Ral	(98)	I V A A C I L A A L S G S A V A D T A A L A A L L L P M M V A A G H D K A K A G G L I A S
YiaN Hin	(98)	I L S A M I M A S L S G S A V A D T A A V A A M L L P M M K T T G Y P I H R S A G L I G T
YgiK Sty	(106)	I L A N T L F G A I S G S R V A S A P A V G G I M S P L Q E K E G Y S P A F S T A I N V A
DctM ₁ Afu	(238)	V V S S A L M G T I S G S S V A N T V T T G A F T I P L M K R V G Y K P E F A G A V E P A
DctM ₂ Afu	(222)	V V A S S L L G T M S G S A T G N V V T T G T F T I P L M K R A G Y K P E T A G A I E A S
DctM ₃ Afu	(220)	V I G S M S V A M C T G S G A A D S A L V G Q F T I P A M R R A G F P A L Y A A A T E G A
Consensus:		- - - - - S G S - V A - - - - G - - - - P - M K - - G Y - - - - A - A - - -

DctM Rasp	(143)	A G I I A P V V P P S V S F V I F G V A
YiaN Hin	(143)	A G I I A P I I P P S I P F I V F G V A
YgiK Sty	(151)	S A P I G L M I P P S N V L I V Y S L A
DctM ₁ Afu	(283)	A S T G G Q I M P P I M G A A A F I M A
DctM ₂ Afu	(267)	A S T G G Q L M P P V L G S A A F I M P
DctM ₃ Afu	(265)	A A L G G L I M P P V M A I A A F L M A
Consensus:		A - - - G - - - P P S - - - - F - - A

Fig. 5. A portion of a multiple alignment for representative bacterial and archaeal members of the DctM family. Abbreviations are explained in Table 3.

Immediately adjacent to the genes encoding the putative DctPQM₂ transporter of *Archaeoglobus fulgidus*, we noted the presence of a gene cluster (*korBADG*) encoding the four subunits of the 2-oxoglutarate:ferridoxin 2-oxidoreductase (Klenk *et al.*, 1997). The clustering of these genes suggests a common function. The proposed function of DctPQM₂ is therefore that of a 2-oxoglutarate uptake system.

Relative degrees of sequence divergence among the three TRAP-T family constituents

We noted that DctM homologues seem to exhibit greater percentage identity values with each other than do DctQ or DctP homologues. We therefore analysed all pairs of

homologues of the complete TRAP-T systems represented in the trees shown in Figs 2(c), 3(c) and 4(c). Percentage identity values were calculated for all pairs of homologues and these values were averaged. The mean percentage identity values for all pairs of DctM, DctP and DctQ homologues were 34.5%, 29.6% and 20.6%, respectively. These results provide quantitative estimates of the relative rates of sequence divergence. They show that DctQ homologues have diverged in sequence more rapidly than DctP homologues, and that DctP homologues have diverged more rapidly than DctM homologues. This fact may well account for our inability to detect DctQ or DctP homologues for some of the DctM homologues in organisms with completely (and incompletely) sequenced genomes (see Discussion).

Fig. 6. Mean hydropathy plots of the three Dct(Q)M (a) and DctP (b) paralogues from *Archaeoglobus fulgidus*. The N-terminal portion of 4 TMSs in the Dct(Q)M proteins comprises the putative DctQ equivalent; the C-terminal portion of 12 TMSs represents the region homologous to bacterial members of the DctM family.

Preliminary evidence that DctM is a member of a large superfamily of ion transporters

We have previously described and classified over 200 families of transporters (see our web site: <http://www-biology.ucsd.edu/~msaier/transport.html>). Use of the PSI-BLAST program (Altschul *et al.*, 1997) suggested that eleven of these previously identified families exhibit common matrices of sequence motifs. These families are listed by name in Table 5 together with their abbreviations, TC numbers and a well-characterized protein example. Database accession numbers are provided for these proteins, allowing easy access to their sequences. All eleven of the families identified function in the transport of organic or inorganic ions. We therefore have designated this putative superfamily the ion transporter (IT) superfamily.

Using the MEME and MAST programs (Bailey & Gribskov, 1998), we have examined these families for common motifs. The results are summarized in Table 6. The Ars family proved to exhibit recognizable motif similarity with four of these families as summarized in the top panel of Table 6. Among these are the GntP and LctP

families, which exhibit extensive motif similarities as revealed in the lower panel of Table 6. The TRAP-T DctM family exhibits an N-terminal motif in common with the DcuC family, but this family is not among those that show motif commonality with the Ars family as revealed by MEME and MAST. The basis for the PSI-BLAST results suggesting common ancestry of the eleven families of the putative IT superfamily is therefore only partially revealed by the output of the MEME and MAST programs.

DISCUSSION

Only a single member of the TRAP-T family of transporters has been both molecularly and functionally characterized (Forward *et al.*, 1997). This system is the three component DctPQM system where DctM and DctQ are integral membrane constituents with 12 and 4 putative TMSs, respectively, and DctP is a periplasmic solute-binding receptor. These proteins have previously been shown to function together, and all of them are required for detectable transport activity (Forward *et al.*, 1997). Our analyses have revealed the presence of complete TRAP-T systems in a large variety of bacteria and archaea, but not in eukaryotes. In *Bacillus subtilis*, a single DctP homologue was identified, but no DctM or DctQ homologue could be found. We therefore suggest that in *Bacillus subtilis*, the DctP homologue functions by a mechanism that differs from that in other bacteria. Adjacent to the *Bacillus subtilis* *ydbE* gene encoding the DctP homologue is the *ydbF* gene encoding a sensor kinase, the *ydbG* gene encoding a response regulator and the *ydbH* gene encoding a DcuA (dicarboxylate transporter) homologue of the DAACS family (TC no. 2.23). In *Agrobacterium tumefaciens*, a periplasmic binding protein, ChvE, interacts with a sensor kinase to effect induction of the *vir* operon (Cangelosi *et al.*, 1990; Winans, 1991). In this case, the binding protein also functions as a constituent of an ABC-type transporter (Kemner *et al.*, 1997) and as a chemoreceptor (Cangelosi *et al.*, 1990). We would like to suggest that the YdbE extracytoplasmic binding protein serves as a receptor for one or more dicarboxylates, and interacts with the YdbF sensor kinase to activate it for phosphorylating the YdbG response regulator that controls transcription of the *ydbH* gene encoding a dicarboxylate transporter. Indeed, hydropathy analyses revealed that YdbF exhibits two putative TMSs centred at residues 20 and 180, suggesting a topology similar to that of VirA of *Agrobacterium tumefaciens*. The intervening region (residues 30–170) presumably binds YdbE. This suggestion is further substantiated by the observation that in *Rhizobium leguminosarum*, the *dctA* gene is controlled transcriptionally by a sensor kinase–response regulator system that renders induction responsive to extracellular dicarboxylates (Huala *et al.*, 1992). We propose that a similar induction mechanism operates in *Bacillus subtilis*.

The genome of the archaeon *Archaeoglobus fulgidus* was shown to encode three DctM homologues and all of these possess N-terminal regions that are homologous

Table 5. Families believed to constitute the IT superfamily

As suggested by PSI-BLAST studies.

Transporter family	Abbreviation	TC no.*	Example†
Gluconate:H ⁺ symporter family	GntP	2.8	GntP of <i>Bacillus subtilis</i> (spP12012)
Citrate-Mg ²⁺ :H ⁺ (CitM)–citrate:H ⁺ (CitH) symporter family	CitMHS	2.11	CitM of <i>Bacillus subtilis</i> (spP55069)
C ₄ -dicarboxylate uptake family	Dcu	2.13	DcuA of <i>Escherichia coli</i> (spP04539)
Lactate permease family	LctP	2.14	LctP of <i>Escherichia coli</i> (spP33231)
NhaB Na ⁺ :H ⁺ antiporter family	NhaB	2.34	NhaB of <i>Escherichia coli</i> (spP27377)
NhaC Na ⁺ :H ⁺ antiporter family	NhaC	2.35	NhaC of <i>Bacillus firmus</i> (gbU61539)
NhaD Na ⁺ :H ⁺ antiporter family	NhaD	2.62	NhaD of <i>Vibrio parahaemolyticus</i> (gbAB006008)
Divalent anion:Na ⁺ symporter family	DASS	2.47	SodiT1 of <i>Spinacia oleracea</i> (gbU13238)
Tripartite ATP-independent periplasmic transporter family	TRAP-T	2.56	DctM of <i>Rhodobacter capsulatus</i> (gbX63974)
C ₄ -dicarboxylate uptake C family	DcuC	2.61	DcuC of <i>Escherichia coli</i> (spQ47134)
Arsenical efflux family	Ars	3.4	ArsA-ArsB of <i>Escherichia coli</i> (ArsA: spP08690; ArsB: spP37310)

* According to the TC system (see our web site and Saier, 1998).

† Accession numbers given in parentheses.

with each other. These N-terminal regions exhibit sizes and topological features that resemble the bacterial DctQ homologues. In *Haemophilus influenzae*, one of the three TRAP-T systems identified exhibits a fused DctQM protein of 16 TMSs. Since the *Archaeoglobus fulgidus* paralogues also exhibit 16 putative TMSs, it is reasonable to propose that the *Archaeoglobus fulgidus* homologues represent the equivalent of both DctQ and DctM of *Rhodobacter capsulatus*. This suggestion is in agreement with our observation that the bacterial DctQ homologues have diverged much more rapidly than have the DctM homologues. Adjacent to each of the Dct(Q)M homologues of *Archaeoglobus fulgidus* is a gene encoding a putative extracytoplasmic solute-binding receptor with significant sequence and motif similarity to the well-characterized glutamine- and glutamate-binding proteins of ABC-type transporters in *Escherichia coli*. These three archaeal proteins exhibit a single N-terminal hydrophobic peak followed by regions of relative hydrophilicity as expected for an extracytoplasmic solute-binding protein. It seems likely that these three *Archaeoglobus fulgidus* proteins function as the solute-binding receptors for the Dct(Q)M homologues of this organism. This suggestion is substantiated by the observation that the genes encoding the four subunits of 2-oxoglutarate:ferridoxin 2-oxidoreductase map immediately adjacent to the *dctPQM*₂ genes of *Archaeoglobus fulgidus*.

The great degree of sequence divergence between the constituents of the bacterial and archaeal TRAP-T systems suggests that they are related by vertical descent, and that horizontal transmission of genetic material between these two domains of living organisms does not account for their occurrences. This observation leads to

the suggestion that the TRAP-T family is very old, dating back to before the split between bacteria and archaea.

Encoded within the fully sequenced genome of *Treponema pallidum*, a DctM homologue was identified. This protein exhibits the same size and apparent topology as the DctQM protein of *Haemophilus influenzae* with the non-homologous putative DctQ moiety at the N-terminus. Moreover, adjacent to the gene encoding this protein were two genes encoding proteins of about 330 amino acid residues which exhibited N-terminal peaks of hydrophobicity. Either of these proteins could be the solute-binding receptor for the Dct(Q)M homologue of *T. pallidum*. Thus *T. pallidum* may possess a complete DctPQM transporter as is apparently true for many other prokaryotes.

DctP, DctQ and DctM occur in many bacteria as distinct polypeptide chains encoded by three contiguous genes, usually in operons with the gene order *dctPQM*. However, we found two types of protein (or domain) fusions. One is DctPQ (Y4 mM from *Rhizobium* sp. strain NGR243) and the other is DctQM (Y147 from *Haemophilus influenzae* and several other organisms). None of the other possible domain orders (i.e. DctQP, DctMQ, DctPM and DctMP) were found. This observation leads to the prediction that P interacts with Q, and Q with M in all DctPQM homologues. DctQ may serve as a structural anchor for DctP, allowing association of P with M, a suggestion that is consistent with the relative rates of divergence of these three TRAP-T protein constituents. It is also interesting to note that no DctPQM fusions have been detected, and that this fact correlates with the fact that in ABC uptake permeases, the extracytoplasmic receptors are seldom

Table 6. Motifs common among members of the proposed IT superfamily as identified by MEME and MAST analyses

Family	Protein*	Motif† (residue no.)‡	TMS§	Motif† (residue no.)‡	TMS§	Motif† (residue no.)‡	TMS§	Motif† (residue no.)‡	TMS§
Ars	ArsB Eco	AANTIGDGLGPKISP		FKEYFSLT		QKxHRISWG		LKIWQPKG	
	ArsB Sxy	YANVIGCDLGPKITP (363)	11	FREYASVM (170)	6	QKNMTISWG (392)	12		
		YANVIGSDLGPKITP (364)	11	FIEYFSRM (169)	6	QKGVKISWG (393)	12		
GntP	GntP Bsu			FKEYFGLS (411)	12				
	GntP Eco			FKEYFNLT (400)	12				
LctP	LldP Eco	AANTS GG VVGKMISP (481)	11					LKEWQPKR (265)	6–7
	LctP Hpy	AANTTGGVTGKMISP (487)	11					LKRWQPV (266)	6–7
DASS	Y672 Mja Ndc2 Rno					QEHHRFSQG (207)	6	FKKWIPKE (216)	6–7
Dcu	DcuB Eco	AASSIGAQMGIISP (133)	5						
Family	Protein*	Motif† (residue no.)§	TMS§	Motif† (residue no.)‡	TMS§	Motif† (residue no.)‡	TMS§	Motif† (residue no.)‡	TMS§
GntP	GntP Bsu	LYIGFPFGAALLEGAGHFGPPH		PAVIVAGPSFA		LFPVLVxLGIDP			
	GntT Eco	LFLGIPMVAAALSVTHGFLPPH (143)	5	PTVLIAGPLFT (190)	6				
	GntV Eco	LYVGVPMVAAALSVTHGFLPPH (136)	5	PTVILAGPVYA (183)	6				
LctP	LctP Eco	LIVGFCFGAFLEGAAGFGAPV (119)	4	PAVVVAGGSFA (231)	6				
	LctP Hpy	LLIGFCFGSFLGAIGFGGPV (118)	4	PAVAVTGFSFA (232)	6				
	YghK Eco	LLIGFSFGALLEGAGFGAPV (119)	4	PAALVAGGSFA (227)	6				
TRAP	YiaN Eco					LMPLVKEAGIDP (343)			11
	DctM Rca					LFPVAVRLGIDP (357)			11
DcuC	DcuC Eco					LFPVMNVGISR (148)			5
	YhcL Eco					LFPVLVSLGVS (145)			5

* ArsB Eco, ArsB of *Escherichia coli* (accession no. spP37310); ArsB Sxy, ArsB of *Staphylococcus xylosus* (spQ01255); GntP Bsu, GntP of *Bacillus subtilis* (spP12012); Gnt Eco, Gnt of *Escherichia coli* (spP39835); GntV Eco, GntV of *Escherichia coli* (spP39344); LctP of *Escherichia coli* (spP33231); LldP Eco, LldP of *Escherichia coli* (gbACC76011); LctP Hpy, LctP of *Helicobacter pylori* (gbAE000535); YghK Eco, YghK of *Escherichia coli* (spQ46839); Y672 MJA, Y672 of *Methanococcus jannaschii* (spQ58086); Ndc2 Rno, Ndc2 of *Rattus norvegicus* (spP70545); DcuB Eco, DcuB of *Escherichia coli* (spP14409); YiaN Eco, YiaN of *Escherichia coli* (spP37675); DctM Rca, DctM of *Rhodobacter capsulatus* (gbX63974); DcuC Eco, DcuC of *Escherichia coli* (spQ47134); YhcL Eco, YhcL of *Escherichia coli* (spP45428).

† Motifs (boxed) were determined using the MEME program (Grundy *et al.*, 1997). Residues in bold print in the boxed motifs were conserved in all proteins analysed. Residues in bold print in non-boxed motifs are common to the members of that family.

‡ Position of the identified motif in a given protein.

§ Number of the TMS of a given protein in which the identified motif resides.

fused to the integral membrane translocator protein (Obis *et al.*, 1999). Possibly this last fact points to additional non-transport-related functions (e.g. chemoreception, regulation) for these proteins.

We have obtained preliminary evidence that DctM may be a member of a large superfamily of ion transporters which we have tentatively designated the ion transporter (IT) superfamily. Among the members of this superfamily are many well-documented secondary transporters that function without the participation of a extracytoplasmic receptor or an integral membrane auxiliary protein. This fact strengthens our conviction that in *Rhodobacter capsulatus*, DctM alone provides the pathway of solute transport, and that DctQ and DctP function in accessory capacities. In confirmation of this suggestion we note that both *Pseudomonas*

aeruginosa and *Synechocystis* sp. strain PCC6803 apparently have a surplus of genes encoding DctP homologues with no corresponding *dctQ*- or *dctM*-like genes mapping nearby. Either these proteins serve as auxiliary binding receptors for the recognized TRAP-T family systems in these organisms, or they serve entirely different functions as we have proposed for *Bacillus subtilis*.

Another member of the putative IT superfamily (see Tables 5 and 6) is the ArsB protein of *Escherichia coli*, a constituent of the Ars family (TC no. 3.4). In this organism, ArsB can function together with ArsA to form an ATP-dependent anion translocator. However, in the absence of ArsA, ArsB transports arsenite and antimonite in a p.m.f.-driven process (Kuroda *et al.*, 1997; Silver *et al.*, 1993). The auxiliary protein, ArsA, has

evidently been superimposed on the integral membrane secondary transporter ArsB, allowing the use of ATP to energize transport. It seems that members of the IT superfamily exhibit the unusual characteristic of allowing superimposition of additional protein constituents on the basic transport process that modify transport characteristics by allowing high-affinity solute reception (e.g. using DctP), by altering the energy coupling mechanism (e.g. using ArsA) or by providing an unknown function (e.g. DctQ). The molecular basis for this unusual characteristic remains to be elucidated.

DctP is a periplasmic solute-binding protein with many characteristics that typify the extracytoplasmic receptors of ABC transporters (Tam & Saier, 1993). For example, DctP is the same size as many of the latter proteins and it confers high-affinity substrate binding and recognition to its transporter (Walmsley *et al.*, 1992). We have noted that DctP exhibits regions of limited sequence similarity to binding receptors of ABC systems including the citrate-binding protein of *Salmonella typhimurium* (Tam & Saier, 1993). We therefore propose that although sequence divergence is very considerable, the DctP proteins, as well as the putative *Archaeoglobus fulgidus* solute-binding receptors, represent authentic constituents of the solute-binding receptor superfamily that includes all extracytoplasmic receptors that feed into ABC transporters (Kuan *et al.*, 1995; Quiocho & Ledvina, 1996; Tam & Saier, 1993). In this connection it is interesting to note that neither ABC uptake systems nor TRAP-T uptake systems, both of which are dependent on extracytoplasmic receptors, are found in eukaryotes. Either binding-protein-dependent transport arose after prokaryotes and eukaryotes diverged from each other during the 'great split', or such a transport mode is inconsistent with eukaryotic life-styles. In either case, it seems that horizontal transmission of genetic material encoding transport proteins between prokaryotes and eukaryotes has been exceptionally rare (Saier, 1998, 1999). This postulate also leads to the possibility that ABC uptake systems arose from ABC-type efflux systems that function independently of binding receptors and are found ubiquitously throughout the three domains of life (Saurin *et al.*, 1999).

Several members of the IT superfamily arose by an intragenic duplication event that gave rise to proteins of 12 TMSs from primordial systems that presumably exhibited 6 TMSs (Saier & Tseng, 1999). Most dramatic is the ArsB protein where the degree of sequence similarity between the halves is sufficient to easily establish homology (Saier & Tseng, 1999; unpublished observation). Attempts to document an internal duplication in DctM led to recognition of sequence similarity between its two halves, but this similarity was insufficient to establish homology (unpublished observation). We suggest either that the two halves of DctM diverged in sequence more rapidly than did those in ArsB or that the duplication event leading to the full-length DctM homologues occurred earlier than the one that gave rise to ArsB homologues. The latter interpret-

ation is in agreement with conclusions reached from studies of another large superfamily, the RND superfamily (Tseng *et al.*, 1999). Regardless of this possibility, however, we predict that all IT superfamily transporters will prove to have arisen by intragenic duplication.

In spite of the fact that an 'orphan' DctP homologue appears to exist in *Bacillus subtilis*, an organism with a fully sequenced genome, and that a few 'extra' receptors may be present in a few other bacteria, our phylogenetic analyses suggest that in bacteria, and probably in archaea as well, the three constituents of complete TRAP-T systems evolved in parallel from a single primordial three-component (or three-domain) transport system. If this contention is correct, it would imply that intact TRAP-T systems did not arise recently by the superimposition of accessory proteins on the putative DctM transporter, but that these three proteins have functioned together for billions of years with minimal shuffling of constituents between systems. This conclusion is in agreement with the observation that such shuffling has seldom occurred during the evolution of the ubiquitous ABC superfamily (TC no. 3.1; Kuan *et al.*, 1995), the bacterial phosphotransferase functional superfamily (TC no. 6.1–6.6; Postma *et al.*, 1993) and the integral membrane constituents of F-type ATPases (TC no. 3.2; Blair *et al.*, 1996). Moreover, the demonstrable sequence similarity between the putative DctP homologues in *Archaeoglobus fulgidus* and the glutamine/glutamate-binding proteins of bacteria leads further to the suggestion that all DctP homologues are part of the binding protein superfamily (Quiocho & Ledvina, 1996). The general picture that seems to be emerging is that once a multicomponent transport system has appeared, it undergoes diversification following gene duplication and during speciation with minimal shuffling of constituents between functional units. Both ABC and TRAP-T uptake systems must have arisen during molecular antiquity but have retained their independent lineages. The molecular, physiological and regulatory constraints imposed upon transporters that prevent shuffling have yet to be elucidated.

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