

Whole Genome Analyses of Transporters in Spirochetes: *Borrelia burgdorferi* and *Treponema pallidum*

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Abstract

The completely sequenced genomes of two spirochetes, *Borrelia burgdorferi* (Bbu) and *Treponema pallidum* (Tpa) were analyzed for the distribution of transporter types. Both organisms exhibited fewer proteins with >7 α -helical transmembrane spanners (TMSs), and fewer identified transport systems per megabase pair of DNA than most other prokaryotes analyzed. Each organism exhibits one recognizable ion channel protein of the MscS family. Tpa has twice as many primary carriers as Bbu but lacks PTS permeases that are plentiful in Bbu. Tpa is the only prokaryote so far sequenced which has two F-type ATPases. Large families of secondary nutrient uptake carriers (MFS and APC) that are prevalent in other organisms are essentially lacking in Spirochetes. The largest Spirochete secondary carrier families consist of efflux systems. While both Bbu and Tpa exhibit an unusual degree of transporter diversity, major differences in specificity exist between these two organisms.

Introduction

Spirochetes represent an evolutionarily discrete cul-de-sac, forming a relatively close-knit group of organisms that, based on 16S RNA analyses, diverged from other bacteria during early evolutionary history (Paster *et al.*, 1991; Olsen *et al.*, 1994). They exhibit unusual prokaryotic characteristics such as linear chromosomes (Fraser *et al.*, 1997), a cytoskeleton (You *et al.*, 1996; Izard *et al.*, 1999) and periplasmic flagella that confer rapid motility and unusual chemotactic capabilities (Greenberg and Canale-Parola, 1977; Ge *et al.*, 1998; Shi *et al.*, 1998). The genomes of two such organisms, *Borrelia burgdorferi* (Bbu) and *Treponema pallidum* (Tpa) have been completely sequenced (Fraser *et al.*, 1997, 1998). Very little functional data are available regarding transporters present in Spirochetes although these organisms have long been known to possess functionally divergent sugar-transporting phosphotransferase systems (Saier *et al.*, 1977) and an

anaerobic glycolytic mode of ATP generation (Breznak and Canale-Parola, 1969, 1972; Janssen and Morgan, 1992; Fraser *et al.*, 1998). Many of them are pathogens in humans, exhibiting antigenic variation for the purpose of immune evasion (Donelson, 1995). However, free-living forms are also known (Breznak and Canale-Parola, 1975; Canale-Parola, 1977).

As part of a comprehensive program to analyze fully sequenced genomes for transport proteins, we have developed computer programs for analyzing membrane protein topology and function based on genome sequence data (Paulsen *et al.*, 1998a,b). In this report we provide detailed analyses of two Spirochetes, Tpa and Bbu. While reading this article, three facts should be kept in mind. First, the conclusions cited are based primarily on computer-based sequence comparisons and not on direct biochemical analyses. Second, although we will state on occasion that we are describing the transport properties of Spirochetes, only two such organisms have been analyzed. Third, many of the transporters present in these organisms are multicomponent systems, rendering assignment of function more difficult than usual. Finally, the functions of many putative transporters could not be deduced, and others are probably not recognized as they belong to protein families not currently known to include transporters. Because of these disclaimers, the reader should interpret the results described with caution.

Distribution of Integral Membrane Topological Types and Transporters in Spirochetes versus *E. coli*

All living organisms exhibit about one third of their proteins as integral membrane proteins, and about one third of these are transport proteins. The distribution of topological types for Bbu, Tpa and *E. coli* (Eco) is summarized in Table 1. While the Spirochetes exhibit a slightly higher percentage of soluble and 1 TMS proteins than is observed for *E. coli*, they exhibit only one quarter the percentage of proteins with 10 or more TMSs.

This fact correlates with the data presented in Table 2 that tabulates the numbers of recognized transport systems per 100 Mbp of DNA. The Spirochetes exhibit over 2-fold

Table 1. Integral Membrane Proteins: Distribution of Topological Types in Spirochetes vs. *E. coli* (%)

# TMSs	Bbu	Tpa	Eco
0	69	69	67
1	16	16	15
2-3	6	6	5
4-6	5	5	5
7-9	3	3	4
≥10	1	1	4

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Table 2. Numbers of Recognized Transporters Relative to Genome Size in Spirochetes vs. *E. coli*

	Bbu	Tpa	Eco
Genome size (Mbp)	1.44	1.14	4.64
# Systems (Total)	37	37	304
# Systems/100 Kbp	2.2*	2.9	6.2
% Transporter genes	7.5	7.2	10.8

*This is the smallest # of transport systems per unit of DNA for any of the 18 prokaryotic organisms analyzed to date (Paulsen et al., 2000).

lower values than for *E. coli*, even though the gene densities for the genomes of all three of these prokaryotes are essentially the same. Because a greater fraction of the Spirochete transporters are multicomponent systems, relative to *E. coli*, the percent of transporter genes is only somewhat reduced (Table 2).

Distribution of Transporter Types in Spirochetes versus *E. coli*

Spirochetes show an unusual complement of transporter types relative to all other organisms for which complete genome data are available (<http://www-biology.ucsd.edu/~ipaulsen/transport/>). As summarized in Table 3, the two Spirochetes analyzed each encode within their genomes just one recognized ion channel protein, both members of the MscS family of ion channels (TC #1.A.23; Booth and Louis, 1999; Levina et al., 1999). These proteins are BB0453 in Bbu and TP0822 in Tpa (Fraser et al., 1997, 1998). Only Bbu (not Tpa) has a channel protein for exchange of small neutral molecules such as glycerol and urea.

Spirochetes also display decreased percentages of secondary active carriers and greatly increased percentages of primary active transporters plus group translocators relative to *E. coli*. Major differences are observed between the two organisms, however. Bbu has more secondary carriers than does Tpa, while Tpa has twice as many primary carriers as does Bbu. Compensating for this last mentioned deficiency in Bbu, this latter organism has a large number of sugar-transporting group translocators of the phosphoenolpyruvate-dependent phosphotransferase system (PTS). No such systems are present in Tpa (Table 3).

Major Families of Primary Active Transporters in Spirochetes

By far the largest family of transporters in Bbu and Tpa is the ATP-binding cassette (ABC) superfamily (Table 4). Bbu

Table 3. Distribution of Transporter Types in Spirochetes vs. *E. coli*

	Bbu		Tpa		Eco	
	#	%	#	%	#	%
Channels	2	5	1	3	7	2
2° Carriers	17	46	14	38	194	64
1° Carriers	10	28	20	54	74	24
PTS	6	16	0	0	22	7
Unknown	2	5	2	5	7	2
Total	37		37		304	

Table 4. Families of 1° Carriers and Group Translocators in Spirochetes vs. *E. coli*

	Bbu	Tpa	Eco
ABC	9	16	67
F-type ATPase	1	2	1
P-type ATPase	0	1	4
NaT-DC	0	1	0
PTS	6	0	22
Total	16	20	94

encodes nine identified members of this superfamily while Tpa encodes 16. Compensating for this difference, Bbu has 6 PTS permeases. Thus, the numbers of ABC + PTS permeases in the two organisms is almost the same.

Table 5 summarizes the proposed substrate specificities of those ABC uptake permeases for which functional predictions can be made on the basis of sequence comparisons. While Bbu has only one putative sugar transporting ABC permease, Tpa has eight. The large number of ABC-type sugar uptake permeases in Tpa presumably compensates for the total absence of sugar-transporting PTS permeases in this organism as mentioned above. Both Bbu and Tpa are expected to derive energy primarily from anaerobic sugar metabolism via the Embden-Meyerhof-Parnas glycolytic pathway (Saier, 1987), accounting for the large percentages of ATP- and PEP-driven uptake systems relative to proton motive force (pmf)-driven systems. We have found that organisms that synthesize ATP primarily by substrate level phosphorylation exhibit a preponderance of ATP-dependent permeases while those that synthesize ATP by oxidative phosphorylation, using the proton motive force across the cytoplasmic membrane, have a greater number of secondary carriers (Paulsen et al., 1998a). Also worthy of note is the fact that while Bbu has more ABC peptide uptake permeases than does Tpa, Tpa has more ABC amino acid uptake permeases. The Tpa genome also encodes high affinity uptake systems for heavy metals (Co²⁺; Mn²⁺) and molybdate, but these are lacking in Bbu. On the other hand, Bbu has an ABC-type phosphate uptake permease that is lacking in Tpa (Table 5).

Tpa has two monovalent cation transporting systems within the F-type ATPase family (actually homologous to V-type enzymes; see below), a P-type heavy metal transporting ATPase, and a Na⁺ transporting oxaloacetate decarboxylase (NaT-DC). By contrast, Bbu has only one F-type ATPase and neither a P-type ATPase nor a Na⁺ translocating decarboxylase. Strikingly, Tpa is the only prokaryote of the 18 we have so far analyzed which has more than one member of the F-type ATPase family.

Table 5. Recognized ABC Transporters in Spirochetes

Bbu	Tpa
Sugars (1)	Sugars (8)
Glycine-betaine (1)	Amino Acids (2)
Peptides (4)	Peptides (1)
Polyamines (1)	Polyamines (1)
Phosphate (1)	Molybdate (1)
	Mn ²⁺ (2)
	Co ²⁺ (1)

Table 6. Major Families of 2^o Carriers in Spirochetes vs. *E. coli*

	Bbu	Tpa	Eco
RND	3	2	9
MATE	3	1	3
CHR	1	1	0
CaCA	1	1	2
Trk	1	1	2
MFS	1	0	66
APC	0	0	22

Because of this unusual characteristic, we have conducted phylogenetic analyses of the catalytic subunits of all F-type ATPases encoded within the genomes of 19 fully sequenced organisms (Figure 1). These include the β -subunits of F-type ATPases as well as the functionally equivalent and homologous A-subunits of V-type (or A-type) ATPases of archaea, bacteria and yeast vacuoles. When all F-type ATPases are examined with respect to phylogenetic position (Figure 1, lower cluster), all are found to exhibit phylogenetic relationships to each other as expected based on organismal (or 16S rRNA) phylogenies. When all of the V-type ATPases (Figure 1, upper cluster) are examined, most of the proteins exhibit phylogenetic relationships to each other as expected. Thus, AtpA Bbu and AtpA Tpa cluster together with AtpA Ctr (from

Chlamydia trachomatis), branching from the spirochete protein branch near the center of the unrooted tree. Moreover, the *S. cerevisiae* homologue (VatA Sce) clusters separately from the four archaeal proteins (lower right-hand side of the V-type ATPase cluster) although all four archaeal proteins cluster tightly together. Interestingly, the catalytic subunit of the second ATPase of *T. pallidum* branches from a point intermediate between the yeast and archaeal branch points. These results suggest either that Tpa acquired this second ATPase early during its evolutionary history by lateral transfer from a primordial organism that might have resembled the precursor of the eukaryotic/archaeal cell, or it may have arisen by a very early operon duplication event that preceded the "great split", giving rise to the three recognized domains of life, the Archaea, Bacteria and Eukarya. In the latter case, this primordial system must have been lost from most prokaryotes.

Major Families of Secondary Carriers in Spirochetes

No family of secondary carriers has large representation in either of the two spirochetes examined (Table 6). The RND superfamily with three members in Bbu and two in Tpa has the largest total representation of any family of secondary carriers in these two organisms combined. The MATE family comes in second place with four members, and the CHR (chromate resistance) and CaCA (calcium:cation antiporter) families tie for third place with several other families (see below). What is interesting is that all of the functionally characterized members of these families are efflux pumps. It seems that spirochetes use secondary carriers in large measure for efflux purposes rather than for uptake. Nutrient uptake is mediated to a disproportionate extent by ABC and PTS permeases as noted above. The large families of secondary carriers in most other organisms (bacteria, archaea and eukaryotes) are lacking or nearly lacking in spirochetes. Thus, only one MFS member is present in Bbu, and this is probably a drug efflux pump. Tpa is the only organism of the 20 we have analyzed that lacks a member of the MFS. The large amino acid/polyamine/organocation (APC) superfamily (Jack *et al.*, 2000) is altogether lacking in both spirochetes (Table 6 below the dashed line).

The largest of the spirochete families of secondary carriers, the RND superfamily (Tseng *et al.*, 1999) was analyzed in greater detail. This superfamily consists of several distantly related families distinguished by organismal origin. One such family includes members derived almost exclusively from Gram-negative bacteria. A second consists of proteins exclusively from Gram-positive bacteria. A third has members from eukaryotes, and a fourth is primarily derived from archaea. Finally, one RND family has members in both archaea and bacteria.

This last mentioned family is the so-called SecDF family which includes the two proteins, SecD and SecF that somehow function together to facilitate export of proteins from prokaryotes via the general secretory (Sec) pathway (Saier *et al.*, 1989). The two trees are very similar in appearance, and in general, the phylogeny of the proteins follows that of the source organisms. Thus, all archaeal proteins cluster together (lower left of Figure 2A and B), and the bacterial proteins cluster separately (upper right in both figures). The two spirochete proteins cluster together in both trees. These results show that the SecD and SecF

F-type ATPase (beta subunit) tree

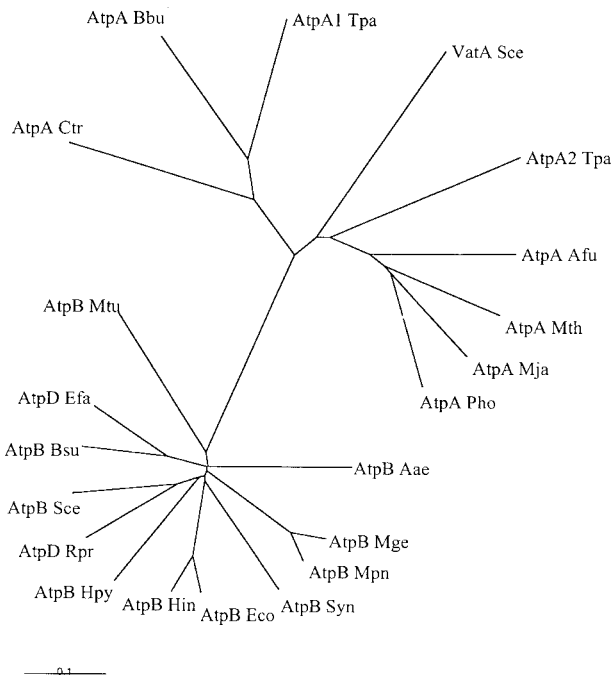


Figure 1. Phylogenetic tree for the catalytic subunits of F- (lower cluster) and V-type (upper cluster) ATPases of the F-ATPase family. Grouping of proteins in each of the two clusters generally follows that of the organisms (*i.e.*, of 16S rRNAs) suggesting vertical transmission. The sole exception is the second *T. pallidum* ATPase catalytic subunit, AtpA2 Tpa, that falls between the eukaryotic protein (VatA Sce) and the cluster of archaeal proteins. The Clustal X program was used to generate the tree.

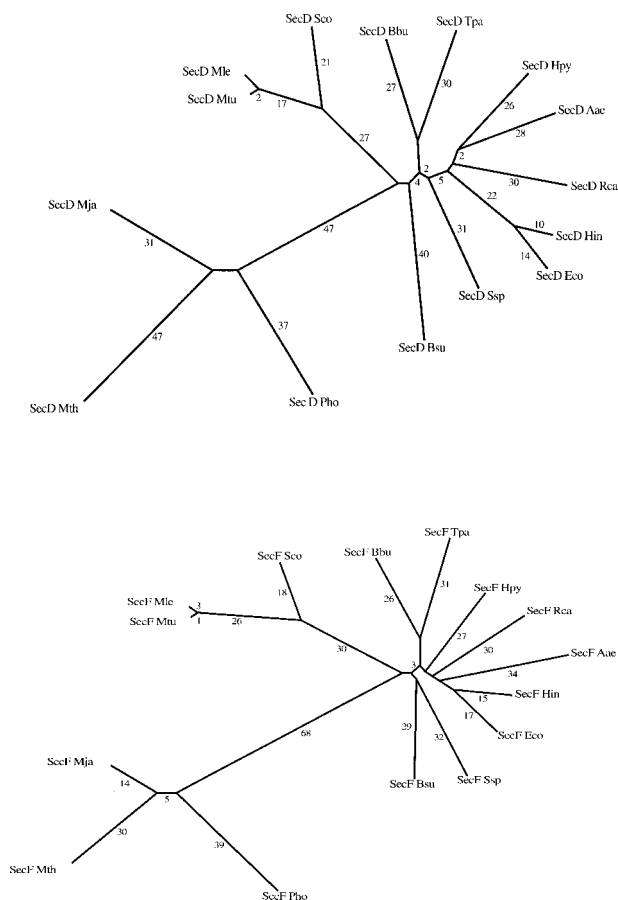


Figure 2. Phylogenetic trees for the SecD proteins (top) and the SecF proteins (bottom) of the SecDF family (TC #2.6.4). Branch lengths, in arbitrary units, are provided adjacent to the branches and are approximately proportional to phylogenetic distance. The TREE program of Feng and Doolittle (1990) was used to generate the tree. Reproduced with permission from Tseng *et al.* (1999), where protein abbreviations can be found.

proteins (each of which is present in each organism in single copy) were inherited vertically with no horizontal transmission of the encoding genetic material between organisms.

The Gram-negative RND superfamily proteins are shown in Figure 3. At the top of the tree is a cluster of multidrug resistance proteins including the well-characterized AcrB protein of *E. coli* and the Mex proteins of *Pseudomonas aeruginosa*. At the bottom of the tree is another well-characterized cluster, a cluster of heavy metal efflux pumps including the CzcA and CnrA proteins of *Ralstonia eutropha* (Tseng *et al.*, 1999).

Finally, the NolGHI protein complex of *Rhizobium meliloti* (lower right) has been implicated in lipooligosaccharide export for purposes of signaling during symbiotic nodule development when the bacteria grow in association with leguminous plants. The single spirochete protein represented in this tree (Orf Bbu, upper left) is so distant from all functionally characterized members of this family that no functional assignment is possible. However, since all RND superfamily members catalyze export, the same can be assumed for this protein. Interestingly, the

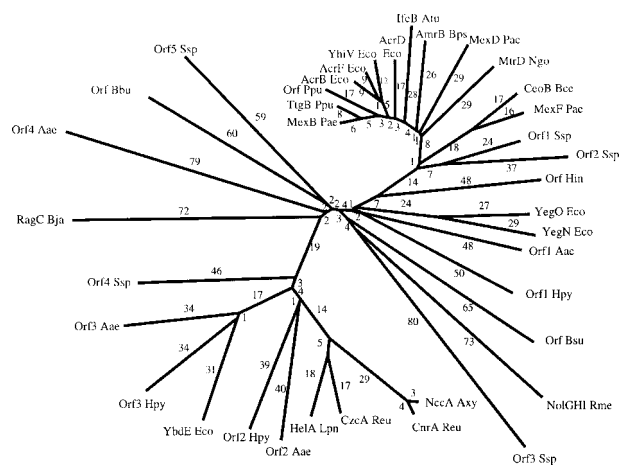


Figure 3. Phylogenetic tree for the Gram-negative bacterial RND family proteins. Protein abbreviations are given in Table 3. The format of presentation and computer program used were as described in the legend to Figure 2. Reproduced with permission from Tseng *et al.* (1999). Protein abbreviations can be found in this reference.

Borrelia gene encoding this RND permease (BB0140) is found in an operon (see Figure 4) that also encodes a membrane fusion protein (MFP; BB0141) and an outer membrane factor (OMF; BB0142) that undoubtedly function together to allow export across both membranes of the spirochete cell envelope (Dinh *et al.*, 1994; Paulsen *et al.*, 1997; Tseng *et al.*, 1999). This is one of the few documented instances of an outer membrane porin protein of β -structure found in a spirochete outer membrane.

Phylogenetic trees for the proteins of the archaeal family of the RND superfamily are portrayed in Figures 5A-C. All characterized RND permeases consist of two repeat units, as noted above for the SecDF complexes (Tseng *et al.*, 1999). One member of the archaeal RND superfamily family, however, has a single repeat unit. This protein is Orf Mja from *Methanococcus jannaschii* (see Figure 5). The halves of the full-length proteins were therefore analyzed separately with the N-terminal halves shown in Figure 5A, the C-terminal halves shown in Figure 5B, and all halves shown in Figure 5C. As expected, the first two trees are very similar in appearance. Noteworthy is the fact that in addition to the archaeal proteins, both Bbu and Tpa have representation.

The tree depicted in Figure 5C is informative with respect to the evolution of this family. The N-terminal halves of the spirochete proteins cluster together as do the C-terminal halves. This fact suggests that the duplication

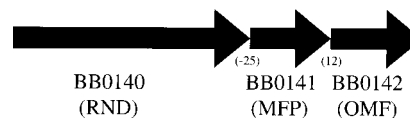


Figure 4. Structure of a putative tricistronic operon encoding an RND permease (BB0140; Tseng *et al.*, 1999), a membrane fusion protein (MFP; BB0141; Dinh *et al.*, 1994), and an outer membrane factor (OMF; BB0142; Paulsen *et al.*, 1997). The first two genes overlap by 25 nucleotides while the last two genes are separated by 12 nucleotides. The promoter precedes BB0140.

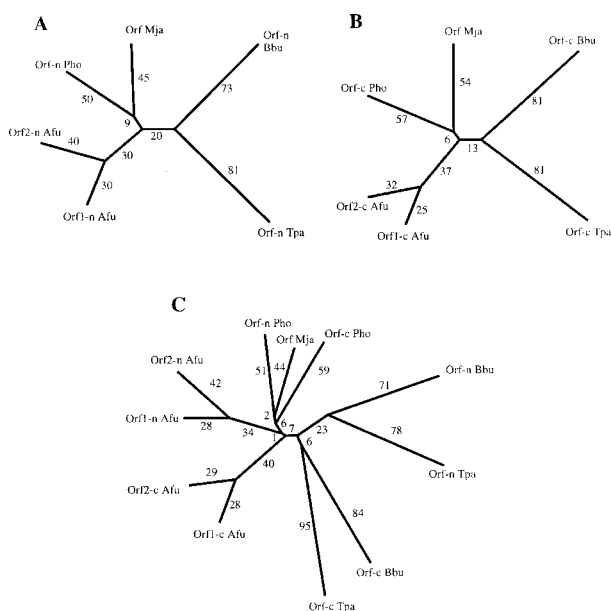


Figure 5. Phylogenetic trees derived from the sequences of the archaeal-spirochete family (HAE3) of the RND superfamily (A, the N-terminal halves; B, the C-terminal halves; C, all repeat units). The single repeat unit of the *M. jannaschii* protein was included in all three trees. The format of presentation and computer program used were as described in the legend to Figure 2. Reproduced with permission from Tseng *et al.* (1999) where protein abbreviations can be found.

event that gave rise to the full-length spirochete proteins occurred before Bbu diverged from Tpa. Similarly, the two proteins from *Archaeoglobus fulgidus*, Orf1 Afu and Orf2 Afu, show clustering of the N-terminal and C-terminal halves. This fact shows that the internal gene duplication event that gave rise to these full-length proteins occurred long before the extragenic duplication events that gave rise to Orf1 and Orf2 from their precursor protein. As all of the protein halves, both from Archaea and Spirochetes, stem from points near the center of the tree, it is possible that the internal gene duplication event that gave rise to the internally duplicated members of this family occurred only once, at a very early stage during the evolution of this family. The function, mode of action and oligomeric structure of the half-length *M. jannaschii* protein will be of considerable interest.

Family Representation in Bbu and Tpa

Table 7 provides information about the numbers of families that have representation in both Bbu and Tpa versus those that are found in only one of these two spirochetes. Nine families are found in both organisms; nine are found only in Bbu, and seven are found only in Tpa. It can therefore be suggested that Bbu and Tpa are as different from each other as they are similar.

Substrates of Spirochete Transporters

The percentages of transporters specific for various classes of compounds were calculated (data not shown). The two spirochetes exhibit a two- to three-fold greater percentage of transporters specific for inorganic cations, relative to

E. coli, but similar distributions of transporters for organic nutrients, drugs, and macromolecules. However, while Tpa appears to acquire amino acids from its environment primarily by taking up free amino acids, Bbu has few amino acid uptake permeases and a much greater potential for peptide uptake. Additionally, Bbu has a far greater potential for drug/hydrophobe efflux than does Tpa.

Tables 8 and 9 summarize the data for inorganic ions and organic nutrients, respectively, in the two spirochetes under study. As summarized in Table 8, each organism has a single K^+ transporting Trk family member, but Bbu has Na^+/H^+ and K^+/H^+ exchange transporters that are lacking in Tpa. Both organisms have a single MgtE Mg^{2+} uptake system and a single CaCA Ca^{2+} efflux system. However, heavy metals are transported in Tpa by ATP hydrolysis-dependent primary active transport while Bbu uses secondary active transport. While both organisms have chromate/sulfate transporters, only Bbu has recognized phosphate permeases while only Tpa has a recognized molybdate transporter (Table 8).

The putative transporters for organic nutrients are listed in Table 9. Only Bbu has a glycerol facilitator of the MIP family; both have a single transporter for quaternary amines (glycine-betaine versus carnitine-choline), and both have tremendous potential for the uptake of sugars. However, as noted above, Bbu takes up sugars primarily via the PTS while Tpa uses ABC permeases for this purpose. With respect to amino acids and peptides, Tpa has a much greater variety of transporters for amino acids while Bbu has more potential for peptide uptake. Finally, both organisms have a putative polyamine uptake permease.

Conclusions

The data presented in this report show that spirochetes exhibit some unique characteristics and provide insight into their physiology and preferred ecological niches. The following observations are particularly noteworthy.

First, both Bbu and Tpa have few proteins with >10 TMSs, relative to most other bacteria, and correlating with this fact, both have fewer identified transporters per unit of genome size than most bacteria. Because they have greater percentages of multicomponent systems, however, they have similar percentages of genes encoding transport proteins.

Second, both Bbu and Tpa lack all but one recognized ion channel proteins. However, Tpa has twice as many primary carriers as Bbu while Bbu has PTS permeases that are lacking in Tpa. Their primary sources of carbon

Table 7. Family Representation in Bbu vs. Tpa

	Common to Bbu and Tpa (9 families)	Bbu-specific (9 families)	Tpa-specific (7 families)
RND	3	2	MFS (1) BCCT(1)
CaCA	1	1	CDF (1) NSS (1)
DAACS	1	2	LctP (1) AGCS (2)
Trk	1	1	SSS (1) LIVS (1)
MATE	3	1	GltS (1) PNaS (1)
ABC	9	16	NhaC (2) P-ATPase (1)
F-ATPase	1	2	CPA2 (1) NaT-DC (1)
CHR	1	1	GlcPTS (4)
MgtE	1	1	FruPTS (2)

Table 8. Transporters for Inorganic ions in Bbu and Tpa

Family	Bbu	Tpa
Monovalent Cations		
MscS	Ions	Ions
NhaC	Na ⁺ /H ⁺ (2)	–
CPA2	Na ⁺ or K ⁺ /H ⁺	–
Trk	K ⁺	K ⁺
F-ATPase	H ⁺	H ⁺ ; Na ⁺ ? (2)
Subtotal	6	4
Divalent Cations		
MgtE	Mg ²⁺	Mg ²⁺
CaCA	Ca ²⁺	Ca ²⁺
ABC	–	Co ²⁺
ABC	–	Mn ²⁺ (2)
P-ATPase	–	Cu ²⁺
CDF	Me ²⁺	–
Subtotal	3	5
Anions		
PnaS	HPO ₄ ⁼	–
ABC	HPO ₄ ⁼	MoO ₄ ⁼
CHR	CrO ₄ ⁼ ; SO ₄ ⁼	CrO ₄ ⁼ ; SO ₄ ⁼
Total # transporters	12	11
Total # families	10	8

for energy production are clearly simple sugars, and both organisms have multiple sugar uptake permeases. Tpa is the only sequenced prokaryote to date with two V-type ATPases, and one of them resembles those found in eukaryotes or archaea more than those of bacteria. Bbu has substantially more secondary carriers than Tpa, correlating with its larger genome size.

Third, the largest families of secondary carriers in most organisms (MFS and APC) are essentially lacking in Spirochetes. The largest families of Spirochete carriers (RND and MATE) are efflux carriers, and these families are not among the larger families in *E. coli*. Only a few recognized families in Spirochetes are lacking in *E. coli*. These include the CHR, MgtE, NSS and PNaS, families all specific for inorganic ions with the sole exception of a single NSS family permease in Tpa that probably transports amino acids and/or their derivatives.

Fourth, the two Spirochetes analyzed have four very substantial differences as follows: (a) While Bbu has ~2x more transporters for monovalent cations than Tpa, Tpa has ~2x more transporters for divalent cations than Bbu. (b) While Bbu has many peptide uptake transporters and few amino acid transporters, the reverse is true for Tpa. (c) Bbu has more drug/hydrophobe exporters than Tpa. (d) Bbu has many PTS permeases but few sugar-specific ABC permeases, while Tpa has many sugar-specific ABC permeases but no PTS permeases. These differences presumably reflect the different lifestyles, homeostatic control mechanisms and metabolic potentials of these two evolutionarily divergent Spirochetes.

Finally, with the sole exception of the ABC superfamily, Spirochetes exhibit exceptional transporter diversity with >90% of represented families having only 1 or 2 members per organism. The vast majority of families have only a single representative in each organism.

We hope that the analyses of Spirochete transporters

reported in this symposium article will provide new insight into the physiology, ecology, and pathology of Spirochetes. They may also provide clues for the development of more effective media for the cultivation of these organisms. The extent to which these studies are relevant to other Spirochetes has yet to be determined. However, it should be kept in mind that all of the conclusions reached in this article are based largely on sequence comparisons and will require experimental verification. Much work lies ahead for the experimental Spirochetoologist.

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References

- Booth, I.R., and Louis, P. 1999. Managing hypoosmotic stress: Aquaporins and mechano-sensitive channels in *Escherichia coli*. *Curr. Opin. Microbiol.* 2: 166-169.
- Breznak, J.A., and Canale-Parola, E. 1969. *Spirochaeta aurantia*, a pigmented, facultatively anaerobic spirochete. *J. Bacteriol.* 97: 386-395.
- Breznak, J.A., and Canale-Parola, E. 1972. Metabolism of *Spirochaeta aurantia*. I. Anaerobic energy-yielding pathways. *Arch. Mikrobiol.* 83: 261-277.
- Breznak, J.A., and Canale-Parola, E. 1975. Morphology and physiology of *Spirochaeta aurantia* strains isolated from aquatic habitats. *Arch. Microbiol.* 105: 1-12.
- Canale-Parola, E. 1977. Physiology and evolution of spirochetes. *Bacteriol. Rev.* 41: 181-204.
- Dinh, T., Paulsen, I.T., and Saier, M.H., Jr. 1994. A family of extracytoplasmic proteins that allow transport of large molecules across the outer membranes of Gram-negative bacteria. *J. Bacteriol.* 176: 3825-3831.
- Donelson, J.E. 1995. Mechanisms of antigenic variation in *Borrelia hermsii*

Table 9. Transporters for Organic Nutrients in Bbu and Tpa

Family	Bbu	Tpa
Carbon Sources		
MIP	Glycerol	–
LctP	Lactate	–
BCCT	–	Carnitine/choline
ABC	Glycine-betaine	–
ABC	Sugars (1)	Sugars (8)
PTS	Sugars (6)	–
Subtotal	10	9
Amino Acids and Derivatives		
NSS	–	?
DAACS	Glu	Glu; neutral Aas
AGCS	–	Ala/Gly (2)
LIVCS	–	Leu/Ile/Val
GltS	Glu	–
ABC	–	Amino acids (2)
ABC	Polyamines	Polyamines
ABC	Peptides (4)	Peptides (1)
Subtotal	7	8
Vitamins		
SSS	Panθοthenate	–
Total # transporters	18	17
Total # families	7	5

- and African trypanosomes. *J. Biol. Chem.* 270: 7783-7786.
- Feng, D.-F., and Doolittle, R.F. 1990. Progressive alignment and phylogenetic tree construction of protein sequences. *Methods Enzymol.* 183: 375-387.
- Fraser, C.M., Casjens, S., Huang, W.M., Sutton, G.G., Clayton, R., Lathigra, R., White, O., Ketchum, K.A., Dodson, R., Hickey, E.K., Gwinn, M., Dougherty, B., Tomb, J.-F., Fleischmann, R.D., Richardson, D., Peterson, J., Kerlavage, A.R., Quackenbush, J., Salzberg, S., Hanson, M., van Vugt, R., Palmer, N., Adams, M.D., Gocayne, J., Weidman, J., Utterback, T., Wathley, L., McDonald, L., Artiach, P., Bowman, C., Garland, S., Fujii, C., Cotton, M.D., Horst, K., Roberts, K., Hatch, B., Smith, H.O., Venter, J.C. 1997. Genomic sequence of a Lyme disease spirochaete, *Borrelia burgdorferi*. *Nature.* 390: 580-586.
- Fraser, C.M., Norris, S.J., Weinstock, G.M., White, O., Sutton, G.G., Dodson, R., Gwinn, M., Hickey, E.K., Clayton, R., Ketchum, K.A., Sodergren, E., Hardham, J.M., McLeod, M.P., Salzberg, S., Peterson, J., Khalak, H., Richardson, D., Howell, J.K., Chidambaram, M., Utterback, T., McDonald, L., Artiach, P., Bowman, C., Cotton, M.D., Fujii, C., Garland, S., Hatch, B., Horst, K., Roberts, K., Sandusky, M., Weidman, J., Smith, H.O., and Venter, J.C. 1998. Complete genome sequence of *Treponema pallidum*, the syphilis spirochete. *Science.* 281: 375-388.
- Ge, Y., Li, C., Corum, L., Slaughter, C.A., and Charon, N.W. 1998. Structure and expression of the FlaA periplasmic flagellar protein of *Borrelia burgdorferi*. *J. Bacteriol.*, 180: 2418-2425.
- Greenberg, E.P., and Canale-Parola, E. 1977. Chemotaxis in *Spirochaeta aurantia*. *J. Bacteriol.* 130: 485-494.
- Izard, J., Samsonoff, W.A., Kinoshita, M.B., and Limberger, R.J. 1999. Genetic and structural analyses of cytoplasmic filaments of wild-type *Treponema phagedenis* and a flagellar filament-deficient mutant. *J. Bacteriol.* 181: 6739-6746.
- Jack, D.L., Paulsen, I.T., and Saier, M.H., Jr. 2000. The APC superfamily of transporters specific for amino acids, polyamines and organocations. *Microbiology.* In press.
- Janssen, P.H., and Morgan, H.W. 1992. Glucose catabolism by *Spirochaeta thermophila* RI 19.B1. *J. Bacteriol.* 174: 2449-2453.
- Levina, N., Töttemeyer, S., Stokes, N.E., Louis, P., Jones, M.A., and Booth, I.R. 1999. Protection of *E. coli* cells against extreme turgor by activation of MscS and MscL mechanosensitive channels: Identification of genes required for MscS activity. *EMBO J.* 18: 1730-1737.
- Olsen, G.J., Woese, C.R., and Overbeek, R. 1994. The winds of evolutionary change: Breathing new life into microbiology. *J. Bacteriol.* 176: 1-6.
- Paster, B.J., Dewhirst, F.E., Weisburg, W.G., Tordoff, L.A., Fraser, G.J., Hespell, R.B., Stanton, T.B., Zablén, L., Mandelco, L., and Woese, C.R. 1991. Phylogenetic analysis of the spirochetes. *J. Bacteriol.* 173: 6101-6109.
- Paulsen, I.T., Park, J.H., Choi, P.S., and Saier, M.H., Jr. 1997. A family of Gram-negative bacterial outer membrane factors that function in the export of proteins, carbohydrates, drugs and heavy metals from Gram-negative bacteria. *FEMS Microbiol. Lett.* 156: 1-8.
- Paulsen, I.T., Sliwinski, M.K., and Saier, M.H., Jr. 1998a. Microbial genome analyses: global comparisons of transport capabilities based on phylogenies, bioenergetics and substrate specificities. *J. Mol. Biol.* 277: 573-592.
- Paulsen, I.T., Sliwinski, M.K., Nelissen, B., Goffeau, A., and Saier, M.H., Jr. 1998b. Unified inventory of established and putative transporters encoded within the complete genome of *Saccharomyces cerevisiae*. *FEBS Lett.* 430: 116-125.
- Saier, M.H., Jr., Newman, M.J., and Rephaeli, A.W. 1977. Properties of a phosphoenolpyruvate:mannitol phosphotransferase system in *Spirochaeta aurantia*. *J. Biol. Chem.* 252: 8890-8898.
- Saier, M.H., Jr., Werner, P.K., and Müller, M. 1989. Insertion of proteins into bacterial membranes: Mechanism, characteristics, and comparisons with the eucaryotic process. *Microbiol. Rev.* 53: 333-366.
- Shi, W., Yang, Z., Geng, Y., Wolinsky, L.E., and Lovett, M.A. 1998. Chemotaxis in *Borrelia burgdorferi*. *J. Bacteriol.* 180: 231-235.
- Tseng, T.-T., Gratwick, K.S., Kollman, J., Park, D., Nies, D.H., Goffeau, A., and Saier, M.H., Jr. 1999. The RND permease superfamily: An ancient, ubiquitous and diverse family that includes human disease and development proteins. *J. Mol. Microbiol. Biotechnol.* 1: 107-125.
- You, Y., Elmore, S., Colton, L.L., Mackenzie, C., Stoops, J.K., Weinstock, G.M., and Norris, S.J. 1996. Characterization of the cytoplasmic filament protein gene *cfpA* of *Treponema pallidum* subsp. *pallidum*. *J. Bacteriol.* 178: 3177-3187.

