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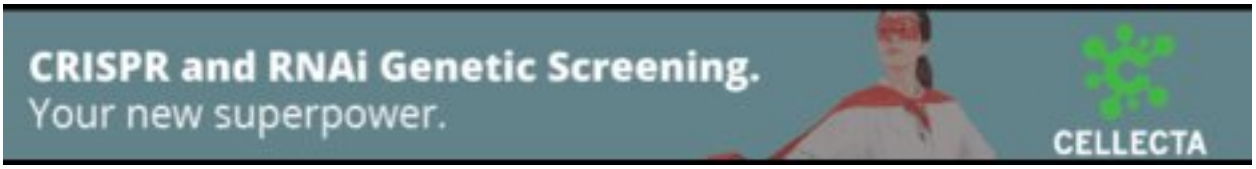

Article

Tropheryma whipplei Twist: A Human Pathogenic Actinobacteria With a Reduced Genome

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The human pathogen *Tropheryma whipplei* is the only known reduced genome species (<1 Mb) within the Actinobacteria [high G+C Gram-positive bacteria]. We present the sequence of the 927,303-bp circular genome of *T. whipplei* Twist strain, encoding 808 predicted protein-coding genes. Specific genome features include deficiencies in amino acid metabolisms, the lack of clear thioredoxin and thioredoxin reductase homologs, and a mutation in DNA gyrase predicting a resistance to quinolone antibiotics. Moreover, the alignment of the two available *T. whipplei* genome sequences (Twist vs. TW08/27) revealed a large chromosomal inversion the extremities of which are located within two paralogous genes. These genes belong to a large cell-surface protein family defined by the presence of a common repeat highly conserved at the nucleotide level. The repeats appear to trigger frequent genome rearrangements in *T. whipplei*, potentially resulting in the expression of different subsets of cell surface proteins. This might represent a new mechanism for evading host defenses. The *T. whipplei* genome sequence was also compared to other reduced bacterial genomes to examine the generality of previously detected features. The analysis of the genome sequence of this previously largely unknown human pathogen is now guiding the development of molecular diagnostic tools and more convenient culture conditions.

[Supplemental material is available online at www.genome.org. The sequence data from this study have been submitted to GenBank under accession no. AE014184. Genome sequence and annotation are also available at http://igs-server.cnrs-mrs.fr/.]

Tropheryma whipplei is the bacterial agent of Whipple's disease, a spectacular chronic disease described in 1907 by Nobel laureate George Whipple (Whipple 1907). Whipple's disease is characterized by intestinal malabsorption leading to cachexia and death if appropriate antibiotic treatment is not given. *T. whipplei* resisted reproducible culture until grown in human fibroblasts in 2000 (Raoult et al. 2000). The bacterium is small (0.3 × 1.5 mm) and Gram-negative on staining (La Scola 2001). It possesses an atypical envelope and a thick cell wall. In culture, it exhibits giant rope-like structures similar to *Mycobacterium tuberculosis*. Very little is known of its physiology (La Scola 2001).

At the time that genome sequencing of *T. whipplei* was initiated, only five genes were identified: 16S rRNA, 5S rRNA, 23S rRNA, *groEL*, and *rpoB* (Wilson et al. 1991; Drancourt and Raoult 1999; Hinrikson et al. 2000; Maiwald et al. 2000). Phylogenetic analyses classified *T. whipplei* within the high G+C content Gram-positive bacteria class (Actinobacteria, including *Mycobacterium tuberculosis* and *Mycobacterium leprae*), albeit not closely related to any known genus (Drancourt et al. 2001; Marth and Raoult 2003). The *T. whipplei* reservoir is suspected to be environmental, as previous studies using PCR experiments revealed its presence in sewage water (Maiwald et al. 1999). In human beings, the bacterium is mostly seen within cells, but its strictly intracellular niche is still debated. *T. whipplei* has been observed within intestinal macrophages and circulating monocytes (Raoult et al. 2001a,b), while Fredricks and Relman (2001) reported extracellular metabolically active bacteria in the intestinal lumen. *T. whipplei* is able to multiply in acidic lysosome-like vacuoles in HeLa cells (Ghigo et al. 2002). In the laboratory, *T. whipplei* has only been cultured within eukaryotic cells. The first subcultures exhibited a slow doubling time of 17 d (Raoult et al. 2000), comparable to 14 d for *M. leprae* (Cole et al. 2001).

Genomes of intracellular/parasitic bacteria undergo massive reduction compared to their free-living relatives. Examples include *Mycoplasma* (Fraser et al. 1995) for Firmicutes (the low G+C content Gram-positive), *Rickettsia* (Andersson et al. 1998) for alpha proteobacteria, and *Wigglesworthia* (Akman et al. 2002) and *Buchnera* (Shigenobu et al. 2000) for gamma proteobacteria. With a genome size of less than 1 Mb, *T. whipplei* offers the prime example of genome reduction among Actinobacteria.

The sequencing of *T. whipplei* genome was undertaken as an efficient way to learn more about this largely unknown fastidious human pathogen, to guide the development of molecular diagnostic tools, and eventually suggest improved culture conditions. These new data also offer an opportunity to reevaluate the generality of features previously proposed to characterize reduced genomes.

RESULTS AND DISCUSSION

Genome Sequence and Predicted Functions

General Features

The 927,303-base pair (bp) circular genome of *T. whipplei* Twist exhibits 808 predicted protein coding genes and 54 RNA genes (Fig. 1A,B; Table 1). The average G+C content is 46%, by far the lowest among the genome sequences available for other high G+C content Gram-positive bacteria. Coding content is 85.6%. On a global scale, *T. whipplei* exhibits no detectable colinearity with any of its close relatives with much larger genomes such as *M. leprae* (1605 open reading frames, ORFs), *Corynebacterium glutamicum* (3040 ORFs), *M. tuberculosis* (3927 ORFs), and *Streptomyces coelicolor* (7897 ORFs; Supplementary Fig. S1, available online at www.genome.org). Predicted gene functions (Fig. 2) indicate that *T. whipplei* is relatively well equipped, with different biological functions compared to other bacteria with reduced genomes (<1 Mb).

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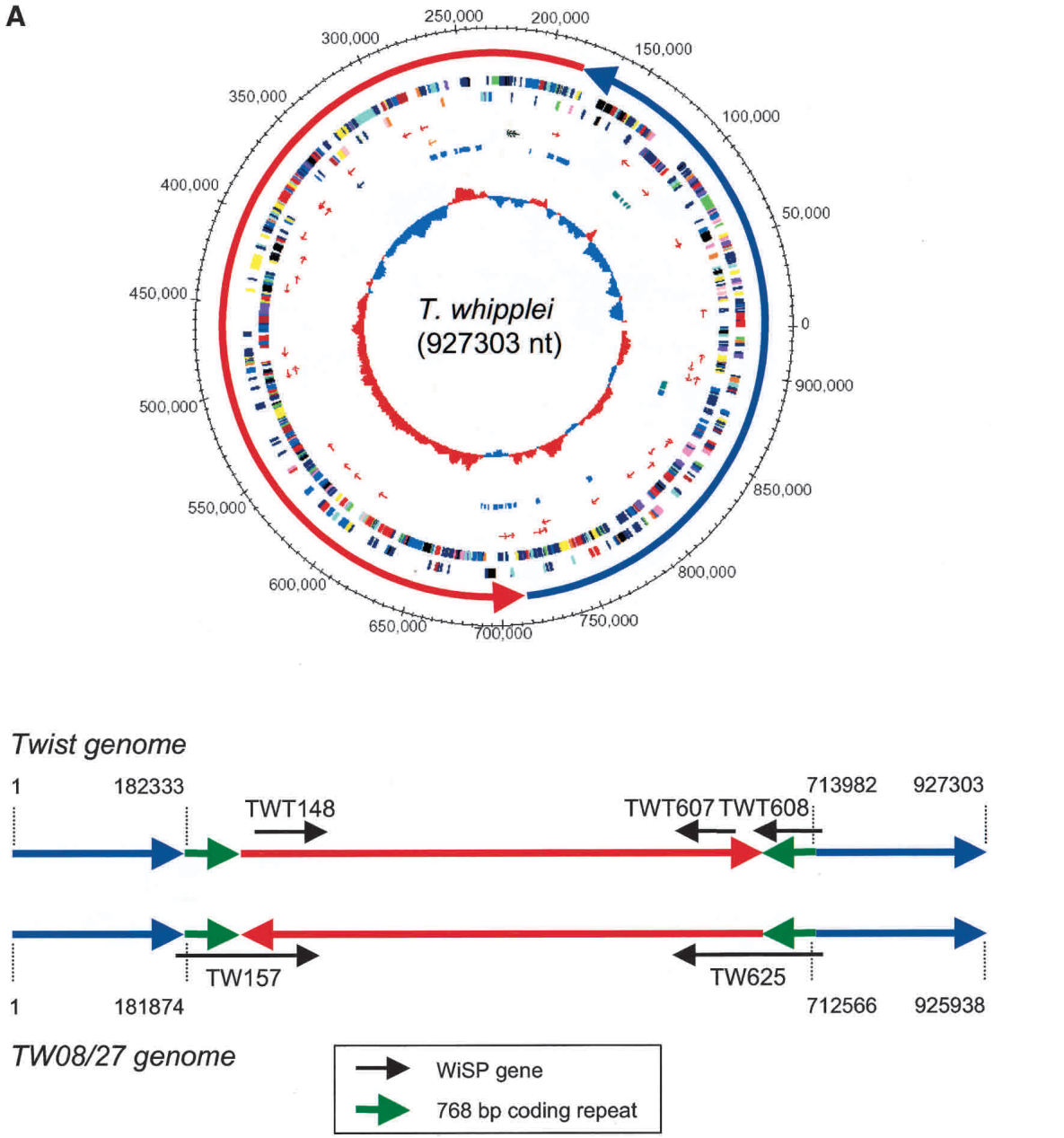

Figure 1 (Continued on next page)

Information Processing

T. whipplei exhibits a complement of information processing genes comparable to that found in other small parasitic bacteria (Fig. 2). The DNA polymerase III complex—the primary replication machinery—is composed of the alpha (*dnaE*), beta (*dnaN*), gamma-tau (*dnaZX*), and putative delta subunits, as found in *Mycoplasma genitalium*. *T. whipplei* lacks homologs for the DNA polymerase *polC*, the second and essential polymerase in *Bacillus subtilis* (Dervyn et al. 2001). In contrast, *T. whipplei* appears to have two DNA gyrases, each made of two subunits, as found in *S. coelicolor*, another Actinobacteria. *T. whipplei*'s TWT006/TWT005 gene pair appears to be the orthologs of the *gyrA/gyrB* subunits common to Actinobacteria; their role is to eliminate positive supercoils at the replication fork. The second gyrase-like copy, TWT491/TWT494, are orthologous to *S. coelicolor* SCO5836/SCO5822 genes that probably encode topoisomerase IV (ParC/ParE); this system is involved in chromosome segregation. Interestingly, an alanine residue was found at position 81 of GyrA and at position 96 of ParC, at which serine residues are usually found. In *Escherichia coli*, mutations at these positions are associated with the acquisition of resistance to quinolone antibiotics (Drlica and Zhao 1997; Drlica 1999). Based on these data, *T. whipplei* is predicted to be resistant to quinolones. This was recently confirmed experimentally (Masselot et al. 2003).

T. whipplei contains two paralogous genes for chromosome partitioning protein ParA. One (*parA*) is apparently orthologous to those of *Mycobacterium* and *Streptomyces*. The other (*parA2*) is rather similar to plasmid-encoded *parA* in the actinomycete *Rhodococcus erythropolis*. In addition, *T. whipplei* exhibits two replicative DNA helicases, *dnaC* and *pcrA*; the latter is involved in plasmid rolling-circle replication and ultraviolet-induced damage repair in *B. subtilis* (Petit et al. 1998). *T. whipplei* contains three

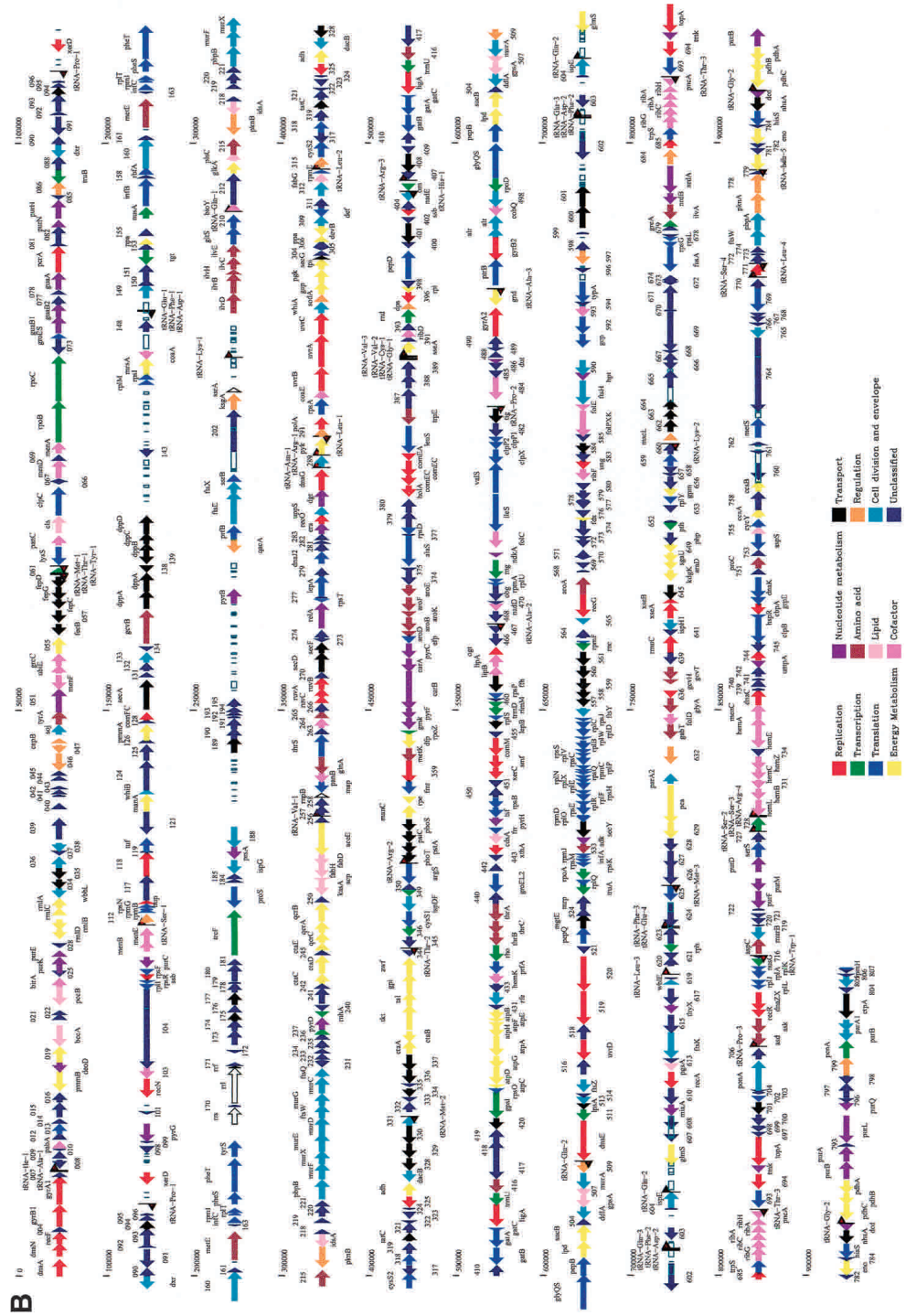

Figure 1 (*A*) Circular representation of the *T. whipplei* Twist genome (*upper panel*), and the alignment of the Twist and TW08/27 genomes (*lower panel*). The origin of replication was predicted to be near the dnaA gene, based on the conservation of dnaA-dnaN-recF gene cluster and the change of AT-skew signal. One leading strand displays a pronounced excess in T vs. A and a slight excess of G vs. C. The location of the oriC was verified by Southern-blot hybridization with a digoxigenin-labeled oriC probe onto the Not I restriction profile. In the *upper panel*, the outermost (1st) circle indicates the nucleotide positions. 2nd circle: The two chromosomal segments, one of which exhibits an inversion (see text). 3rd and 4th circles: The ORF locations on the plus and minus strands, respectively. Functional categories are color-coded (see Fig. 1B). 5th and 6th circles: tRNAs. 7th circle: The locations of three rRNAs are indicated by black arrows. 8th circle: The repeat locations for the two largest families. The most internal circle shows the AT skew (A − T/A+T) computed with a sliding window size of 10 kb. The *lower panel* shows the detailed structure of the chromosomal inversion (red arrows), flanked by repeated sequences coding for WND-domains (green arrows). Black arrows: WISP protein-coding genes at the extremities of the inverted segments. (*B*) Linear view of the complete circular genome of *T. whipplei*. Arrows = genes, with color-coded functional categories; small red flags = TRNAs; open arrows = other RNAs; open boxes = repeats belonging to the two major categories.

Table 1. General Features of the *T. whipplei* Genome

Genome size	927,303 bp
G + C content	46.3%
Coding content	85.6%
ORFs	808
Median size	287 aa
Annotated	535 (66%)
Best database match	678 (84%)
High G + C gram-positive bacteria	552
Streptomyces coelicolor	292
Mycobacterium tuberculosis	95
Corynebacterium glutamicum	72
Mycobacterium leprae	36
Other species	57
Low G + C Gram-positive bacteria	39
Proteobacteria	47
Other bacteria	19
Archaca	13
Eucarya	7
Viruses (*Equine herpesvirus 1*)	1
ORFans	130 (16%)
RNA	54
rRNA	3 (1 set)
tRNA	49 (20 species)
tmRNA	1
M1 RNA (RNase P)	1

competence-related genes homologous to *B. subtilis comEA*, *comEC*, and *comFC*. *T. whipplei* might thus naturally take up DNA from its environment, although this remains to be confirmed. In addition, we identified two putative site-specific integrase/recombinase genes (*xerC* and *xerD*) that may be involved in the genome rearrangements discussed below.

Four RNA polymerase subunit genes were identified: alpha (*rpoA*), beta (*rpoB*), beta′ (*rpoC*), and omega (*rpoZ*). As for the translation apparatus, *T. whipplei* exhibits 53 ribosomal protein genes and 20 genes for aminoacyl-tRNA synthetases (aaRSs). All amino acids are represented among these aaRSs, except for glutamine and asparagine. The *gatCAB* operon for the glutamyl-$tRNA^{Gln}$ amidotransferase found in the genome probably compensates for the lack of glutaminyl-tRNA synthetase (GlnRS) and asparaginyl-tRNA synthetase (AsnRS), as in *Chlamydia trachomatis* (Raczniak et al. 2001).

Energy and Small-Molecule Metabolisms

T. whipplei shows a limited complement of genes related to energy metabolisms. *T. whipplei* appears capable of producing energy by glycolysis, the pentose-phosphate cycle, and oxidative phosphorylation. All glycolysis genes were identified except for those encoding 6-phosphofructokinase (Pfk) and fructose-bisphosphate aldolase (Fba). In contrast, none of the genes corresponding to the tricarboxylic acid cycle was found. The lack of the two glycolysis enzymes is compensated for by the complete pentose phosphate pathway, which on one hand generates $NADPH+H^+$, required for the biosynthesis of fatty acids, and on the other hand, the ribose-5-phosphate required for nucleic acids biosynthesis. The gene complement for oxidative phosphorylation is limited. We identified 21 genes corresponding to the components of complex III, IV, V, plus one NADH dehydrogenase gene (*ndh*). *T. whipplei* has a putative transport system for L-arabinose (and/or D-xylose). Given the abundance of these sugars in plants, this suggests a possible environmental origin for *T. whipplei*. Overall, these predicted metabolic pathways indicate that *T. whipplei* could use glucose, fructose, maltose, and L-arabinose as primary energy sources.

Among parasitic bacteria with reduced genomes, *T. whipplei* has the most complete biosynthetic pathways for purine and pyrimidine nucleotides, fatty acids, several cofactors, and other small molecules (Fig. 2). In contrast, a detailed comparison of *T. whipplei* with the *M. tuberculosis* metabolic pathways in the KEGG database indicates rather important deficiencies in amino acid metabolisms (Fig. 3). Biosynthetic pathways appear to be lacking nine amino acids (histidine, tryptophan, leucine, arginine, proline, lysine, methionine, cysteine, and asparagine). In addition, partial deficiencies are predicted for seven other amino acids (glutamate, glutamine, aspartate, threonine, valine, isoleucine, and phenylalanine). The lack of asparagine synthetase (AsnB) might not affect protein synthesis, because of the presence of the amidotransferase (GatCAB) as described above. To compensate for the other defective biosynthetic pathways, the missing amino acids must be obtained from the environment or the host. This might involve membrane transport systems such as ABC transporters for amino acids and peptides, two of which were identified in the *T. whipplei* genome sequence. This suggests that enriching the medium in amino acids might improve the growth of *T. whipplei* in laboratory culture (P. Renesto, N. Crapoulet, H. Ogata, B. La Scola, G. Vestris, J.-M. Claverie, and D. Raoult, in prep.).

Both *T. whipplei* and *Buchnera* (Shigenobu et al. 2000) have retained approximately half of the amino-acid biosynthetic pathways. It is thought that *Buchnera* species specifically retained the pathways corresponding to amino acids essential for their insect hosts. As no such symbiotic association is known for *T. whipplei*, its residual biosynthetic capacity might point out the amino acids that are in the most limited supply from its environment or host.

T. whipplei lacks clear orthologs for thioredoxins (Trx) and thioredoxin reductase (TrxR) of *M. tuberculosis*, whose genome encodes three Trx (Rv3914, Rv1470, Rv1471) and one TrxR (Rv3913) in two operons. TrxR is a ubiquitous enzyme that reduces Trx, which in turn acts as electron donor in various essential redox reactions in the cell. TrxR- and Trx-encoding genes have been found in all bacterial genomes sequenced so far (Fig. 4). This includes *Coxiella burnetii*, which shares the same intracellular acidic vacuoles niche as *T. whipplei*. Because this would represent the first case of a bacterium without a functional thioredoxin system, this matter was investigated in greater detail. Protein motif searches using the PROSITE database entries (PS00194 for Trx; PS00573 for TrxR) failed to identify any ORFs with the sequence signatures in the *T. whipplei* genome. Another search using the TIGRFAM database (TIGR01068 for Trx; TIGR01292 for TrxR) again failed to identify any significantly similar sequences (above the noise cutoff). However, we further analyzed some candidates listed below this threshold. One was ORF TWT756, similar to *B. subtilis* thioredoxin-like protein gene *resA*. Another was ORF TWT210, exhibiting a significant sequence similarity to proteins of the pyridine nucleotide-disulfide oxidoreductases class II family. TWT210 might thus encode the thioredoxin reductase function, despite its weak similarity to known thioredoxin reductases. The glutaredoxin system serves similar roles as the thioredoxin system. *M. tuberculosis* has one copy of glutathione reductase gene (Rv2855), but the glutaredoxin system appears incomplete because of the lack of glutaredoxin genes. *T. whipplei* exhibits a distant homolog TWT629 to glutathione reductase, but shows no evidence of glutaredoxin genes. In conclusion, experiments are necessary to confirm that *T. whipplei* might be the first example of a bacterium without the—usually—essential thioredoxin pathway.

Interaction With the Environment

We identified 40 genes for transporters, which probably comprise 10 to 15 different transport systems. These include two systems for amino acids transport, one for L-arabinose, two for iron, and one for phosphate. We identified two putative membrane pro-

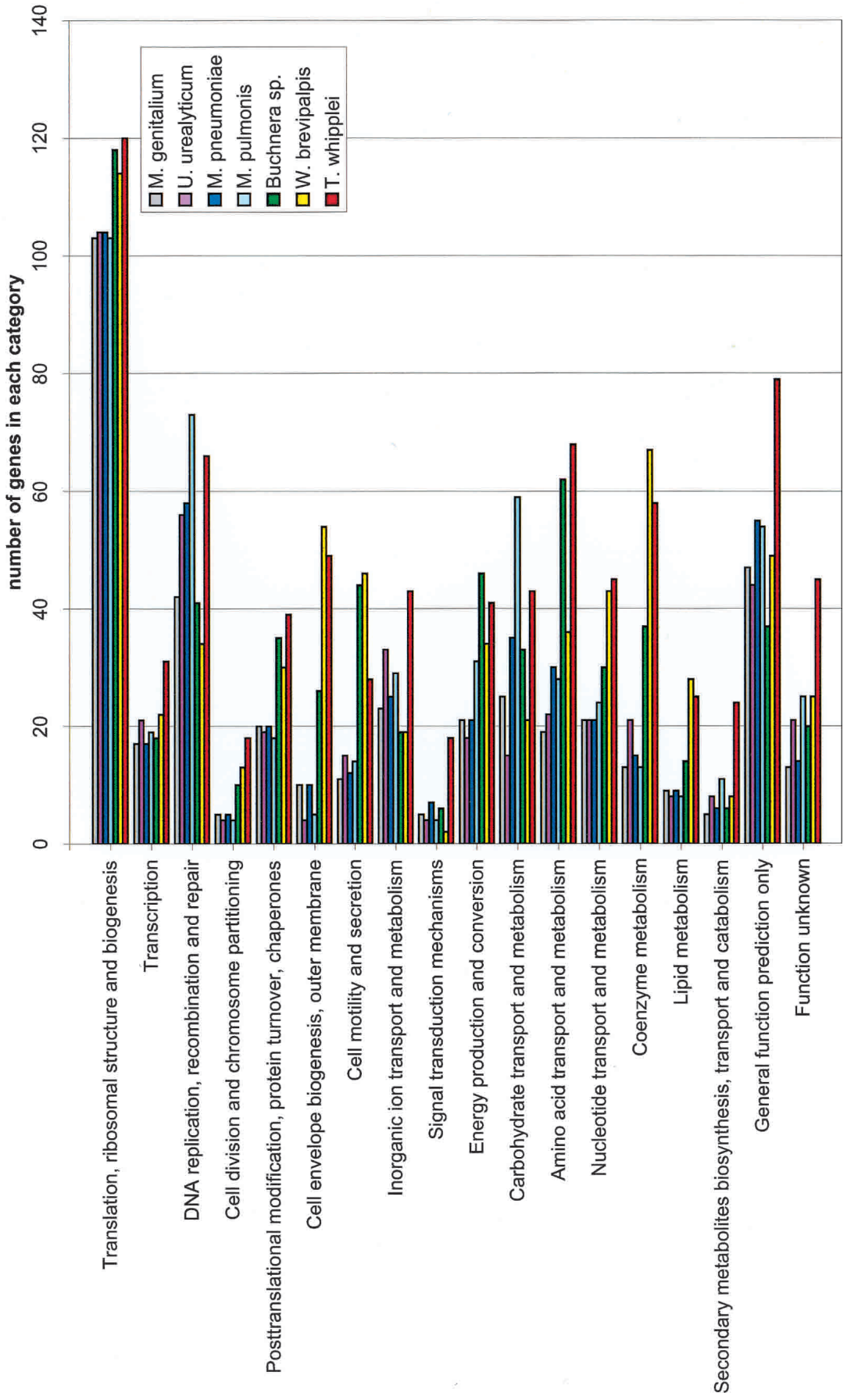

Figure 2 Comparative analysis of the number of genes present in each functional category as defined by the COG database. For the sake of comparing bacterial genomes of largely different sizes, a percentage graph is provided in Suppl. Fig. S2.

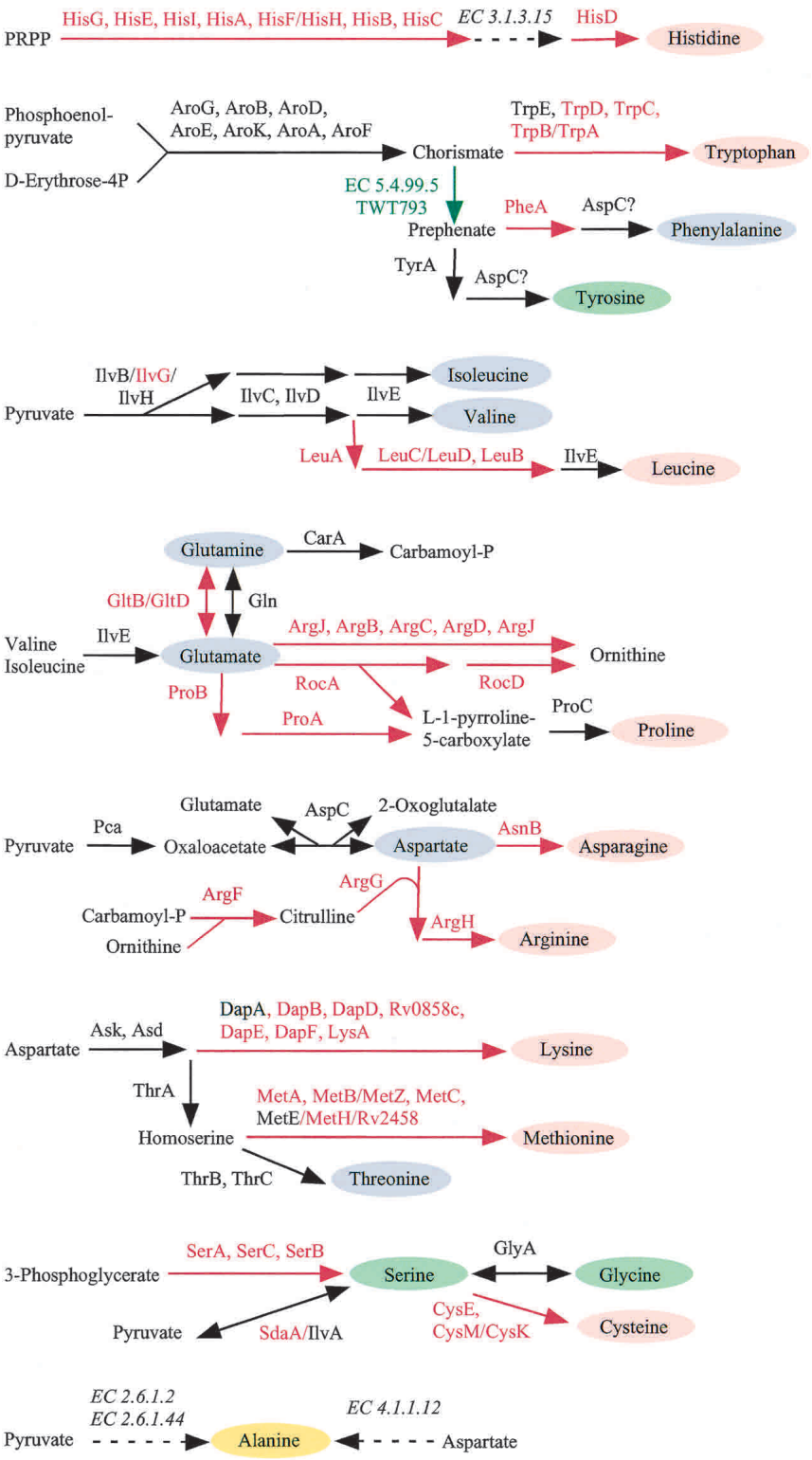

Figure 3 Predicted amino acid metabolisms of *T. whipplei* based on the *M. tuberculosis* metabolisms described in KEGG. Metabolic pathways were predicted to be lost for nine amino acids (pink ovals). Additional deficiencies were predicted for other seven amino acids (blue ovals). Metabolic pathways for three amino acids (green ovals) were retained. Enzymes for the alanine (yellow oval) biosynthesis were not identified in both *M. tuberculosis* and *T. whipplei*. Enzymatic steps predicted to be present in both *M. tuberculosis* and *T. whipplei* are shown in black. Enzymatic steps present only in *M. tuberculosis* or in *T. whipplei* are shown in red and green, respectively. Enzymatic steps absent in both *M. tuberculosis* and *T. whipplei* are shown with dashed black lines.

teins related to drug efflux proteins that may play a role in modulating antibiotic susceptibility. *T. whipplei* also exhibits a gene for dimethyladenosine transferase (*ksgA*), known to be related to kasugamycin resistance in *E. coli*. Those genes may help *T. whipplei* resist various antibiotics. A cold-shock protein gene *capB* was found, suggesting that the lifestyle of *T. whipplei* may include some cold periods and arguing against obligate human parasitism. The genes for two sensor histidine kinase/response regulator systems were also identified. Two protein translocation gene sets were identified, a Sec system and a twin-arginine translocase (TAT).

Finally, *T. whipplei* possesses *whiA* and *whiB*, two regulatory (possibly transcriptional) factors essential for the sporulation of *S. coelicolor* (Molle et al. 2000), and another *whiB* homolog (*wblB*). Spores have not yet been observed for *T. whipplei*, but the presence of these genes suggests that they may arise in environments not yet mimicked in laboratory culture conditions.

Gene Families

We identified 38 families of predicted gene paralogs (Suppl. Table S1). Of these, eight are lineage-specific families (exhibiting higher similarities within the family than to any other genes from other bacteria). They include WiSP membrane proteins (Bentley et al. 2003), other predicted membrane proteins, exodeoxyribonuclease III, inorganic pyrophosphatases, and iron ABC transporters.

Comparative Genome Analysis

The genome sequence of another *T. whipplei* isolate (strain TW08/27; GenBank: BX072543) recently became available (Bentley et al. 2003). The two genomic sequences of the different *T. whipplei* strains are mostly identical (>99% identity at the nucleotide sequence level), and encode quasi-identical gene complements. However, the Twist and TW08/27 genome organizations differ by the inversion of a large chromosomal segment (Twist coordinates: 182333–713982; Fig. 1A). Such inversions, approximately symmetrical to the origin of replication, are frequently observed in interspecific bacterial genome comparisons (Eisen et al. 2000; Hughes 2000; Makino and Suzuki 2001). However, such a difference between otherwise almost identical strains is an indication of a very active genome rearrangement process in *T. whipplei*. As already suggested for several Gram-negative species (*Salmonella*, *Neisseira*, *Pseudomonas*, and *Bordetella*), such genome rearrangement might be the consequence of the host-bacteria interaction (Hughes 2000). Interestingly, the boundaries of *T. whipplei* genome inversion are within the coding regions of two genes of

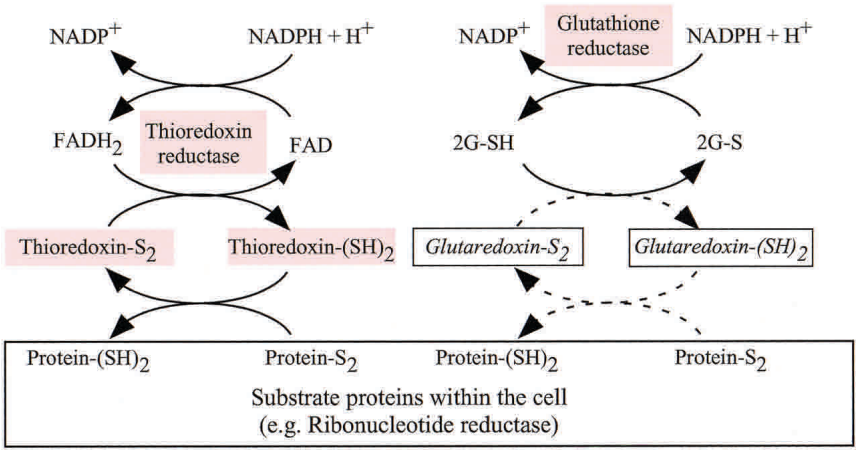

Figure 4 Thioredoxin and glutaredoxin systems. Proteins in pink rectangles are predicted to be present in *M. tuberculosis*, for which only remote homologs are detected in *T. whipplei*. Glutaredoxin gene is absent in both *T. whipplei* and *M. tuberculosis* (see text). 2G-SH and 2G-S represent reduced and oxidized forms of glutathione, respectively.

the WiSP membrane protein family. More precisely, they involve two virtually identical nucleotide sequences, corresponding to the N-terminal WND-domains of the WiSP proteins. In the re combination process, the WiSP genes in the TW08/27 isolate (TW157, TW625) were significantly altered in the Twist isolate. The TW08/27 genome exhibits eight copies of WND-domain sequences that are up to 99% identical over an 800-nucleotide span. In contrast, the rest of these WiSP genes are all quite different. This strongly suggests that WND-domain sequences act both as coding regions and as DNA repeats promoting genome recombination. PCR experiments were performed to validate the genome organization of our Twist isolate, using primers precisely defining the boundaries of the inversion. While the experiments confirmed the Twist genome organization for both extremities of the inverted segment, a PCR product compatible with one extremity of the TW08/27-like arrangement was also observed. This intra-isolate variation, as well as the differences between the Twist and TW08/27 genomes, suggests that *T. whipplei* exhibits a large genomic plasticity mediated by the highly conserved WND-domain repeats. We also found a paralogous gene family for another type of predicted membrane spanning protein (TWT569, TWT580, TWT653) that exhibits highly conserved bipartite segments separated by a variable sequence.

It is tempting to speculate that the frequent genome rearrangements mediated by such "coding" repeats lead to significant changes in the set of proteins exposed at the surface of the bacteria, and might constitute an adaptive response to the host defense or various environmental conditions.

T. whipplei gene content was classified into functional categories (Fig. 2) and compared with the other bacteria with reduced (<1Mb) genomes (*Mycoplasma* species, *Ureaplasma*, *Buchnera*, and *Wigglesworthia*). Overall, *T. whipplei* exhibits a larger complement of genes for most functional categories compared to these other bacteria. However, *Wigglesworthia* exhibits more genes relevant to lipid metabolism, cell envelope biogenesis, the outer membrane, and coenzyme metabolism. For the categories of carbohydrate transport and metabolism and DNA replication and repair, *Mycoplasma pulmonis* is better equipped. Finally, *Buchnera* exhibits a larger gene complement for energy production and conversion. This variability indicates that small bacterial genomes are not the result of a universal and unique reductive evolution pathway.

Genomic Features of Reduced Genomes

A certain number of genome reduction "rules" have been enunciated, following the analysis of the available genomes of parasitic/intracellular bacteria. Characteristic features of reduced genomes may be associated with the adaptive strategies linked to their strict or facultative association with hosts (Koonin et al. 2001; Doolittle 2002). The genome sequence of *T. whipplei* provides the first opportunity to examine these rules for a high G+C Gram-positive bacteria.

G+C Content

Within each clade, reduced genome bacteria tend to exhibit the lowest G+C content. *Rickettsia*, *Buchnera*, *Wigglesworthia*, *Mycoplasma*, and *Ureaplasma* species are all in the range of 22%–33% except for *Mycoplasma pneumoniae* (40%). At 46%, the G+C content of *T. whipplei* is by far the lowest of the Actinobacteria, including *M. leprae* (58%), *M. tuberculosis* (66%), and *S. coelicolor* (72%). The cause for this general trend remains unknown, although it has been linked to a mutational bias due to the loss of repair and recombination functions (Moran 1996; Moran and Wernegreen 2000). Consistently, the genome of *T. whipplei* is lacking most of the base excision repair genes found in *M. tuberculosis*. We also noticed that the genes most conserved (ubiquitous) across distant clades tend to resist this trend. For example, the average G+C content of the *T. whipplei* ORFs for 53 ribosomal proteins is 50%, whereas that for all of the ORFs is 47%.

Horizontal Gene Transfer

Gene acquisition by horizontal transfer appears less frequent for small genome parasitic bacteria such as *M. genitalium* and *Chlamidia* (Ochman et al. 2000; Brinkman et al. 2002) than for free-living bacteria. One trivial explanation might be found in their less promiscuous lifestyles, offering much fewer opportunities to exchange DNA with other microorganisms. The analysis of two *Rickettsia* genomes (Ogata et al. 2001) confirmed this tendency by revealing the lack of horizontal gene transfer over a period of 40–80 million years. The reduced *T. whipplei* genome appears to follow the same rule. Potentially foreign genes can be identified by their atypical G+C content (Lawrence and Ochman 1998). In the *T. whipplei* genome, only two such ORFs are identified (TWT513 and TWT613). Indeed, the G+C content of the *T. whipplei* ORFs varies little (the standard deviation is 3%), as in other obligate intracellular bacteria (Brinkman et al. 2002). Horizontally transferred genes are also pointed out by inconsistencies in phylogenetic trees. This approach identified nine candidate genes likely to have been acquired by horizontal gene transfer (Suppl. Table S2), thus amounting to about 1% of the genome. Of these nine, five are aminoacyl-tRNA synthetases, including a valyl-tRNA synthetase of the archaeal-type, also observed in *Rickettsia* (Woese et al. 2000). Four of these anomalous phylogenies with different topologies are shown in Figure 5, for two aminoacyl-tRNA synthetases (AspRS, ValRS), and two enzymes (PurB, PyrB) of the nucleotide metabolism. It should be noted that some of those anomalous phylogenies might also originate from ancient gene duplication followed by lineage-specific loss of paralogs.

Repeated Sequences

Another proposed rule is that repeated sequences should be less frequent in the reduced genomes of parasitic bacteria (Frank et al. 2002). A possible cause is again that the sequestered lifestyle of these bacteria diminishes their exposure to foreign selfish DNA elements (bacteriophages and transposons). Concurrently, even-

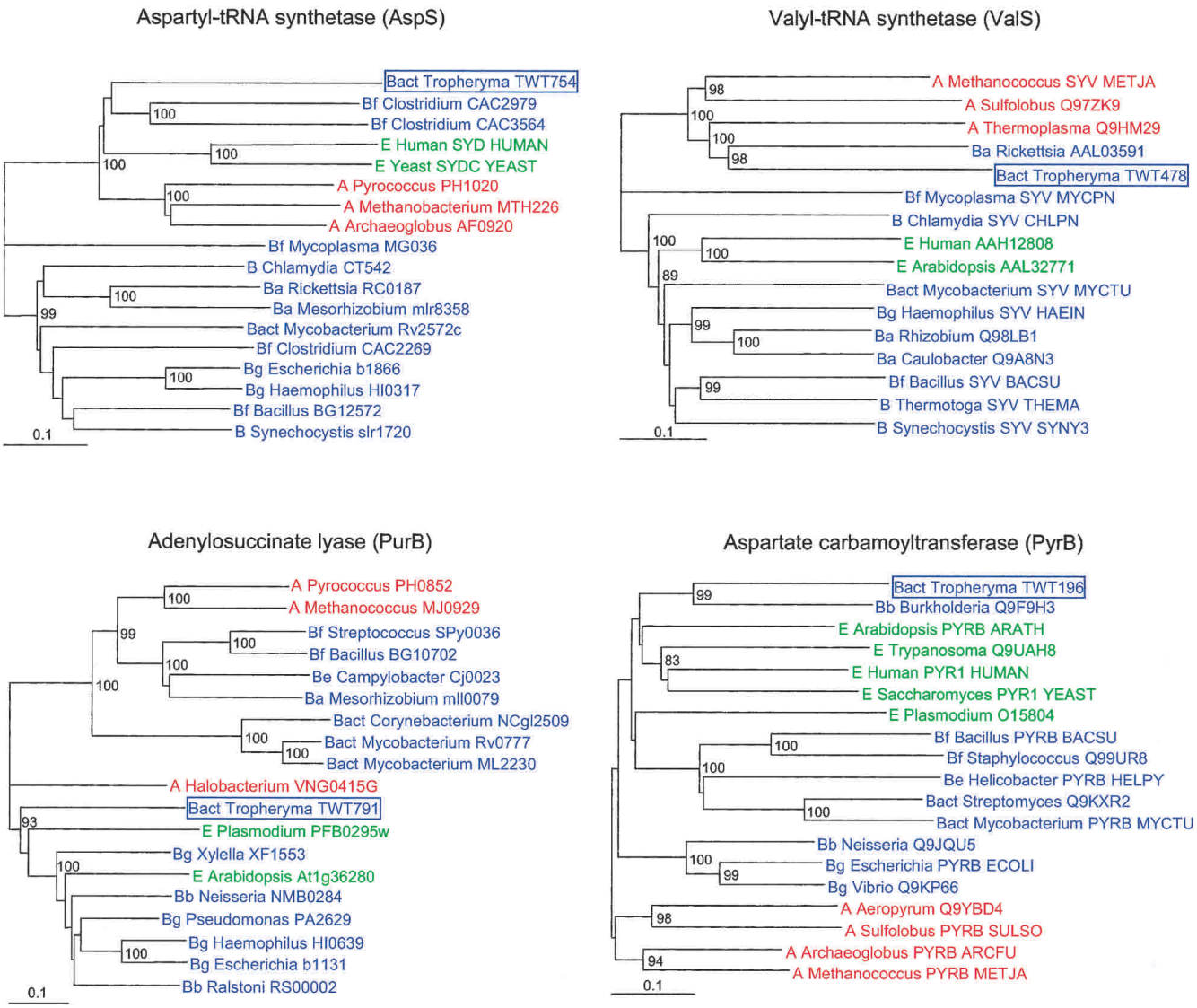

Figure 5 Typical examples of anomalous phylogenies exhibited by *T. whipplei* genes. Trees were constructed using CLUSTAL W alignments and the neighbor-joining method (Thompson et al. 1994). Bootstrap values are indicated when larger than 80%. Other examples are provided in Supplemental Figure S3.

tual repeated sequences are eliminated from these genomes by recombination. However, there are already some strong exceptions to that rule. *M. pneumoniae* exhibits a large number of repeats (Rocha et al. 1999). We observed many repeated sequences in an obligate intracellular parasite *Rickettsia conorii* (Ogata et al. 2002; Claverie and Ogata 2003). *T. whipplei* is another counter-example, with a high frequency of repeats. Thirty-eight repeat families were identified representing 6% of the *T. whipplei* genome. The two largest families account for 29,850 nt (3.22%) and 11,137 nt (1.20%) respectively (Fig. 1A,B). The family of the longest repeat consists of a pair of nearly identical sequences (99.91% identity over 4692 nucleotides). The positions of the repeats frequently correspond to abrupt changes in the AT-skew profile (Fig. 1A), suggesting their involvement in frequent local genome rearrangements (Petes and Hill 1988).

Genome Degradation

Finally, "on-going genome degradation" (Andersson and Andersson 2001; Lawrence et al. 2001) has been proposed to be induced by the sheltered and isolated lifestyle of parasitic bacteria. Accordingly, *R. prowazekii* (Andersson et al. 1998) and *M. leprae* (Cole et al. 2001) both exhibit a number of pseudogenes in their genomes, which appear under the process of elimination. However, this tendency is not so clear for other reduced genome bacteria such as *Chlamydia*. Moreover, two closely related *Rickettsia* exhibit very different levels of genome degradation (Ogata et al. 2001). Here, the *T. whipplei* genome exhibits a coding content (86%) comparable to the one of free-living bacteria, and shows few pseudogenes and little sign of on-going degradation.

Conclusion

An important finding derived from *T. whipplei* Twist (this work) and TW08/27 (Bentley et al. 2003) genome sequences is the existence of frequent genomic instability mediated by protein-coding repeats within genes of membrane proteins. This active recombination process probably causes the bacteria to expose different sets of proteins at their surface, in response to the host defense or various environmental conditions. At the same time,

frequent recombinations homogenize the repeat sequences and maintain or amplify their capacity to be involved in forthcoming genome rearrangements.

A related phenomenon has been reported to explain the variation of the MSP2 outer membrane protein of *Anaplasma marginale*, a rickettsial pathogen. Gene conversions between functional *msp2* genes and their pseudogenes (Brayton et al. 2001) have been invoked as the most likely mechanism. The *msp2* genes also exhibit highly conserved DNA segments, as found in *T. whipplei* WiSP genes. UV-induced genomic inversions mediated by highly conserved lipoprotein-like ORF sequences were also recently reported (Uchida et al. 2003) in *Streptomyces griseus*, an environmental actinobacterium. Intra-ORF genome inversions might thus be a process generally used by Actinobacteria to modulate the expression of their surface proteins in response to their environment.

The *T. whipplei* genome sequence now provides important and practical information on a poorly characterized bacterium, isolated only three years ago. In an attempt to improve the current molecular diagnosis for Whipple's disease, new PCR primers were designed according to the sequence of the highly conserved WND-domain repeats. The primers (Tw53–3F: 5′- TGT GTC TGT GGT TGG GGT AA-3′ / Tw53–3R: 5′-CCT CCT GCT CTA TCC CTC CT-3′) were tested against diluted *T. whipplei* cultures, and detected 10 to 100 more cells than *rpoB*-based primers (Drancourt et al. 2001).

The prediction of the *T. whipplei* resistance to quinolone antibiotics, which was later confirmed (Masselot et al. 2003), suggested the avoidance of the use of these compounds in the treatment of Whipple's disease.

Finally, the detailed analysis of the predicted metabolism of *T. whipplei* suggests useful clues on how to rationally modify the current culture conditions and improve our capacity to grow and study this extremely fastidious bacterium in the laboratory (P. Renesto, N. Crapoulet, H. Ogata, B. La Scola, G. Vestris, J.-M. Claverie, and D. Raoult, in prep.).

METHODS

Source and Preparation of DNA

T. whipplei Twist strain, a type 2A, was cocultivated with HEL cells in 150-cm^2 flasks as described (Raoult et al. 2000) to the 20th passage. Purified bacteria (analyzed using electron microscopy) were submitted to pressure shock using a French-pressure device (Bioritech), and lysed bacteria were mixed with 1% low melting point agarose (FMC Bio Products) in TE to form plugs in a mold at 37°C. DNA purity was assessed after logarithmic dilutions of purified *T. whipplei* DNA were subjected to a competitive PCR, targeting both human β-globin and a portion of the *rpoB* gene encoding the β-subunit of the RNA polymerase of *T. whipplei*. There was a 10:6 ratio between the concentrations of DNA of *T. whipplei* and that of the cells in which the organism was cultured. Digestion was performed by incubation of plugs in 25 mL of Tris-Sodium EDTA (6 mM Tris-HC1, pH 7.5, 1 M NaCl, 0.2 M EDTA, pH 8.0) and 1.25 mL of 10% sodium lauryl sarcosine solution (Sigma) to which 30 mg lysozyme (Boehringer Mannheim) was added and incubated at 37°C for 16 h on a roller. After two gentle washings in TE, the plugs were incubated overnight in a solution of EDTA-sarcosine-proteinase (30 mL 0.5 M EDTA, pH 8.5, 1.5 mL 10% sodium lauryl sarcosine, 60 mg proteinase K [Euromedex]) at 50°C. This operation was repeated three times, and the plugs were finally washed gently three times. Digested plugs were stored in EDTA 0.2 M, pH 8.0 at 4°C until used for cloning.

Genome Sequence Analysis

A first library A (5-kb inserts obtained by mechanic shearing, cloned in pcDNA 2.1 with Bst XI adaptors) was constructed, and a preliminary analysis of 96 clones disclosed <10^{-4} HEL cell DNA contamination. Plasmid clones were sequenced at both ends of the insert with flanking vector sequences as primers. Dye primer reactions were analyzed on an L1-COR 4200L, and six genomic equivalents were sequenced. We generated a second library B by cloning 20-kb fragments in pCNS with BstXI adaptors. Dye primer reactions were analyzed on a capillary ABI3700. The whole-genome assembly was performed by means of the PHRED, PHRAP, and CONSED 11.0 software packages (Ewing and Green 1998; Ewing et al. 1998; Gordon et al. 1998). A total of 6340 (6.34 X) and 5286 (2.93 X) end sequences (X's indicate genome equivalents) respectively, from libraries A and B were incorporated into contigs. Cloning gaps and several sensitive regions were resolved and confirmed by sequencing-duplicated PCR products. In addition, regions with lower quality were optimized using dye terminator reaction analyzed on a capillary ABI3100. The final sequence includes 99.9% positions with PHRED scores over 40. The coherence of the assembly was verified by comparison of the Spe I and Not I restriction site pattern previously obtained by pulsed-field gel electrophoresis. Also, the assembly was confirmed by sequencing PCR products obtained by incorporating primers designed on the basis of the scaffold of the molecule.

Informatics

Nucleotides in the *T. whipplei* genome were numbered according to the *M. tuberculosis* genome, where position one corresponds to the predicted origin of replication. Genes for tRNA were identified with the use of tRNAscan-SE (Lowe and Eddy 1997). Other RNAs were identified using BLAST (Altschul et al. 1997). ORFs at least 50 amino acid residues-long were predicted using SelfID (Audic and Claverie 1998). Additional ORFs shorter than 50 aa residues were identified by homology searches. ORFs overlapping with other ORFs were removed retrospectively. Repeated sequences were delineated using RepeatFinder (Volfovsky et al. 2001). ORF functions were predicted based on the homology searches by BLAST against the SWISS-PROT and TrEMBL sequence databases (Boeckmann et al. 2003). Metabolic pathways were analyzed using KEGG (Kanehisa et al. 2000). Preliminary sequence data for *C. burnetii* were obtained from The Institute for Genomic Research (http://www.tigr.org/). The functional classifications of the genes were performed in reference to the COG database (Tatusov et al. 2001) except for *T. whipplei*. COG categories for *T. whipplei* were determined by homology to COG database sequences.

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