Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 1160–1164, February 1985
Evolution

Construction of the mycoplasma evolutionary tree from 5S rRNA sequence data

(acholeplasma/anaeroplasma/spiroplasma/ureaplasma)

M. J. ROGERS*, J. SIMMONS*, R. T. WALKER*, W. G. WEISBURG†, C. R. WOESE†, R. S. TANNER†, I. M. ROBINSON‡, D. A. STAHL§, G. OLSEN§, R. H. LEACH¶, AND J. MANILOFF||**

*Department of Chemistry, University of Birmingham, P.O. Box 363, Birmingham B15 2TT, United Kingdom; †Department of Genetics and Development, University of Illinois, 515 Morrill Hall, Urbana, IL 61801; ‡National Animal Disease Center, Agricultural Research Service, Ames, IA 50010; §Department of Molecular and Cellular Biology, National Jewish Hospital and Research Center, Denver, CO 80206; ¶Public Health Laboratory Service Mycoplasma Reference Laboratory, Bowthorpe Road, Norwich NR2 3TX, United Kingdom; and ||Department of Microbiology, University of Rochester, Medical Center, Rochester, NY 14642

Communicated by Robert K. Selander, October 10, 1984

ABSTRACT **The 5S rRNA sequences of eubacteria and mycoplasmas have been analyzed and a phylogenetic tree constructed. We determined the sequences of 5S rRNA from *Clostridium innocuum*, *Acholeplasma laidlawii*, *Acholeplasma modicum*, *Anaeroplasma bactoclasticum*, *Anaeroplasma abactoclasticum*, *Ureaplasma urealyticum*, *Mycoplasma mycoides mycoides*, *Mycoplasma pneumoniae*, and *Mycoplasma gallisepticum*. Analysis of these and published sequences shows that mycoplasmas form a coherent phylogenetic group that, with *C. innocuum*, arose as a branch of the low G+C Gram-positive tree, near the lactobacilli and streptococci. The initial event in mycoplasma phylogeny was formation of the *Acholeplasma* branch; hence, loss of cell wall probably occurred at the time of genome reduction to ≈1000 MDa. A subsequent branch produced the *Spiroplasma*. This branch appears to have been the origin of sterol-requiring mycoplasmas. During development of the *Spiroplasma* branch there were several independent genome reductions, each to ≈500 MDa, resulting in *Mycoplasma* and *Ureaplasma* species. Mycoplasmas, particularly species with the smallest genomes, have high mutation rates, suggesting that they are in a state of rapid evolution.**

The wall-less prokaryotes are grouped in the class Mollicutes and are referred to as mycoplasmas (1). Mycoplasma genera (Table 1) are diverse in terms of their biochemistry, ecological niches, and genome structure. In addition, some of these organisms are significant animal, plant, and insect pathogens. Two aspects of mycoplasma biology are of particular interest. First, some mycoplasmas (i.e., *Spiroplasma*, *Mycoplasma*, *Ureaplasma*, and some *Anaeroplasma* species) are the only prokaryotes known to require cholesterol for growth; and, second, mycoplasmas have the smallest amounts of genetic information of any free-living organisms. Mycoplasma DNAs contain a low percentage of G+C.

Mycoplasma genomes fall into two size classes. *Acholeplasma*, *Spiroplasma*, and *Thermoplasma* genomes are ≈1000 MDa, a size that is rare but not unknown among eubacteria (reviewed in ref. 2). *Mycoplasma* and *Ureaplasma* genomes are ≈500 MDa, the smallest reported cellular genomes. This is only slightly larger than the theoretical minimal amount of genetic information for a cellular system (4), indicating that the complexity of mycoplasma cells must be constrained by their limited genome content.

The origin and phylogenetic relationships of mycoplasmas have been of interest because these cells are the result of natural selection operating within the constraint of limited genome complexity. In the past decade, a number of studies indicated that particular biochemical properties of some *Mycoplasma* and *Acholeplasma* species are closer to those of Gram-positive than to Gram-negative eubacteria (reviewed in refs. 2 and 3). A more complete view of mycoplasma evolution came from a recent comparative analysis of 16S rRNA oligonucleotide catalogs of *Acholeplasma laidlawii*, *Spiroplasma citri*, *Mycoplasma capricolum*, *Mycoplasma gallisepticum*, and *Thermoplasma acidophilum* with those of eubacteria (3). This showed that *Acholeplasma*, *Spiroplasma*, and *Mycoplasma* form a phylogenetically related group. Two clostridia, *Clostridium innocuum* and *Clostridium ramosum*, which form one of about five clostridia branches within the low G+C Gram-positive eubacteria, are related to the mycoplasma group. This cluster of the mycoplasma group and two clostridia species originated as a deep phylogenetic branching in the *Bacillus–Lactobacillus–Streptococcus* branch of the Gram-positive evolutionary tree. *Thermoplasma* is an archaebacterium and has no specific relationship to other mycoplasmas or eubacteria.

To get a more detailed picture of mycoplasma phylogeny, a molecular probe was needed that could be more readily applied to a larger number of organisms. Therefore, we used 5S rRNA sequences to examine mycoplasma molecular phylogeny. Analysis of 5S rRNA sequence homology has proven to be a reasonably accurate method for the construction of phylogenetic trees of prokaryotes and eukaryotes (e.g., see refs. 5–9).

We report here on the 5S rRNA sequences for *C. innocuum* and eight mycoplasmas. These have been analyzed, together with published sequences for three other mycoplasmas. Phylogenetic analysis confirms that mycoplasmas, together with *C. innocuum*, form a cluster that arose as a branch of the low G+C Gram-positive tree. The branching order within the mycoplasma cluster has been determined, with the surprising result that genome reductions appear to have occurred more than once. Some of the branches of the mycoplasma tree are characterized by relatively high mutation rates.

MATERIALS AND METHODS

Cells and Media. *Clostridium innocuum* was grown as described (10).

Acholeplasma laidlawii strain K2 was grown in tryptose broth (11).

Acholeplasma modicum type strain Squire (NCTC 10134) was grown in Hayflick's medium (12).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

**To whom reprint requests should be addressed.

Table 1. Properties of mycoplasma genera

Genus	Cholesterol required for growth	Genome % G+C	Genome Size, MDa	Habitat
Acholeplasma	No	31–34	950–1110	Animals and plants
Anaeroplasma	Most spp.	29–40	—	Bovine and ovine rumens
Spiroplasma	Yes	26–29	900–1210	Arthropods and plants
Mycoplasma	Yes	23–41	400–530	Animals
Ureaplasma	Yes	27–30	410–480	Animals
Thermoplasma	No	44–47	840–1000	Burning coal refuse piles

Refs. are cited in Maniloff (2). *Thermoplasma* is an archaebacterium and is not on the mycoplasma phylogenetic branch (3).

Anaeroplasma bactoclasticum strain 5LA and *Anaeroplasma abactoclasticum* strain 6-1, cholesterol-requiring anaeroplasmas, were grown as described by Robinson and Allison (13).

Ureaplasma urealyticum type strain 960-(CX8) (NCTC 10177), verified by serotyping as serotype 8, was used. A series of expanding-volume subcultures was made in Hayflick's medium until a 500-ml culture was obtained. The medium was as described (12), except thallous acetate and glucose were deleted, pH was adjusted to 6.5, and 0.1% (wt/vol) urea was added.

Mycoplasma mycoides mycoides was grown as described (14).

Mycoplasma pneumoniae type strain FH (NCTC 10119) was grown as cell sheets on glass surfaces in six "medical flat" (500 ml) screw capped bottles, each containing 20 ml of Hayflick's medium (12). Each bottle was laid on its side and incubated at 37°C. The resultant growing sheets of mycoplasma cells were fed by replacement of medium at 2- to 3-day intervals until growth was seen to be confluent.

Mycoplasma gallisepticum strain A5969 was grown in MBB medium (15).

Isolation and Sequencing of 5S rRNA. Cells were harvested (usually from 500- to 1400-ml cultures), and nucleic acids were isolated and fractionated as described (16, 17). The low molecular size RNA was analyzed by polyacrylamide gel electrophoresis and the 5S rRNA band was eluted and used for sequencing. General electrophoresis conditions were 1.0 kV for 7 hr in an 8% polyacrylamide gel (40 cm × 20 cm × 0.2 mm); if the 5S rRNA showed heterogeneity at the 3′ terminus, an 80-cm gel was used and electrophoresis time was increased.

The 5S rRNA was end-labeled *in vitro* with ^{32}P and sequenced by one of the following methods: (*i*) nuclease T1 treatment followed by electrophoresis and homochromatography (18), with the 3′-labeled material being previously treated with alkaline phosphatase to remove observable heterogeneity in the enzymatic digestion products; (*ii*) rapid gel sequencing methods using RNase T1, U2, Phy M, and *Bacillus cereus* digestion (19); and (*iii*) rapid gel sequencing using chemical degradative methods (20).

RESULTS AND DISCUSSION

5S rRNA Sequences. The sequences of *C. innocuum*, *A. laidlawii*, *A. modicum*, *An. bactoclasticum*, *An. abactoclasticum*, *U. urealyticum*, *M. mycoides mycoides*, *M. pneumoniae*, and *M. gallisepticum* 5S rRNAs were determined and are shown in Fig. 1. The sequence of *M. mycoides mycoides* 5S rRNA was almost identical to that reported for *M. mycoides capri* (14): the only differences are near the termini; at position 4, *M. mycoides capri* has a gap and *M. mycoides mycoides* has a C, and at position 121, *M. mycoides capri* has a U and *M. mycoides mycoides* has a G. *A. modicum* 5S rRNA had a unique size, but there was heterogeneity in the nucleotide at the 5′ terminus, which was A or U. *U. urealyticum* 5S rRNA could be fractionated into two species on gels; these differed only in length, with one species having an additional nucleotide at the 3′ terminus. The two *Anaeroplasma* species had identical 5S rRNA sequences.

For comparative purposes, Fig. 1 also shows sequences reported for three other mycoplasmas: *Spiroplasma* sp. strain BC3 (14), *M. capricolum* (21), and *M. mycoides capri* (14); and one Gram-negative, *Escherichia coli* (22), and five Gram-positive eubacteria, *Bacillus subtilis* (23), *Lactobacillus brevis* (24), *Streptococcus faecalis* (24), *Streptococcus cremoris* (25), and *Clostridium pasteurianum* (26). Hence, Fig. 1 contains all available 5S rRNA sequence data for *Streptococcus*, *Clostridium*, and mycoplasma species.

The 5S rRNA sequences were aligned to the positions of the 69 conserved nucleotides (residues conserved in 90% of reported sequences) and the positions of the five secondary structure helices; A-A′, B-B′, C-C′, D-D′, and E-E′ (28). A minimum number of gaps was inserted into the sequences, when necessary, to facilitate this alignment.

Hori and Osawa (6) have noted that 5S rRNAs from Gram-negative eubacteria have 120 nucleotides, whereas those from Gram-positive eubacteria have 116–117 nucleotides. The three mycoplasma 5S rRNAs previously sequenced (14, 21) were reported to be distinctly shorter. The eight mycoplasmas and *C. innocuum* sequenced in this study were also found to be shorter than typical eubacterial 5S rRNAs. The lengths of these shorter 5S rRNAs are as follows: *C. innocuum*, 114; *A. laidlawii*, 112; *A. modicum*, 109; *An. bactoclasticum* and *An. abactoclasticum*, 113; *Spiroplasma* sp. strain BC3, 107 (14); *M. capricolum*, 108 (ref. 21; see also Fig. 1 legend); *M. mycoides mycoides*, 108; *M. mycoides capri*, 107 (14); *M. pneumoniae*, 108; *M. gallisepticum*, 106; and *U. urealyticum*, 104 nucleotides. From the alignment (Fig. 1), the shorter lengths are seen to be due to small deletions in the regions of the RNAs comprising helix E-E′ and the single-stranded loop formed by helix E.

Based on the small number of previously reported mycoplasma 5S rRNA sequences, it was suggested that the shorter length of these RNAs is due to one or two large deletions in the area of helix E or the loop at its base (5, 9, 14, 25). With the larger number of sequences now available, it can be seen that, if small deletions are used to account for the shorter RNA lengths, a pattern of conserved residues emerges in the area of helix E and its loop for *C. innocuum* and mycoplasma 5S rRNAs (Fig. 1): e.g., purine tracks at positions 84, 89, 91, 92, and 96, and pyrimidine tracks at positions 85, 87, 88, 90, 94, and 97. Since this alignment allows maximal conservation of primary and secondary structure, the shorter lengths of *C. innocuum* and mycoplasma 5S rRNAs probably are a result of accumulation of independent small deletions in the area of helix E and its loop, presumably reflecting relaxed constraints on this region of the 5S rRNA molecule during evolution of these organisms.

Mycoplasma Phylogeny. Table 2 shows the percentage homology and number of base changes for every pair of 5S rRNA sequences in Fig. 1. The number of base changes is used as a metric for construction of phylogenetic trees.

General phylogenetic conclusions based on these data (detailed below) are the same as those from 16S rRNA studies (3): mycoplasmas form a phylogenetically related cluster, and this cluster is associated with the Gram-positive rather than the Gram-negative eubacteria. The proximity of mycoplasmas to Gram-positive eubacteria also can be seen from a

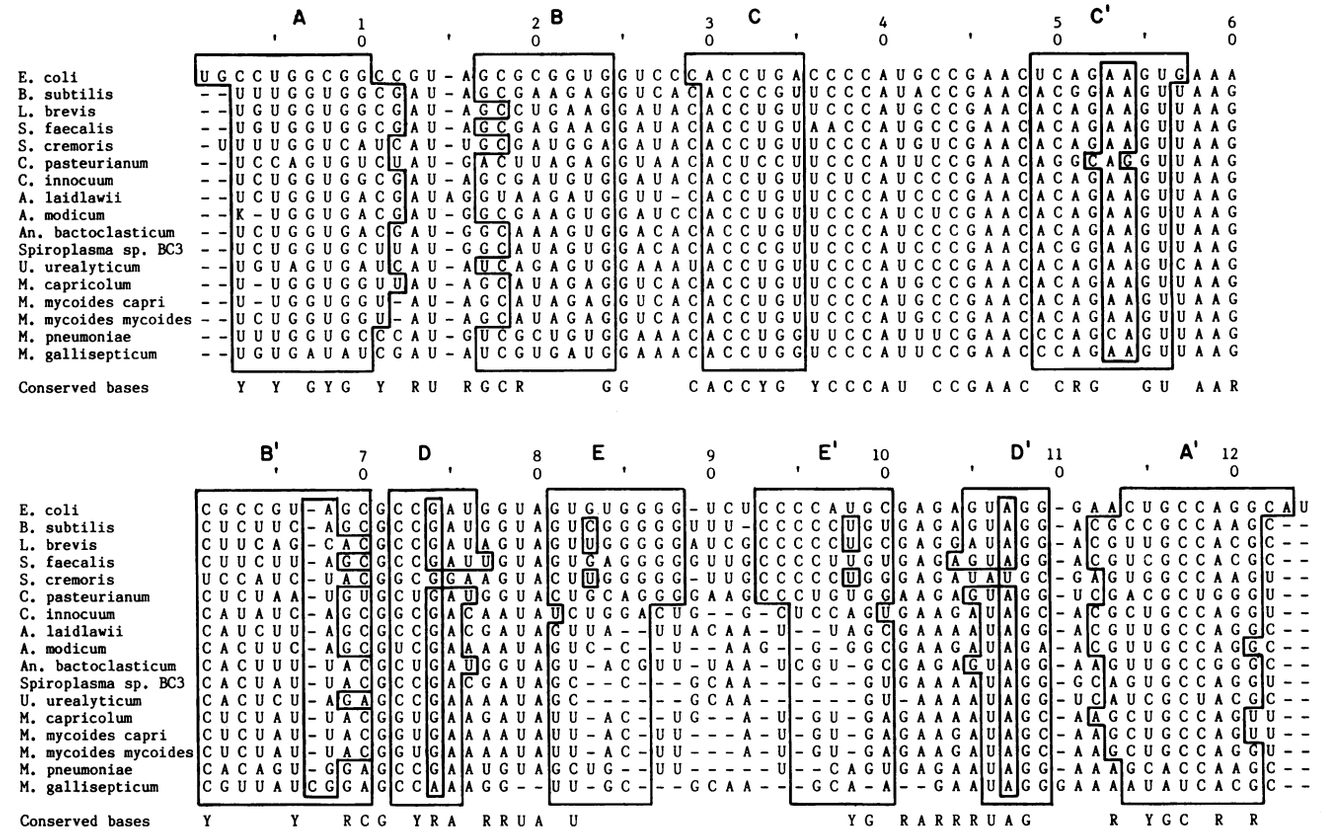

FIG. 1. Sequences of 5S rRNAs used to construct the mycoplasma phylogenetic tree. The data for *E. coli* (22), *B. subtilis* (23), *Lactobacillus brevis* (24), *Streptococcus faecalis* (24), *S. cremoris* (25), *C. pasteurianum* (26), *Spiroplasma* sp. strain BC3 (14), *M. capricolum* (21), and *M. mycoides capri* (14) have been reported previously. The residue U has been inserted into position 8 of the published *M. capricolum* sequence, based on recent sequence data on the *M. capricolum* 5S rRNA gene (A. Muto, personal communication). The sequences for *C. innocuum*, *A. laidlawii*, *A. modicum*, *An. bactoclasticum* strain 5LA, *An. abactoclasticum* strain 6-1, *U. urealyticum* serotype 8, *M. mycoides mycoides*, *M. pneumoniae*, and *M. gallisepticum* were determined in this study. There are only two differences between the two *M. mycoides* subspecies, at positions 4 and 121. Both *Anaeroplasma* species had identical sequences, so only one is shown here. The first nucleotide of the *A. modicum* sequence, designated K, is an A or a U. Bottom line shows positions of conserved bases: those residues conserved in 90% of the reported sequences (27). Double-stranded areas A-A', B-B', C-C', D-D', and E-E' are boxed. A minimum number of gaps (−) has been inserted to align conserved primary and secondary structures.

consideration of five 5S rRNA signature positions (30). In Gram-positive eubacteria, these are A_{28}, U_{35}, A_{49}, U_{57}, and G_{60}; and for Gram-negative eubacteria, they are C_{28}, A_{35}, U_{49}, G_{57}, and A_{60} (using the numbering scheme in Fig. 1). All mycoplasma 5S rRNAs have Gram-positive signature sequences. In a few cases, a couple of residues have changed, as they have for some Gram-positive eubacteria (30).

The sequences in Table 2 have evolved at different rates. This can be seen by considering the number of base changes between each organism and an organism on a distant phylogenetic branch. For example, the number of base changes between *E. coli* and other organisms (column 1 in Table 2) varies by a factor of >2. The matrix of base changes (lower left half of Table 2) can be "corrected" for unequal mutation rates by the method of Li (31). Equivalent transformations for various base change rates have been described by Klotz and Blanken (32) and by Blanken *et al.* (33).

Li (31) described a procedure of determining, for a matrix as in Table 2, which organisms are on either side of a phylogenetic node. In a clustering with *E. coli* or *B. subtillis* 5S rRNA sequences, all mycoplasmas cluster with *B. subtilis*, in agreement with their being related to Gram-positive eubacteria. In a clustering with *S. faecalis* or *M. mycoides* sequences (using a "corrected" matrix), all mycoplasmas cluster with *M. mycoides*. This confirms the conclusions of Woese *et al.* (3) that mycoplasmas form a coherent phylogenetic branch, and it rules out models requiring specific relationships between only some mycoplasmas and eubacteria (e.g., see ref. 34).

The topology of the mycoplasma phylogenetic tree was determined by two methods, that of Farris (35) as modified by Tateno *et al.* (36), and that of Li (31). The modified Farris method makes no assumptions about homogeneity of evolutionary rates of different organisms, and it was used with the data in Table 2. For the Li method, repeated cycles of clustering were done, using a "corrected" matrix, to subdivide each branch. Both methods yield the same topology, shown in Fig. 2. The branch lengths in Fig. 2 are proportional to the corrected matrix values and, therefore, should be related to evolutionary distance. The number of base changes on each branch was calculated by the Li method (31); similar values were obtained using the modified Farris method (36).

As previously reported (3), the mycoplasma cluster (*Acholeplasma, Anaeroplasma, Spiroplasma, Mycoplasma*, and *Ureaplasma*) is a branch of the tree formed by Gram-positive eubacteria with low G+C DNA (Fig. 2). The mycoplasma branch is specifically related to a branch formed by *C. innocuum* (Fig. 2; see also ref. 3) and *C. ramosum* (3). This branch, in turn, is somewhat closer to the lactobacilli and streptococci than to bacilli.

Divergence of the *C. innocuum* and mycoplasma branches appears to have been slightly more recent than separation of the *B. subtilis* and *C. pasteurianum* branches (Fig. 2), in agreement with the tree derived from 16S rRNA data (37).

Table 2. Relationships between 5S rRNA sequences

	Eco	*Bsu*	*Lbr*	*Sfa*	*Scr*	*Cpa*	*Cin*	*Ala*	*Amo*	*Aba*	*Sbc*	*Uur*	*Mca*	*Mmy*	*Mpn*	*Mga*
Eco	—	72	69	72	59	54	62	61	57	66	58	53	58	57	57	51
Bsu	41	—	77	82	69	67	71	64	66	72	65	59	65	63	62	52
Lbr	46	32	—	85	69	59	72	69	66	69	61	58	64	66	58	60
Sfa	41	24	20	—	67	61	72	70	64	71	59	60	62	60	60	57
Scr	68	46	46	50	—	51	66	54	61	63	53	51	58	58	58	46
Cpa	82	50	68	63	93	—	50	51	50	62	59	53	56	53	47	47
Cin	62	43	40	41	52	96	—	72	74	68	66	61	65	67	61	52
Ala	65	57	46	45	84	92	41	—	76	78	71	66	68	67	63	59
Amo	72	52	54	57	64	96	36	34	—	75	72	68	68	70	62	55
Aba	52	41	46	43	60	61	48	31	35	—	75	64	75	75	63	57
Sbc	72	55	63	70	84	70	52	42	39	34	—	75	75	71	62	59
Uur	87	70	72	65	93	84	62	51	46	56	31	—	63	61	64	60
Mca	72	55	57	61	72	77	55	49	45	34	33	57	—	95	57	56
Mmy	75	59	54	66	70	87	50	50	42	34	40	60	5	—	57	53
Mpn	72	62	72	68	70	105	64	58	60	58	60	55	71	70	—	64
Mga	94	86	67	76	114	110	91	70	78	74	67	65	75	85	56	—

Organisms are designated as follows: *Eco, E. coli; Bsu, B. subtilis; Lbr, L. brevis; Sfa, S. faecalis; Scr, S. cremoris; Cpa, C. pasteurianum; Cin, C. innocuum; Ala, A. laidlawii; Amo, A. modicum; Aba, An. bactoclasticum* and *An. abactoclasticum; Sbc, Spiroplasma* sp. strain BC3; *Uur, U. urealyticum; Mca, M. capricolum; Mmy, M. mycoides capri; Mpn, M. pneumoniae; Mga, M. gallisepticum.* Upper right half matrix shows percentage homology for each pair of 5S rRNA sequences in Fig. 1. Homology was calculated from percentage mismatches between two sequences, beginning at position 3 and ending at position 121 (to exclude terminal variation beyond the A-A′ helix). A gap in one sequence opposite a base in the other sequence was counted as a mismatch. Lower left half matrix shows number of base changes between each pair of 5S rRNA sequences, calculated from percentage homology for each pair of sequences using the formula of Jukes and Cantor (29): this formula assumes equal rates for transition and transversion type substitutions (27).

Hence, most, if not all, of mycoplasma origin and phylogeny may have occurred after evolution of an oxidizing atmosphere on the earth. Oxidizing conditions were required for evolution of sterol-requiring mycoplasmas, because oxygen is essential for sterol synthesis (38).

The general order of the mycoplasma branch (Fig. 2), *Acholeplasma* to *Spiroplasma* to *Mycoplasma* and *Ureaplasma*, had been suggested on biochemical grounds (2).

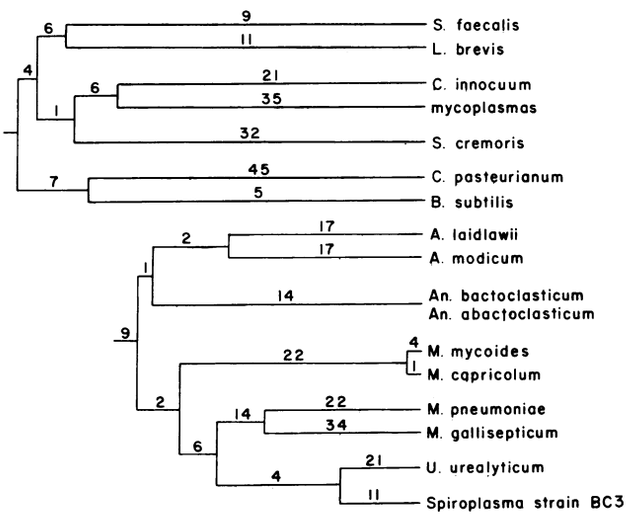

FIG. 2. Phylogenetic tree for mycoplasmas and some Gram-positive eubacteria based on 5S rRNA sequences. Tree topology was determined by the Farris (35) method as modified by Tateno *et al.* (36), and by the Li (31) method. Branch lengths are proportional to the corrected matrix values; hence, lengths should be a direct function of evolutionary distance. The number on each branch is the number of base changes in the 5S rRNA molecule on that branch, calculated as described by Li (31). Similar numbers were calculated by the modified Farris method (36). (*Upper*) Tree for Gram-positive eubacteria having DNA with low percentage G+C, showing position of mycoplasma branch. (*Lower*) Mycoplasma branch, including *Acholeplasma, Anaeroplasma, Spiroplasma, Mycoplasma*, and *Ureaplasma* branches.

However, the details of this branch were completely unexpected. As indicated by the 5S rRNA data, divergence of mycoplasmas from the *C. innocuum* branch of the Gram-positive eubacteria, which must have involved chromosomal deletions, probably led to the ancestral *Acholeplasma* branch of wall-less organisms with genomes of ≈1000 MDa. A splitting of this branch led to sterol-requiring organisms ancestral to *Spiroplasma*. The *Anaeroplasma* branch is close to the node between the *Acholeplasma* and *Spiroplasma* branches, and there still must be some uncertainty about its exact position. Recently the genome size of *Anaeroplasma* has been found to be 980–1140 MDa (unpublished data). This is the size expected from the phylogenetic tree derived here (Fig. 2). Quite surprisingly, there appear to have been repeated independent genome reductions, each to ≈500 MDa, during evolution of the *Spiroplasma* branch, with *Mycoplasma* or *Ureaplasma* species resulting from these events. The three branches from *Spiroplasma* identified in these studies are the *M. capricolum–M. mycoides, M. pneumoniae–M. gallisepticum*, and *Ureaplasma* branches. Since the *Spiroplasma* branch must contain a number of *Spiroplasma* species, *Mycoplasma* and *Ureaplasma* species may represent divergences from different *Spiroplasma* species.

The biochemical and ultrastructural diversity of *Mycoplasma* and *Ureaplasma* species must reflect the multiple origins of these organisms. The idea of separate origins for *Mycoplasma* branches is consistent with the recent observation that *Mycoplasma* lipid structures are so heterogeneous that they appear to have evolved following different pathways (39). A special aspect of evolution of the *M. pneumoniae–M. gallisepticum* branch must have been development of the cellular organization and terminal structures that are found in these organisms (40).

The decrease in 5S rRNA length through gradual accumulation of small deletions follows the mycoplasma phylogenetic tree (Fig. 2). The 116- to 117-residue molecule in Gram-positive eubacteria would be reduced to 114 residues in *C. innocuum*, 109–113 residues in the *Acholeplasma–Anaeroplasma* branch, and 104–108 residues in the *Spiroplasma, Mycoplasma*, and *Ureaplasma* branches.

The model for mycoplasma evolution presented here (Fig. 2) is based on 5S and 16S rRNA sequence analysis and is

consistent with all other reported data on mycoplasma composition, biochemistry, and metabolism (e.g., see refs. 2 and 3). This allows two earlier models of mycoplasma evolution to be ruled out. One model suggested that mycoplasmas were not a coherent phylogenetic group and had multiple origins from a variety of eubacteria; in particular, that *Acholeplasma* (but no other mycoplasmas) were specifically related to streptococci (34). There are no macromolecular phylogenetic data to support this model and, as can be seen from Fig. 2, it is a far too limited view of the relationship of *Acholeplasma* to other mycoplasmas and streptococci. The other model suggested that mycoplasmas are descendants of primitive organisms that preceded eubacteria in the evolutionary progression (41). This model focuses on the genome size data, postulating genome doublings from 500-MDa genomes to produce typical eubacterial size genomes. Since the root, or direction of time's arrow, cannot be absolutely determined for a tree as in Fig. 2, the primitive mycoplasma model would suggest that one could invert the mycoplasma branch in Fig. 2, thereby making a small genome mycoplasma the root of the eubacterial tree. Interpretation of the primitive mycoplasma phylogenetic tree would be far more complicated than the one in Fig. 2, requiring a variety of biochemical assumptions and genome size increases. Although this model requires the mycoplasma branch to be ancestral to all eubacteria, there are no data specifically relating any mycoplasma biochemical property to Gram-negative raher than Gram-positive eubacteria. Hence, in the absence of any supporting data, this model can also be ruled out.

In conclusion, the tree in Fig. 2 is consistent with all available data on mycoplasma phylogeny and presents an overall picture explaining many disparate observations (e.g., see ref. 2). This model poses many interesting questions that need to be experimentally investigated, particularly regarding mechanisms of genome reduction and gene conservation.

Mycoplasmas have a high mutation rate, as indicated by the observation that many 16S rRNA sequences that are highly conserved in eubacteria are absent in mycoplasmas (3). Fig. 2 shows that the rate may be highest for mycoplasmas with the smallest genome sizes. The upper bound on a population's mutation rate must be determined by the requirement for error avoidance in information transfer, and the lower bound is determined by the need for a pool of variants. For cells with small genomes, such as mycoplasmas, the upper bound should be relatively high and, because small genome cells might require a large pool of variants, the lower bound could also be high. This high mutation rate would allow mycoplasmas to explore areas of evolutionary phase space requiring multiple compensating mutations (2, 3). Necessary and sufficient conditions for rapid evolution are an increased mutation rate and environmental duress (unpublished observations). The small genome that allows the potential for increased mutation rate may also keep the genetic capacity so limited that mycoplasma cells are always in a state of environmental duress. Therefore, mycoplasmas may be useful systems for the study of molecular events during periods of rapid evolution.

M.J.R. is the recipient of a CASE award with the Public Health Laboratory Service. Some of this work was supported by grants from the Sciences and Engineering Research Council to R.T.W., the National Aeronautics and Space Administration to C.R.W., and the National Institutes of Health (Grant GM32442) to J.M.

1. Krieg, N. R. & Holt, J. G., eds. (1984) *Bergey's Manual of Systematic Bacteriology* (Williams & Wilkins, Baltimore), Vol. 1, pp. 740–793.
2. Maniloff, J. (1983) *Annu. Rev. Microbiol.* **37**, 477–499.
3. Woese, C. R., Maniloff, J. & Zablen, L. B. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 494–498.
4. Morowitz, H. J. (1967) *Prog. Theor. Biol.* **1**, 35–58.
5. Hori, H. (1975) *J. Mol. Evol.* **7**, 75–86.
6. Hori, H. & Osawa, S. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 381–385.
7. Küntzel, H., Heidrich, M. & Piechulla, B. (1981) *Nucleic Acids Res.* **9**, 1451–1461.
8. Sankoff, D., Cedergren, R. J. & McKay, W. (1982) *Nucleic Acids Res.* **10**, 421–431.
9. Küntzel, H., Piechulla, B. & Hahn, U. (1983) *Nucleic Acids Res.* **11**, 893–900.
10. Johnson, J. L. & Francis, B. S. (1975) *J. Gen. Microbiol.* **88**, 229–244.
11. Haberer, K., Klotz, G., Maniloff, J. & Kleinschmidt, A. K. (1979) *J. Virol.* **32**, 268–275.
12. Hayflick, L. (1965) *Tex. Rep. Biol. Med.* **23**, Suppl. 1, 285–303.
13. Robinson, I. M. & Allison, M. J. (1975) *Int. J. Syst. Bacteriol.* **25**, 182–186.
14. Walker, R. T., Chelton, E. T. J., Kilpatrick, M. W., Rogers, M. J. & Simmons, J. (1982) *Nucleic Acids Res.* **10**, 6363–6367.
15. Ghosh, A., Das, J. & Maniloff, J. (1977) *J. Mol. Biol.* **116**, 337–344.
16. Walker, R. T. & RajBhandary, U. L. (1975) *Nucleic Acids Res.* **2**, 61–78.
17. Stahl, D. A., Luehrsen, K. R., Woese, C. R. & Pace, N. R. (1981) *Nucleic Acids Res.* **9**, 6129–6137.
18. Silberklang, M., Gillum, A. M. & RajBhandary, U. L. (1979) *Methods Enzymol.* **59**, 58–75.
19. Donis-Keller, H. (1980) *Nucleic Acids Res.* **8**, 3133–3142.
20. Peattie, D. A. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 1760–1764.
21. Hori, H., Sawada, M., Osawa, S., Murao, K. & Ishikura, H. (1981) *Nucleic Acids Res.* **9**, 5407–5410.
22. Brownlee, G. C., Sanger, F. & Barrell, B. G. (1968) *J. Mol. Biol.* **34**, 379–412.
23. Marotta, C. A., Varricchio, F., Smith, I., Weissman, S. M., Sogin, M. L. & Pace, N. R. (1976) *J. Biol. Chem.* **251**, 3122–3127.
24. Woese, C. R., Luehrsen, K. R., Pribula, C. D. & Fox, G. E. (1976) *J. Mol. Evol.* **8**, 143–153.
25. Neimark, H., Andersen, J. & Delihas, N. (1983) *Nucleic Acids Res.* **11**, 7569–7577.
26. Pribula, C. D., Fox, G. E. & Woese, C. R. (1976) *FEBS Lett.* **64**, 350–352.
27. Kimura, M. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 454–458.
28. Erdmann, V. A., Wolters, J., Huysmans, E., Vandenberghe, A. & De Wachter, R. (1984) *Nucleic Acids Res.* Suppl. **12**, 133–166.
29. Jukes, T. H. & Cantor, C. R. (1969) in *Mammalian Protein Metabolism*, ed. Munro, H. N. (Academic, New York), Vol. 3, pp. 21–132.
30. Delihas, N., Andersen, J. & Singhal, R. P. (1984) *Prog. Nucleic Acid Res. Mol. Biol.* **31**, 161–190.
31. Li, W.-H. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 1085–1089.
32. Klotz, L. C. & Blanken, R. L. (1981) *J. Theor. Biol.* **91**, 261–272.
33. Blanken, R. L., Klotz, L. C. & Hinnebusch, A. G. (1982) *J. Mol. Evol.* **19**, 9–19.
34. Neimark, H. & London, J. (1982) *J. Bacteriol.* **150**, 1259–1265.
35. Farris, J. S. (1972) *Am. Nat.* **106**, 645–668.
36. Tateno, Y., Nei, M. & Tajima, F. (1982) *J. Mol. Evol.* **18**, 387–404.
37. Fox, G. E., Stackebrandt, E., Hespell, R. B., Gibson, J., Maniloff, J., Dyer, T. A., Wolfe, R. S., Balch, W. E., Tanner, R. S., Magrum, L. J., Zablen, L. B., Blakemore, R., Gupta, R., Bonen, L., Lewis, B. J., Stahl, D. A., Luehrsen, K. R., Chen, K. N. & Woese, C. R. (1980) *Science* **209**, 457–463.
38. Bloch, K. (1979) *CRC Crit. Rev. Biochem.* **7**, 1–5.
39. Langworthy, T. A. (1983) *Yale J. Biol. Med.* **56**, 385–390.
40. Maniloff, J. (1981) *BioSystems* **14**, 305–312.
41. Wallace, D. C. & Morowitz, H. J. (1973) *Chromosoma* **40**, 121–126.