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Subcellular structures of mycoplasmas

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1. ABSTRACT

Although the field of prokaryotic cell biology is well-advanced now, mycoplasmas were the first bacteria in which the existence of a cytoskeleton was postulated. Despite this head-start, the cytoskeletons of mycoplasmas are presently less well understood than those of other bacteria. This deficit is principally attributable to three factors: the novel nature of most of the cytoskeletal elements as compared with other bacteria, which have the advantage of being related to eukaryotic cytoskeletal proteins; differences among the cytoskeletons of different mycoplasma species; and the fastidiousness of mycoplasmas, which complicates efforts to perform protein biochemistry. In better studied mycoplasmas like *Mycoplasma pneumoniae*, a major component of the cytoskeleton is associated with the attachment organelle, a polar structure that is essential for adherence to host cells, involved in gliding motility, and associated with cell division. *Mycoplasma mobile* also has structures that appear to be involved in gliding motility, though in contrast to the structures of *M. pneumoniae*, these are extracellular. Some other species also have distinct subcellular structures.

2. INTRODUCTION

Before molecular criteria established that mycoplasmas are a variety of bacteria (1), the observation of gliding motility in several mycoplasma species (2-4) prompted investigation of the possibility of cytoskeletal structures in these small cells. Additionally, the morphology of mycoplasma cells was known to differ both among and within species, including irregular cocci, multicellular filaments, and highly derived forms with special features (5), further suggesting that, analogous to the cytoskeletons of eukaryotic cells, intracellular structures might contribute to mycoplasma cell shape, particularly in the absence of a cell wall.

Bacterial cytoskeletal systems have become increasingly well-understood in recent years. Actin-related MreB forms helical filaments underlying the plasma membrane of non-coccoidal bacterial cells (6-8) and is involved in coordination of peptidoglycan deposition during cell wall synthesis (9) and positioning of replication origins during chromosome segregation (10). Most bacteria also produce the tubulin homolog FtsZ (11), which forms a cytoskeletal filament that is essential for membrane

Figure 1. Comparison of *M. pneumoniae*, *M. gallisepticum*, and *M. mobile* as viewed by scanning electron microscopy. A, *M. pneumoniae*. Arrow, AO. B. *M. gallisepticum*. Arrow, AO. C. *M. mobile*. Arrow, neck region between head structure (above the arrow) and cell body (below the arrow). Scale bar, 500 nm.

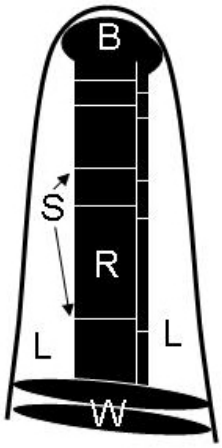

Figure 2. Schematic of *M. pneumoniae* attachment organelle. Two parallel rods (R) with cross-striations (S) and a button-like swelling at one end (B) occupy an electron-lucent space (L). At the cytoplasmic end is a wheel-like structure (W). Not to scale.

constriction and coordination of septal peptidoglycan synthesis (12). Finally, crescentin, which is homologous to intermediate filament proteins, produces a polymeric filament in *Caulobacter crescentus* that is essential for cell curvature (13).

Prior to the appreciation of bacterial cytoskeletons, electron microscopy revealed that both *Mycoplasma pneumoniae* (14) and *Mycoplasma gallisepticum* (15) have differentiated intracellular structures associated with the specialized area of the mycoplasma cell that interacts with host cells, the terminal organelle. Observations of *M. pneumoniae* cells extracted with the nonionic detergent Triton X-100 (TX) demonstrated that these structures, along with other filaments throughout the cells, were detergent-insoluble, analogous to the eukaryotic cytoskeleton (16,17), though early suggestions that mycoplasmas contained actin-like proteins (18,19) were dispelled by both genomic (20) and proteomic studies (21). Instead, as revealed by genetic and biochemical studies, a novel set of proteins restricted to *M. pneumoniae*, *M. gallisepticum*, and their closest relatives constitutes this cytoskeleton (22).

Although features of comparable function and properties have not been identified in other mycoplasmas, subcellular structures have been observed in some species. Extracellular filamentous structures with potential roles in adherence and gliding motility have been visualized in *Mycoplasma mobile* (23), and under osmotic stress various strains of *Mycoplasma mycoides* form rigid rods with characteristic structural features (24).

Some mycoplasmas have a gene encoding FtsZ (25); its function in cell division has not been demonstrated in mycoplasmas, and in many species that do have such a gene, its sequence is significantly divergent. Furthermore, in *M. pneumoniae* there is very little *ftsZ* mRNA (26) and very little FtsZ protein (unpublished data), raising the possibility that mycoplasmas do not actually use FtsZ for cell division. Interestingly, in *M. pneumoniae*, the TX-insoluble structures of the terminal organelle are involved in cell division (27,28), suggesting functional substitution of one type of cytoskeleton for another. Neither actin-like nor intermediate-filament like proteins are encoded in genomes of the *Mycoplasma* genus. Clearly, mycoplasmas and their relatives have subcellular structures that are distinct from those of other bacteria. Furthermore, there is a diversity of cytoskeletal systems among these organisms despite their close phylogenetic relationship.

3. TERMINAL ORGANELLES

Some mycoplasmas have specialized polar appendages called terminal organelles. They are dispersed throughout the phylogeny of mycoplasmas; their disparate features suggest they have arisen in evolution more than once. Terminal organelles may take the form of attachment organelles (AOs), as in *M. pneumoniae* and *M. gallisepticum*, in which it is historically called the terminal bleb, or head structures, as in *M. mobile* (Figure 1).

Largely because of its importance as a human respiratory tract pathogen (29), *M. pneumoniae* is a model organism among mycoplasmas. The means of its attachment to host cells, or cytadherence, has been the subject of considerable study. Essential for normal, productive *M. pneumoniae* cytadherence is the localization of adhesin proteins to a polar structure, the AO (Figure 1) (22), which is present in virtually every wild-type cell as viewed by electron microscopy (J.M. Hatchel & M.F. Balish, unpublished observations). Within the cytoplasm of this structure is an electron-dense core (30) consisting of a bipartite rod with a regular pattern of striations perpendicular to its long axis (Figure 2) (27). The core, which is insoluble in TX (16,17), is surrounded by an electron-lucent space (30). At its cell-proximal side is a perpendicular structure that has been interpreted as a wheel with spokes (27), though it has never been seen in isolation. Though morphologically somewhat distinct, a similar feature, called the terminal bleb but also functioning as an AO, is present in the avian respiratory tract and ocular

pathogen *M. gallisepticum* (Figure 1) (31,32). Less well understood is a similar yet distinct polar protrusion, the head structure, in *M. mobile* (Figure 1). Though it is morphologically similar to the AO and also found on every cell (unpublished observations), it is apparently not used for attachment to host cells (33).

3.1. Functions

3.1.1. Cytadherence

The *M. pneumoniae* AO and its homologs in other species have principally been studied in terms of their roles in cytadherence. *M. pneumoniae* cells intimately contact host cells through the AO (14). Some host ligands have been identified (34-36), but interactions between these ligands and the known adhesins have yet to be demonstrated. AOs are not the only factors mediating attachment to the host, as a variety of surface proteins distributed over the entire cell surface have been implicated in binding to fibronectin (37), mucin (38) and surfactant protein A (39) in either *M. pneumoniae* or its close relative, the human urogenital tract pathogen *Mycoplasma genitalium* (40). However, a properly functioning AO, containing both adhesins and the TX-insoluble proteins that are essential for its formation, is required for virulence (14).

The primacy of the AO in cytadherence for some species that are closely related to *M. pneumoniae* has been confirmed or suggested. *M. gallisepticum* also has a long history of study at the ultrastructural level. Its terminal bleb is functionally analogous to the AO of *M. pneumoniae* in that it is the site of adherence to host cells (15), and sialic acid-containing carbohydrates are among its host cell ligands (41). *Mycoplasma penetrans*, which was isolated from both the urogenital tract of HIV-infected individuals (42) and the respiratory tract and blood of a patient with primary antiphospholipid syndrome (43), also has a structure resembling an AO that functions in attachment as well as invasion of host cells (44).

Among mycoplasma species more distantly related to *M. pneumoniae*, some also have polar structures resembling AOs. It has not been established that these structures play any roles in cytadherence. *M. mobile*, a fish pathogen (45), has such a structure but is generally visualized in association with host cells through the other pole of the cell (33). A comparable structure in the rodent pathogen *Mycoplasma pulmonis* (46) also seems unassociated with cytadherence (47).

3.1.2. Motility

Gliding motility, or motility on surfaces that is not mediated by visible appendages (48), is a characteristic of several species of mycoplasmas with AOs or similar structures, including *M. pneumoniae* (3), *M. gallisepticum* (49), *M. pulmonis* (50), *M. genitalium* (51), and *M. mobile* (52). In all cases, gliding is unidirectional with the polar structure leading, suggesting that the motor activity is contained within this organelle irrespective of a role in adherence.

Some crucial proteins that are also associated with adherence have been identified in both *M. pneumoniae* (53) and *M. mobile* (54,55). The gliding-associated proteins of each of the two organisms are absent from the other. For *M. pulmonis*, only fresh isolates are motile (50). In such an isolate, genes orthologous to the *M. mobile* gliding-associated proteins are present, but these genes are mutated in non-motile *M. pulmonis* strains (55). Other than the identities of some of the proteins, the molecular means for motility is not understood, although it appears to be powered by ATPase activity, not ion motive force, in *M. mobile* (56).

The specific roles of gliding motility in the life cycles of mycoplasmas are not yet understood, though it is possible that motility functions in host colonization. Unfortunately, study of this relationship has been hampered by the overlap between motility proteins and cytadherence proteins, making it difficult to obtain fully cytadherent isolates that are non-motile.

3.1.3. Cell division

In both *M. gallisepticum* (57) and *M. pneumoniae* (3) a special relationship between cell division and AO duplication has been observed wherein the AO is apparently replicated prior to cell division. This is evidenced in *M. gallisepticum* by the appearance of smaller cells with a single structure and larger, often dividing cells with two structures approximately diametrically opposed (57). In *M. pneumoniae* the process of AO duplication has been suggested by time-lapse microcinematography (3) at relatively low resolution. It is hypothesized based on both these data and images of presumptive intermediate stages of cell division in *M. pneumoniae* (58) that the onset of cell division is marked by the appearance of a second AO immediately adjacent to the first. Migration of one AO to the other pole is followed by binary fission.

The appearance of a separation between the paired rod elements of the electron-dense core of the AO (27) and a mutant in which bifurcated AOs are abundant (28) suggest that splitting of the two parallel elements of the electron-dense core underlie the mechanism for AO duplication. Another *M. pneumoniae* mutant, which lacks protein HMW3, which appears to be a component of a series of fibers that are wrapped around the electron-dense core (59), has AOs in which the paired elements of the electron-dense core are separated (60), implicating HMW3 in regulation of splitting. By analogy with the splitting of DNA strands to form templates for synthesis of new DNA, this is referred to as the semiconservative model for AO duplication (14).

There is evidence in both *M. gallisepticum* and *M. pneumoniae* that AO duplication is linked with DNA replication. In *M. gallisepticum*, a large portion of newly-synthesized DNA is present in a biochemical fraction enriched for AOs, suggesting a physical connection between the DNA replication machinery and the polar structure (61). This raises the possibility of a signal linking the two processes. In *M. pneumoniae*, the number and position of AOs correlates with the amount of DNA in a manner that suggests that AO duplication is coordinated with DNA replication initiation (58). Again, the nature of the coordinating signal is unknown.

3.2. Ultrastructural elements

3.2.1. *Mycoplasma pneumoniae*

A hallmark of the eukaryotic cytoskeleton is a network of filaments and associated proteins that, because of its size and, presumably, an abundance of large ionic interfaces among component proteins, is insoluble in the nonionic detergent TX-100 (62). This property of TX insolubility, together with superficial actin-like properties and a presumed role in gliding motility, was used to justify describing the TX-insoluble material of *M. pneumoniae* as cytoskeletal (17,18,63). However, since the genomic era gave rise to the revelation that *M. pneumoniae* does not contain actin or any other components homologous to eukaryotic cytoskeletal proteins other than the distant tubulin relative FtsZ (20), deciding whether these TX-insoluble structures are cytoskeletal has rested upon establishing their cellular roles and properties.

Incubation of *M. pneumoniae* cells grown attached to Formvar- and carbon-coated electron microscopy grids with 0.5% or 1.0% TX resulted in the removal of membranes and other soluble material and revealed irregular fibrous structures and a rod of regular dimensions, approximately 300 nm long and 40-80 nm wide (16,17). The use of lower concentrations of TX indicated that the rod was present in the AO, corresponding to the electron-dense core seen *in situ*, and had a thickened region at the distal end (17) composed of small particulate material (16). Extraction with 2% TX resulted in the loss of most of the fibrous material, with the core structure and small amounts of attached membrane-like material as the most prominent component of the insoluble fraction (21,27).

Examination of the *M. pneumoniae* cores by transmission electron microscopy revealed some aspects of their shape (Figure 2) (27). They appear to have both a broad side and a narrower edge, suggestive of a flattened, rectangular bar. Cross-striations are clearly visible in a regular pattern across the broad side. Observations of the electron-dense core *in situ* indicate further structural features, including the paired nature of the two bar-like components (27,60), and their apparent splitting during duplication of the AO (27). Fine filaments might link the core to the cell membrane (27). The wheel-like structure at the cell-proximal end of the core does not appear clearly in TX-insoluble fractions (21,27). It has been suggested that rotation of the wheel contributes to motor activity (27), but other than the presence of partly twisted rods in TX-insoluble preparations there has been no evidence of rotation.

The protein composition of the TX-insoluble fraction of *M. pneumoniae* was investigated by two-dimensional gel electrophoresis coupled with mass spectrometry (21). This fraction, which, though dominated by the electron-dense core, also contains other smaller, less regular structures, was found to consist of known AO proteins (see below), some proteins with unknown function, and some metabolic proteins. There are two principal reasons to expect to identify metabolic enzymes among the TX-insoluble material of *M. pneumoniae*: 1) some are present in large complexes that are likely to be TX-insoluble for the same reasons as cytoskeletal filaments are (62); and 2) some might be bifunctional, with a metabolic form and a structural form. For example, elongation factor Tu and the pyruvate dehydrogenase E1 beta-subunit bind fibronectin (37), and glyceraldehyde 3-phosphate dehydrogenase binds mucin (36). These proteins are partially exposed on the mycoplasma cell surface in addition, presumably, to functioning metabolically in the cytoplasm. This dual duty, as has been proposed for specificity of mycoplasma sugar kinases (64), likely compensates for the substantial gene loss during reductive evolution of mycoplasmas from a *Clostridium*-like or *Lactobacillus*-like ancestor (1).

Although TX-insoluble structures have not been examined in other species of the *M. pneumoniae* phylogenetic group, electron microscopy reveals some shared features among many of them. *M. genitalium* is both ultrastructurally and genomically very similar to *M. pneumoniae* (20,65) and is similar with respect to the structures underlying its AO. Electron-dense cores have also been clearly seen in *Mycoplasma alvi* (66), despite their being non-motile in culture (4); *Mycoplasma pirum*, which also has a double cap structure associated with the distal end of the core (67); and *Mycoplasma imitans* (68). *M. gallisepticum* also possesses electron-dense structures within its AO, though the core seems shortened as compared with *M. pneumoniae* and other species (69); limited evidence suggests that *Mycoplasma testudinis* (70) may be similar. However, no such structures have been observed in more distantly related members of the *M. pneumoniae* group, including *M. penetrans* (44), which has an AO, *Mycoplasma muris* (71), for which such a structure has not been described, and *Mycoplasma fastidiosum* (72) and *Mycoplasma cavipharyngis* (73) or their relatives, the hemoplasmas, which are obligately erythrocyte-associated (74).

3.2.2. *Mycoplasma mobile*

M. mobile, a species of the *Mycoplasma hominis* phylogenetic group, is much more distantly related to *M. pneumoniae* than *M. penetrans* and *M. fastidiosum* (75). An ultrastructural examination of the TX-insoluble fraction of *M. mobile* has not been reported. However, at least one TX-insoluble protein, Gli349, appears to be required for attachment of *M. mobile* to glass and gliding motility have been identified (54). This transmembrane protein is located on the cell surface in the region of the cell at the base of the head structure, designated the neck (54; Figure 1); the same is true for two other apparent gliding proteins, Gli521 (55) and Gli123 (76). Freeze-fracture electron microscopy reveals that spike structures protrude from the cell membrane in this region of the *M. mobile* cell (23) These structures are absent in the *M. mobile* gliding mutant m12 (23), which exhibits irregular morphology, no motility, and no capacity to adhere to erythrocytes (77). The spikes are approximately 51 nm in length and 4 nm in width (23), roughly concordant with structural predictions for Gli349 (78).

Although many mutants with irregularities in morphology and gliding motility have been isolated (77),

M. mobile lacks genetic tools, making it impossible at present to demonstrate the roles of candidate proteins by complementation of these mutants. Nonetheless, evidence in favor of the importance of these proteins in attachment- and gliding-related phenomena is accumulating, and future structural work will be informative as well. Interestingly, Gli349, Gli521, and Gli123 have orthologs in *M. pulmonis* but their coding genes are disrupted in non-motile strains (55), reinforcing the hypothesis that these proteins contribute to gliding motility and demonstrating the importance of examining multiple species. The species of the *M. pneumoniae* group lack these genes, as do the non-motile *Mycoplasma hyopneumoniae*, also in the *M. hominis* group, and *M. mycoides* subsp. mycoides SC, which belongs to a third phylogenetic group of mycoplasma species (76).

4. *MYCOPLASMA PNEUMONIAE* ATTACHMENT PROTEINS

The *M. pneumoniae* TX-insoluble fraction, which includes structures of the AO, was analyzed proteomically and found to include proteins known to be localized to the AO and to function in cytadherence (21). While many of the identified proteins are unlikely to function in cytadherence, some of them are also of unknown function. Of those whose function is known from other studies, the proteins fall into two classes: adhesins, which are responsible for binding ligands and mediating attachment to host cells *in vivo* and glass and plastic surfaces *in vitro*, and cytadherence accessory proteins, whose functions are likely structural. Genes encoding these proteins are found only in the species most closely related to *M. pneumoniae*, including *M. genitalium* and *M. gallisepticum*, and are absent from all others, including *M. penetrans* and *M. mobile*. Thus their distribution is the same as the electron-dense core. It is likely that they were acquired or innovated by a common ancestor of *M. pneumoniae* and *M. gallisepticum* after the lineage leading to *M. penetrans* had diverged.

4.1. Adhesins

Numerous lines of experimentation led to the identification of protein P1 as an important adhesin protein of *M. pneumoniae*. Protease-induced loss and reacquisition of cytadherence to respiratory epithelial cells was correlated with loss and reacquisition of P1 (79). P1-specific antibodies inhibit attachment (80), and P1-lacking mutants are negative for hemadsorption (HA) (81), a characteristic which correlates well with mycoplasma cytadherence (82). P1, which is surface-exposed (79,80), is clustered at the AO though present at lower concentrations on the rest of the cell surface as well according to immunocytochemical data (58,80,83,84). P1 orthologs are essential for cytadherence in *M. genitalium* (85) and *M. gallisepticum* (86); in *M. gallisepticum* there are two P1 orthologs, but the role of one of them is not known (87).

P1 is a component of the TX-100-insoluble material of *M. pneumoniae* (21,88), though the degree to which it is present in this fraction is variable and preparation-specific (unpublished observations). Repeated extraction of the *M. pneumoniae* TX-insoluble fraction results in progressive solubilization of P1 (R.H. Waldo III, M.F. Balish & D.C. Krause, unpublished data), suggesting that P1 is only loosely associated with the *M. pneumoniae* cytoskeleton.

The requirements for P1 clustering at the AO have been analyzed through analysis of mutants. Two principal factors seem critical: assembly of a normal core, which requires proteins HMW1, HMW2, and HMW3 (60,89; J.L. Jordan, M.F. Balish & D.C. Krause, unpublished data); and the irreversible insolubilization of HMW1 (90). If these criteria are not met, P1 is stable but delocalized; evidence from *M. genitalium* suggests that in that species the P1 ortholog P140 is unstable under the same circumstances (91).

The likely configuration of protein P1, as determined by a combination of structure prediction and epitope accessibility, is that of a two-pass transmembrane protein with most of the volume on the extracellular surface, including domains that are closely apposed to the membrane (92). Portions of the extracellular regions of the *M. gallisepticum* P1 ortholog GapA resemble carbohydrate-binding domains (86), suggesting potential ligand-binding regions of P1.

CrmA, an *M. gallisepticum* ortholog of the precursor of proteins B and C, also has such domains (86). Pre-B/C appears to be cleaved only in *M. pneumoniae*; its gene is cotranscribed with that of P1 (93), and both proteins are required for cytadherence (81) and have the same localization pattern as P1 (89). Furthermore, proteins P1, B, and C can be cross-linked (94), suggesting that they form a functional complex. Thus, proteins B and C are likely to constitute part of the adhesin activity associated with P1, though direct evidence for this is lacking.

Antibody inhibition has also implicated protein P30 as an adhesin (95). This protein is absent in a class of *M. pneumoniae* cytadherence mutants (96) that are impaired in terms of morphology (97) and gliding motility (53) but not formation of the electron-dense core or clustering of P1, B, and C at the AO (89,97). Although P30 is also a transmembrane protein (98), it is not distributed over the entire cell surface like P1, B, and C but is located exclusively at the AO (58) and possibly at the opposite pole in some cells (53). Thus, it is unclear whether P30 functions together with P1, as proposed for proteins B and C, or independently of it, but both are essential for virulence. *M. genitalium* has a P30 ortholog (99), and *M. gallisepticum* has two (100,101), though the absence of one, PvpA, from the virulent *M. gallisepticum* strain R_{low} (87) signifies that the other, Mgc2, is sufficient for pathogenesis.

4.2. Cytadherence accessory proteins

Proteins that are required for cytadherence but are not adhesins themselves are defined as cytadherence accessory proteins. The lack of homology with proteins from better-characterized systems prevents sequence-based characterization, but studies of the characteristics of both

these proteins and *M. pneumoniae* strains that lack them have resulted in the sense that most cytadherence accessory proteins function structurally (22). Most *M. pneumoniae* cytadherence accessory proteins have been identified through analysis of HA mutants. Although some of the specific interactions among these proteins have been proposed (22), defining specific roles for each has not been possible.

The most illustrative example is *M. pneumoniae* mutant I-2, a spontaneous HA-negative mutant (81) with a disruption in the gene encoding protein HMW2 (102). Transposon insertion mutants into this gene result in the same phenotype, with wild-type properties reemerging upon transposon loss (103). HMW2 is a TX-insoluble (104) AO component (28) with predicted alpha-helical coiled coil properties (103); its coding gene also encodes a smaller protein, P28, presumably directed by an internal translation start site (102), but its role in cytadherence remains unclear. In the absence of HMW2, cells are irregular in morphology, lacking electron-dense cores (89) and appearing filamentous, without obvious AOs (90).

In the absence of HMW2, cytadherence accessory proteins HMW1, HMW3, and P65 are unstable (105,106). HMW1, HMW3, and P65 all possess a large acidic, proline-rich (APR) domain, whose purpose is unknown (62); the remainder of each protein consists of novel domains unrelated to other proteins. All three are located at the AO (59,106,107). Stability of P65 is directly dependent on HMW3 (60) as well as the P30 adhesin (53). The nature of the dependence of HMW3 on HMW2 is unknown; the instability of HMW1 in the absence of HMW2 is associated with failure to become stably incorporated in the cytoskeleton, which appears to be a two-step process that requires HMW2 for the second step (108). Accumulation of HMW1, normally a surface-exposed protein (108), in the TX-soluble fraction, in which form it is not localized (M.F. Balish, K.K. Lee & D.C. Krause, unpublished data), results in degradation (108).

Furthermore, in mutant M6, which lacks HMW1, HMW2 is also unstable and delocalized (109). This mutant also lacks an electron-dense core (89), fails to cluster P1, and has severe morphological abnormalities (110). Thus, HMW1 and HMW2 appear to be central players in AO assembly (22); the presence of only HMW1 on the cell surface has led to the hypothesis that HMW2 is a structural element of the core itself (90). However, direct evidence of this remains to be demonstrated, pending the purification of cores.

Structure-function analysis of HMW2 suggests that its HMW1 stabilization activity lies somewhere within the central 81% of the protein and is independent of its HMW3 stabilization activity, which is located elsewhere (90). *M. pneumoniae* cells expressing a recombinant allele of HMW2 lacking this central portion are morphologically normal and partially HA-positive but fail to stabilize HMW1 and fail to cluster the P1 adhesin at the AO (90), implicating the stabilized form of HMW1 in P1 localization. Additionally, a transposon insertion strain producing an HMW2 variant lacking the C-terminal 43% of the HMW2 protein is phenotypically identical to mutant I-2. Truncated HMW2 is stable but not completely TX-insoluble, implicating the C-terminal half of HMW2 in cytoskeletal assembly (90). Structure-function analysis of HMW1 implicates portions of the C-terminus of HMW1 in cytoskeletal stabilization, localization, and P1 clustering (109). It is conceivable that a transient interaction between the C-terminal domain of HMW1 and the central domain of HMW2, occurring prior to the transport of HMW1 to the cell surface by an unknown mechanism, allows formation of a functional and morphologically normal AO.

The *M. pneumoniae* HMW3 mutant has a more subtle phenotype than those of the HMW1 or HMW2 mutants, with intermediate HA levels, nearly morphologically normal cells, and partial clustering of P1 (60; J.L. Jordan, M.F. Balish & D.C. Krause, unpublished observations). Taken together with the localization of HMW3 to filamentous structures associated with the electron-dense core (59) and the predominance of inappropriately split cores in cells lacking HMW3 (60), a role for HMW3 in core dynamics at the time of duplication is postulated. According to this model, HMW3 prevents core splitting at inappropriate times (22). In the absence of HMW3, normal interaction between the paired elements of the electron-dense core is less likely to occur. In cells in which the core is in a functional conformation, then adhesins will be localized to the AO and cells will be nearly morphologically normal. However, in cells in which the paired elements of the rod separate, which is normally prevented from happening at inappropriate times by the filaments containing HMW3, then the AO is non-functional and P1 is not clustered.

Other possible electron-dense core components are P41 and P24, which are located at the base of the AO (111), in contrast to HMW2 and P65, the other proteins with which P41 and P24 are cotranscribed (103), which are at the distal end of the structure (111). P65, however, is at least partly surface-exposed (112) and dependent upon both HMW3 (60) and P30 (53) for stabilization and normal localization. While nearly all cytadherence accessory proteins are encoded by *M. genitalium* and *M. gallisepticum*, P24 is only found in *M. pneumoniae*.

5. OTHER SUBCELLULAR STRUCTURES IN MYCOPLASMAS

M. gallisepticum contains in its cell body a set of fibrous structures of uncertain composition (113). The conventional view of these structures is that they arise under stress conditions (114) and are helical arrays of ribosomes (115). A dissenting viewpoint is that they are microtubule-like structures that label with antibodies against tubulin (116). However, genome studies have since revealed that there is no tubulin-like protein in *M. gallisepticum* aside from FtsZ (87). Regardless of what these structures actually are and how they function, it is striking that they are restricted to one species.

Numerous strains of *M. mycoides*, including the small colony and large colony variants of the mycoides

subspecies as well as subspecies capri, exhibit a morphological variant form, the rho-form, upon growth in suboptimal media. The rho-form is characterized by filaments with disc-like swellings along the length and a short terminal structure at each end (24). Throughout the length of the filament and passing through the swellings is a rho-fiber, which is helical or straight depending upon the width of the fiber. The rho-fiber, which is variable in width, is regularly cross-striated prominently and also exhibits more subtle longitudinal striation. The terminal structure is wider than the fiber and of distinct appearance. A membrane protein and a soluble protein are induced in cultures exhibiting rho-forms; only the soluble component, designated protein B, is present in the actual fiber (117). Fibers similar to native rho-fibers can be reconstituted from purified protein B in the presence of divalent cations. Neither protein A nor protein B has been identified to date. Although nutritional status has something to do with the formation of the rho-fiber, the significance of the rho-form of *M. mycoides* is uncertain.

6. CONCLUSION

Despite genomic reduction, metabolic fastidiousness, and the absence of a cell wall, mycoplasmas are ultrastructurally complex. Moreover, at the subcellular level, groups of mycoplasmas are as disparate in comparison to one another as they are in comparison to all other bacteria, with unique structural assemblages not found in other organisms. In evolutionary terms, how they arrived at this state is likely a consequence of their history. The closest relatives of the mycoplasmas, represented by *Spiroplasma citri* and *Spiroplasma melliferum*, retain several copies of the actin-like MreB protein (118), which might play a role in their long, helical shape and their corkscrew motility (119). These are present in addition to still other novel cytoskeletal elements (120), suggesting that cell wall loss preceded the innovation of new cytoskeletal systems while species retained vestiges of the old ones, perhaps reapplying them for new purposes. In the mycoplasmas, these became obsolete. It will be informative to learn what cytoskeletal elements are present in earlier branching wall-less relatives of the mycoplasmas, such as the acholeplasmas.

7. ACKNOWLEDGMENTS

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Abbreviations: AO: attachment organelle, TX: Triton X-100

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