Mycoplasma pneumoniae, an Underutilized Model for Bacterial Cell Biology

Mitchell F. Balish
Department of Microbiology, Miami University, Oxford, Ohio, USA

In recent decades, bacterial cell biology has seen great advances, and numerous model systems have been developed to study a wide variety of cellular processes, including cell division, motility, assembly of macromolecular structures, and biogenesis of cell polarity. Considerable attention has been given to these model organisms, which include Escherichia coli, Bacillus subtilis, Caulobacter crescentus, and Myxococcus xanthus. Studies of these processes in the pathogenic bacterium Mycoplasma pneumoniae and its close relatives have also been carried out on a smaller scale, but this work is often overlooked, in part due to this organism’s reputation as minimalist and simple. In this minireview, I discuss recent work on the role of the M. pneumoniae attachment organelle (AO), a structure required for adherence to host cells, in these processes. The AO is constructed from proteins that generally lack homology to those found in other organisms, and this construction occurs in coordination with cell cycle events. The proteins of the M. pneumoniae AO share compositional features with proteins with related roles in model organisms. Once constructed, the AO becomes activated for its role in a form of gliding motility whose underlying mechanism appears to be distinct from that of other gliding bacteria, including Mycoplasma mobile. Together with the FtsZ cytoskeletal protein, motility participates in the cell division process. My intention is to bring this deceptively complex organism into alignment with the better-known model systems.

Mycoplasmas have a long-standing reputation for being something they are not: exceptionally simple. The common term “mycoplasma” encompasses the bacteria of the genera within the phylum Tenericutes and the class Mollicutes (1). These organisms are most distinctively characterized by the absence of any cell wall material, with the cell envelope consisting of a single lipid bilayer and its associated proteins and carbohydrates. Having undergone reductive evolution from Firmicutes ancestors (2), mycoplasma genomes are less complex than those of other bacteria that are capable of being grown axenically in a laboratory setting, which has led to their service as model organisms for studying genomes in a minimal setting (3) and as platforms for synthetic biology (4).

This reduced genomic complexity has led to a perception that mycoplasmas are aberrantly uncomplicated organisms whose value to researchers, beyond dealing with the diseases that they cause, is only as simplified models for cell structure and genome analysis. But since the small genome size results from loss of major anabolic pathways and reduction in the complexity of gene expression regulation, mycoplasmas can still serve as models for other kinds of complex cellular processes. These include genesis of cell polarity, assembly of macromolecular structures, cell division, adherence, and motility.

The human pathogen Mycoplasma pneumoniae (5) is particularly instructive. M. pneumoniae cells feature an attachment organelle (AO) or terminal organelle, a polar extension of the cell involved in adherence, motility, and cell division (6) (Fig. 1). Its construction, mechanisms, and regulation have been studied to various extents. These processes, which are important aspects of the basic biology as well as the pathogenicity of this bacterium, lie at the heart of the major cell biological issues concerning M. pneumoniae.

In this minireview, I argue that the AO is a structure that shares features with important polar structures and surface appendages of other bacteria. I describe the contents and composition of the AO in terms of the relationships of these features to those of other polar structures and also highlight novel aspects of the AO. Finally, I discuss the processes of AO assembly and the roles of the AO in protein localization, gliding motility, and cell division, drawing attention to how M. pneumoniae studies have the potential to inform cell biologists about these processes in their own study organisms. I occasionally reference work on some close relatives of M. pneumoniae, especially Mycoplasma genitalium (7), whose genome essentially consists of orthologs of a subset of M. pneumoniae genes (8), and Mycoplasma gallisepticum.

CELL POLARITY AND THE AO

Cell polarity models. Cell polarity, broadly defined as uneven distribution of molecules and functions over the cell surface, is exhibited by some prokaryotic and eukaryotic cells, for which it is generally essential to their normal function and development. In many bacteria, polarization results in the development of distinct structures at cell poles, such as the endospore of Bacillus subtilis (9) or the stalk, holdfast, pili, and flagellum of Caulobacter crescentus (10). Bacterial cell polarization results from processes and features that include the locations of critical morphogenetic genes along the chromosome, interactions of proteins with cytoskeletal polymers, and membrane curvature (11–13). With its simplified cell envelope, M. pneumoniae can serve as an excellent model for understanding the basic principles underlying the biogenesis and maintenance of cell polarity.

The M. pneumoniae AO. In M. pneumoniae and other polarized mycoplasmas, cytadherence requires adhesins that are localized to all or part of the AO membrane (14–17). The AO parallels
the C. crescentus stalk in the sense that it is a prostheca-like structure whose contents are distinct from that of the cell body and whose synthesis is targeted to a particular cell pole. Additionally, the AO interior contains a specialized proteinaceous structure, the core, which serves structural, cytoskeleton-like functions (18–20). Like a pilus or a flagellum, the core is constructed in an orderly fashion from several different protein components. Thus, the AO is a hybrid between these two types of cellular structures. The AO mediates not only cytoadherence but also the closely linked function of gliding motility. Most AO-bearing species, including M. pneumoniae, exhibit gliding motility in vitro (21, 22), with the AO at the leading end, containing the motor (23, 24).

### THE AO CORE AND ITS COILED-COIL AND PROLINE-RICH COMPONENTS

**AO organization.** The electron-dense core in the AO interior is organized as a double rod with a proximal base and a distal terminal button (Fig. 2); an electron-lucent space surrounds this rod, even in mutants in which the core fails to form an AO by protruding from the cell body, suggesting the presence of material in that region that is difficult to image (19, 20). The core is insoluble in Triton X-100 nonionic detergent (25, 26), a characteristic which has long served as a partial definition of the eukaryotic cytoskeleton. In mutants that fail to form a core, the AO adhesins P1 (MPN141) and P30 (MPN453) are mislocalized and/or unstable (18), and cells tend to be filamentous or branched or both, suggesting nonlethal compromise with respect to the cell division process (27). The cores of M. pneumoniae relatives are quite similar in appearance, with overall similar organizations but different relative sizes of substructures (28–30). Understanding how its homologous components differ across species is sure to provide valuable information about the architecture of the M. pneumoniae core as an example of the assembly of large, multiprotein structures.

**Alpha-helical coiled coils and proline.** The proteins that constitute the AO core have compositional and functional similarities to cytoskeletal proteins from other organisms which might be superficial but could be significant. Like bactofilins (31), the core interacts with the membrane, functioning as a scaffold for the AO. Predicted alpha-helical coiled-coil motifs, which are widespread but are also essential components of intermediate filament proteins, are highly represented among core proteins, with 216-kDa protein HMW2 (MPN310) consisting mostly of these elements (18, 32).

Proline-rich regions also feature prominently among M. pneumoniae AO core proteins, reminiscent of the polar protein PopZ of C. crescentus (33) or of some bactofilins (31, 34). In fact, acidic, proline-rich (APR) domains with compositional simplicity and great variability in length and sequence among orthologs are prominent features of the core proteins (18, 35). Although the functions of these regions are unclear, it is intriguing to speculate that some aspect of richness in proline drives or is at least particularly compatible with protein polarization. Deletion of the APR domain from M. genitalium protein MG312 results in instability, suggesting that it confers normal folding or stabilizing interactions with other cellular components (36).

Some APR domain-containing core proteins also have at least one copy of an ~30-amino-acid sequence motif enriched in aromatic and glycine residues, designated the EAGR box (37). EAGR boxes are not found in any other known proteins. A recombinantly generated EAGR box exhibits a novel fold and crystallizes as a dimer, suggesting involvement in protein-protein interactions (38). Although deletion of the EAGR box from either of two AO proteins does not prevent them from localizing to the AO, deletion from both causes motility impairment (29, 38). These data might suggest a specific role in motility or that disruption of EAGR box-mediated protein-protein interactions within the core interferes with motility.

**AO SYNTHESIS: ASSEMBLY OF A MACROMOLECULAR APPENDAGE**

**Assembly cues.** The assembly of large proteinaceous structures is important in many aspects of biology, including bacterial pathogenesis. Various secretion systems, pili, and flagella must be assembled properly, either constitutively or in response to environmental cues, at appropriate cellular locations (39–42). Like many of these appendages, the M. pneumoniae AO core is composed of...
several proteins that are assembled in a particular order (18) and is constitutively present on wild-type cells. How new AOs form and the nature of the relationship between the new and old AOs are complicated issues. One could imagine that components of the old AOs are used in construction of the new one, either by splitting or as a template. Alternatively, the new structure could be formed independently of the old one, with the coincidence of positions being related to some other factor, such as the location of molecules involved in signaling the start of AO synthesis. For example, DNA is associated with the base of the AO core (28); perhaps newly replicated DNA provides a spatial signal for AO synthesis. If so, then synthesis of the new AO would be able to be uncoupled from the position of the old one by alteration of the spatial position or the timing of critical cues. Indeed, there is evidence for AO synthesis both coupled to and uncoupled from the preexisting AO, suggesting that both these options are available.

**Coupled AO synthesis.** Occasionally, images of *M. galaxiicola* cores show branching, with two terminal buttons emanating from individual cores (30). Some specimens have two terminal buttons emanating from a single rod, whereas in others, the two terminal buttons are at the ends of individual stalks that fused part of the way down the length of the rod. Likewise, cryoelectron micrographs of the *M. pneumoniae* AO also occasionally reveal a complete core immediately adjacent to a partially formed one (20). These data are consistent with AO duplication taking place by progressive synthesis of a new core adjacent to an old one, beginning at the terminal button and proceeding toward the base (Fig. 2).

**Uncoupled AO synthesis.** Synthesis and subcellular location of the AO are significantly impacted by loss of P24 (MPN312), a protein that arrives early in the process of AO assembly (24) and appears to be important in dictating both the timing and positioning of new AO synthesis. In a mutant strain lacking P24, there is an increase in the appearance of AOs on lateral surfaces of cells as well as a general retardation of AO synthesis (43). These data suggest the availability of a *de novo*, P24-independent mode of AO synthesis that is less efficient than template-driven AO synthesis. Assembly of both flagella and pili similarly involves factors that regulate their synthesis both temporally and spatially (42, 44). Understanding how P24 functions in the AO assembly process could be quite informative to those who are interested in the biogenesis of these other macromolecular appendages.

TopJ (MPN119), the only AO protein with clear homology to proteins from other types of organisms, consists of a J domain, which is associated with protein folding (45), an APR domain, and several EAGR boxes (46). In the absence of TopJ, all other known AO proteins are stable except for P24, and cores are normal in appearance, but the core frequently fails to protrude from the cell body either fully or at all (46, 47). Mutations of amino acids in TopJ that are required for chaperone function in its homolog DnaJ generally recapitulate phenotypes associated with TopJ loss (48), suggesting that chaperone activity lies at the heart of this aspect of AO positioning. When TopJ is absent, the P1 adhesin adopts a partly trypsin-resistant form (47). Again, mutation of chaperone-associated amino acids is sufficient to achieve this phenotype. Intriguingly, DnaJ is essential for flagellin synthesis in *Escherichia coli* (49), and a J domain protein in *Vibrio alginolyticus* is involved in determining the correct location of that organism’s flagellum (50). The roles of DnaJ homologs in macromolecular assembly processes may be underappreciated, and *M. pneumoniae* TopJ provides an excellent example for study of these roles.

**Phosphoregulation of AO activity.** Protein phosphorylation is significant in a variety of cellular functions, including bacterial morphogenesis (51, 52). This phosphorylation is frequently carried out in the context of two-component regulatory systems or phosphorelays that involve histidine kinases, which are absent in mycoplasmas. Serine/threonine protein kinases also have major roles in bacterial cell division and morphogenesis (52, 53). An important role for phosphorylation of AO proteins has been revealed by deletion of the serine/threonine protein kinase PrkC (MPN248), whose *B. subtilis* homolog has a variety of substrates and is presumed to be involved in a number of cellular processes (54). In mutant *M. pneumoniae* cells lacking PrkC, many of the proteins most markedly reduced in their phosphate content are AO components (55, 56). Both PrkC and its cognate phosphatase, PrpC (MPN247), are required for normal gliding motility frequency, with deletion of the phosphatase resulting in more frequent gliding (57). These data suggest that homeostasis of phosphorylation and dephosphorylation of AO components is critical for normal AO function, although it is unclear whether the phosphate is regulatory or structural or both. PrkC and PrpC homologs are widespread among bacteria but not very well characterized. With its reduced phosphoproteome (58), *M. pneumoniae* presents a useful platform for study of the specificity and regulation of these proteins and other kinases and phosphatases.

**CYTOSKELETON-MEDIATED POLAR LOCALIZATION OF ADHESINS**

The interactions that are responsible for localization of adhesins P1 and P30 to the *M. pneumoniae* AO are poorly understood but involve other cytoskeletal proteins. The process of localization is likely to relate to other examples of bacterial proteins that use cytoskeletal cues to localize to specific subcellular regions, such as the interaction of Min system proteins with DivIVA in *B. subtilis* (59, 60), type IV pilus components of *Myxococcus xanthus* with a bactofilin (61), *C. crescentus* crescentin with MreB (62), and peptidoglycan precursor biosynthetic enzymes with MreB in a variety of bacteria (63–65). Further study of these protein-protein interactions in *M. pneumoniae* is therefore of potential value to a variety of bacterium-cell biologists.

P1 is distributed over the entire *M. pneumoniae* cell surface but is concentrated at the AO (14, 15). In the absence of HMW2, a Triton X-100-insoluble core protein with an extensive alpha-helical coiled-coil structure that is required early in the core assembly process for AO formation, P1 is present in clusters located at seemingly random locations in the cell membrane (14, 18). HMW2 is required for the permanent incorporation of AO protein HMW1 (MPN447) into the Triton X-100-insoluble fraction (37). When a recombinant variant of HMW2 lacking the central 81% is produced in an HMW2 null mutant, morphologically normal AOs are produced, but HMW1 is not stabilized and P1 is not localized, suggesting an interaction between P1 and the HMW2-stabilized form of HMW1 (66).

In contrast to P1, P30 is restricted to the AO tip (16). Retention of P30 at the AO relies on the presence of P65 (MPN309), a Triton X-100-insoluble protein that requires P30 for localization to the AO and stabilization (67). Remarkably, when P65 is disrupted by transposon insertion such that only the N-terminal region of the protein is produced, P30 is initially localized normally to the AO,
but bodies containing P30, but not core proteins, are shed from the cell (68). These data implicate P30 interactions with core components such as P65 in maintenance of P30-containing membrane structures in the AO.

**YET ANOTHER GLIDING MOTILITY MECHANISM**

A variety of bacteria move using gliding motility (69). The mechanisms underlying this smooth movement of cells across surfaces differ greatly among phyla, leading to the conclusion that gliding is actually the outcome of several entirely unrelated processes. These processes are often instructive regarding other biological mechanisms. For example, an unexpected sharing of components between *M. xanthus* gliding motility and cell envelope modification during myxospore formation illustrates the potential for modularity among biological machines (70). Likewise, recent revelations about the use of a secretion apparatus in the gliding motility of *Bacteroides* species provide opportunities to study secretion of virulence factors in related organisms (71). At the same time, the unrelatedness of the mechanisms limits researchers’ abilities to draw specific conclusions from model organisms. Therefore, whatever we come to understand about mycoplasma gliding beyond the most general principles must derive from study of mycoplasmas. Nonetheless, it is likely that doing so will provide insight into unexpected areas of research.

**Directionality.** Gliding motility is generally coupled with taxis machinery to promote better feeding, but there is no strong evidence that mycoplasmas are able to make directional choices. Interestingly, *M. genitalium* has a curved AO (Fig. 1) and tends to glide in circular paths, but when MG217, the ortholog of *M. pneumoniae* P65, is deleted, both the AO and the gliding paths become less curved, suggesting that direction of movement is related to AO architecture and allowing the possibility that cells can control their direction by modifying key proteins (72). Recent evidence suggests that *M. pneumoniae* gliding is important for penetration of the mucus layer to reach host cell surfaces (73), although how or even whether this organism can sense direction in doing so is entirely unknown. Further study of this phenomenon might reveal alternatives to currently understood chemotaxis systems.

**Difficulty in determining the motility mechanism.** Gliding motility in *M. mobile*, in which an ATPase is proposed to power conformational changes in adhesin molecules that ratchet the cell forward in coordination with cyclic binding to and unbinding from the substratum (22), is probably not a good model for *M. pneumoniae* motility because of substantial differences between the two organisms. *M. pneumoniae* lacks homologs of *M. mobile* motility proteins, including the adhesin and all the components of the core, including proteins proposed to transduce energy to the adhesin (22, 74). It is conceivable that *M. mobile* and *M. pneumoniae* have simply diverged very greatly, rendering homology among P30 orthologs in *M. pneumoniae* and its relatives (81). A P30 mutant with alteration to a 16-amino-acid segment on the cell surface supports adherence but gives rise to very slow and infrequent gliding (78), thereby implicating P30 as a component of the motility apparatus independently of its role in adherence. Finally, 51 of the 125 amino acid residues at the surface-exposed C-terminal end of *M. pneumoniae* P30 are prolines, mostly organized into imperfect, 6-amino-acid repeats (82). Mutants lacking a number of these repeats exhibited greatly reduced adherence, and the adherent cells themselves glided very slowly (78, 83). Progressive truncation of the C-terminal region of P30 results in decreasing steady-state levels of P30 (84) and concomitant speed reduction. These data suggest a model in which the proline-rich repeats stabilize P30, perhaps through promoting interactions with components of the motor.

Interestingly, substitution of the *M. genitalium* ortholog of P30 for *M. pneumoniae* P30 results in full motility and adherence (85), despite the fact that the proline-rich region of the *M. genitalium* protein has a completely different composition, with about half as many prolines, as well as distinctly less repeat regularity (86). These data suggest that the overall structures of *M. pneumoniae* P30 and its *M. genitalium* ortholog are similar enough to allow the same stabilizing interactions with other cell components, raising the issues of what the structure of P30 is and what its important binding partners are.

**Regulation of motility.** Links between AO assembly and gliding motility are not unanticipated, given the central role of the AO in motility. Indeed, both the frequency of cells in the population that glide and their gliding velocity are substantially reduced in the absence of P24, suggesting a role for this protein in catalyzing activation of the motility function (43). Protein phosphorylation, as described above, is also likely to play roles in regulation of motility, but distinguishing between roles for phosphorylation in AO assembly and in motility requires more work.
CELL DIVISION: THE COMBINED EFFORTS OF ADHESINS, MOTILITY, AND FtsZ

Most of what is understood about bacterial cell division takes place in the context of the relationship between the FtsZ cytoskeletal protein and peptidoglycan synthesis, but M. pneumoniae and its wall-less relatives provide a different perspective. A popular model of how FtsZ functions in other organisms involves simultaneous constriction of the membrane and direction of peptidoglycan synthesis proteins to a location behind the constricting membrane, causing local cell wall synthesis and thereby preventing reversal of invagination (87). However, study of FtsZ-mediated division in bacterium-like entities that lack peptidoglycan has been instructive with respect to seeing FtsZ as part of a bigger picture. In plastids and, in some eukaryotic lineages, mitochondria, FtsZ-mediated constriction within the organelle is linked to constriction of proteinaceous structures on the organelle surface (88, 89). On the other hand, in artificial systems that lack partners for FtsZ, membrane constriction generally reverses without a net effect of division. Thus, FtsZ must be coupled with some other component and/or process in order to effectuate cell division efficiently. M. pneumoniae and its motile mycoplasma relatives provide interesting evidence of the need for this partnering.

Remarkably, whereas FtsZ is essential in most walled bacteria, an ftsZ knockout mutant of M. genitalium is viable (90). Cell division is slowed but not blocked in this mutant. However, spontaneous adherence mutants, which appear quite frequently among wild-type cells, do not occur in appreciable numbers. This observation suggests synthetic lethality of loss of FtsZ and AO functions, implying that the AO is a partner of FtsZ in these organisms. Interestingly, nonadherent M. pneumoniae mutants are branched or filamentous (27), reinforcing the idea of the participation of the AO in the cell division process.

An understanding of the role of the AO in cell division comes from studies of AO assembly during the M. pneumoniae cell cycle. The best-described paradigm for M. pneumoniae AO biosynthesis (Fig. 3) features a new, adherence-active AO constructed adjacent to the existing one at the onset of DNA replication (27). Before the new one matures into a motility-active structure, the old AO pulls the cell body past it such that the two AOs come to mark opposite poles (91). Cell division ensues, with the daughter cells having either the old AO or the new one. Eventually, the new AO becomes motility active and the cell that bears the new AO begins to move. Often this occurs in overlapping cycles, with further AOs appearing before division has separated the cells bearing the first two.

Synthesis of the FtsZ and AO data leads to a model in which cytokinesis is effectuated by the combination of the presence of FtsZ and motility, which itself can take place only while the cell is adherent to a surface. In this model, FtsZ constricts the membrane, and motility pulls the cells apart before the constriction can reverse (Fig. 3). Thus, FtsZ and AO-mediated motility can individually carry out inefficient cell division, but both processes are required for efficient division. Furthermore, using motility as a source of power for cell division is quite unusual and discovery of such a process is certainly unprecedented among prokaryotes.

Considering the information derived from study of walled bacteria, eukaryotic organelles, and mycoplasmas, a picture emerges in which FtsZ activity alone is poorly effective at allowing unidirectional cell division but can be coupled with any number of other forces—including peptidoglycan synthesis, constriction of surface-localized machinery, and motility—to support unidirectional membrane constriction and result in successful cytokinesis. This insight could be valuable for deepening understanding of bacterial and organellar division and for imagining novel mechanisms of division that could be used in synthetic biological systems.

FUTURE DIRECTIONS

For researchers to understand the complex cellular features of M. pneumoniae, better tools for genetic manipulation must be developed. M. pneumoniae does not stably support plasmids, leaving researchers to rely on the cumbersome process of random transposon insertion, although an ordered transposon mutant library...
has been created and is available (92). Likewise, inducible promotors have not been developed for use in M. pneumoniae. Production of M. pneumoniae proteins in other organisms is complicated by the use of UGA as a frequently occurring tryptophan codon rather than a stop codon, although molecular workaroundes have been reported (93, 94). Success in expressing functional, complete versions of AO core proteins has not been reported, likely because of their unusual composition and requirements for coassembly with other AO proteins.

The entire cell biology research community, engaged in studies of parallel cellular processes in other organisms, stands to benefit from advances in mycoplasmal cell biology. Further work focused on the structure, function, and interactions of M. pneumoniae AO proteins in both AO assembly and gliding motility will illuminate such topics for those who are engaged in understanding both M. pneumoniae pathogenicity and, more generally, the principles underlying macromolecular assembly and cell movement. In particular, FtsZ from M. pneumoniae and other motile mycoplasmas is likely to have special adaptations that enable it to function coordinately with the gliding motility apparatus and therefore warrants special attention. Additionally, the study of cell division in nonmotile mycoplasmas would provide an opportunity to learn more about the mechanism by which FtsZ functions in the absence of a cell wall. Recent work on wall-less L forms of other bacteria has drawn attention to alternative mechanisms for cell division, involving processes such as membrane synthesis that are not well studied in the context of cell division in mycoplasmas (95, 96). Whether these processes parallel mycoplasma cell division remains unknown and is worth studying.

ACKNOWLEDGMENTS

This work was supported by the National Institutes of Health (Public Health Service grant R15 AI073994). I thank Ryan Relich and the Miami University Center for Advanced Microscopy and Imaging for the images in Fig. 1.

REFERENCES


26. Nakane D, Miyata M. 2009. Cytoskeletal asymmetrical dumbbell structure of a gliding mycoplasma, Mycoplasma gallisepticum, revealed by neg-


