

Division without Binary Fission: Cell Division in the FtsZ-Less *Chlamydia*

 Scot P. Ouellette,^a Junghoon Lee,^a John V. Cox^b

^aDepartment of Pathology and Microbiology, College of Medicine, University of Nebraska Medical Center, Omaha, Nebraska, USA

^bDepartment of Microbiology, Immunology, and Biochemistry, University of Tennessee Health Science Center, Memphis, Tennessee, USA

ABSTRACT *Chlamydia* is an obligate intracellular bacterial pathogen that has significantly reduced its genome size in adapting to its intracellular niche. Among the genes that *Chlamydia* has eliminated is *ftsZ*, encoding the central organizer of cell division that directs cell wall synthesis in the division septum. These Gram-negative pathogens have cell envelopes that lack peptidoglycan (PG), yet they use PG for cell division purposes. Recent research into chlamydial PG synthesis, components of the chlamydial divisome, and the mechanism of chlamydial division have significantly advanced our understanding of these processes in a unique and important pathogen. For example, it has been definitively confirmed that chlamydiae synthesize a canonical PG structure during cell division. Various studies have suggested and provided evidence that *Chlamydia* uses MreB to substitute for FtsZ in organizing and coordinating the divisome during division, components of which have been identified and characterized. Finally, as opposed to using an FtsZ-dependent binary fission process, *Chlamydia* employs an MreB-dependent polarized budding process to divide. A brief historical context for these key advances is presented along with a discussion of the current state of knowledge of chlamydial cell division.

KEYWORDS *Chlamydia*, FtsZ, MreB, binary fission, cell division, penicillin, peptidoglycan, polarized budding

Chlamydia is a genus of obligate intracellular bacterial pathogens that undergo an unusual biphasic developmental cycle in the course of propagating and disseminating from one cell to another (see reference 1 for a review). The elementary body (EB) is infectious but nondividing, whereas the reticulate body (RB) is noninfectious but dividing. An EB infects a cell and differentiates to an RB, which multiplies within a membrane-bound parasitic organelle called an inclusion (2). After multiple rounds of division, RBs differentiate to EBs and are released from the cell. In adapting to their obligate intracellular niche, these pathogens have significantly reduced their genome size and content, which includes the elimination of genes that would otherwise be essential in most bacteria (3). Some examples of such genes include those involved in cell division, such as the canonical organizer of the division site, *ftsZ* (4). Recently, there have been multiple reports concerning the mechanism of chlamydial cell division as well as the role of peptidoglycan (PG) in this process. After providing historical context for these studies, this review will update the current state of the field in three parts: peptidoglycan, cell division proteins, and the mechanism of division.

Early clues: the effect of penicillin on *Chlamydia*. With Fleming's discovery of penicillin and its inhibitory action against various bacteria, many studies from the 1940s began basic characterizations of the effect of penicillin on different bacterial species (5). This was also true for *Chlamydia* (e.g., see references 6 to 9), which at that time was still classified as a virus. One of the first systematic studies of the mechanism of action of penicillin on *Chlamydia* was performed by Tamura and Manire in 1968 (10). In this

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Address correspondence to Scot P. Ouellette, scot.ouellette@unmc.edu.

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report, the authors made several key and noteworthy observations. First, they observed that adding penicillin at later times during infection failed to inhibit recovery of infectious forms. This indirectly suggested that the primary target of the antibiotic was the RB and not the EB because, once EB production had started, penicillin failed to inhibit growth. Second, the authors observed that measurements of protein, nucleic acid, and cell envelope composition of the penicillin-treated organisms suggested characteristics of an RB. This indirectly indicated that replication, transcription, and translation were globally unaffected by penicillin, some of which has been noted more recently (11). The authors also mentioned that no muramic acid was detectable and, should it be present, was at levels comprising less than 0.2% of the dry weight of the cell envelope. By comparison, peptidoglycan can constitute up to 10% of the dry weight of Gram-negative bacteria (12). Third, the authors observed that penicillin, when added at the time of infection, could be removed from the culture to restore infectious progeny production. This indirectly alluded to the reversibility of the effect in what would later be referred to, in other experimental systems, as persistence (13). Finally, the authors observed small, internal membrane structures from the membrane preparations of penicillin-treated organisms. This would be indirectly explained by later morphologic studies examining structural changes to the RB upon penicillin treatment (see below).

A subsequent electron micrographic study from Matsumoto and Manire in 1970 further examined the impact of penicillin on the morphology of *Chlamydia* (14). Here, the authors observed gross morphological changes in the RB after penicillin treatment, whereby organisms were aberrantly enlarged. This was direct evidence to suggest that penicillin treatment caused a blockage in chlamydial cell division. Intriguingly, the authors also observed internal membrane structures within the aberrant RBs, as previously noted by Tamura and Manire (10). Perhaps more importantly, the authors also examined what changes occurred after penicillin removal from the culture medium. This led the authors to note that as the aberrant forms recovered from penicillin, smaller RBs underwent a “budding process” as they emerged from the aberrantly enlarged RB that had been previously blocked from dividing. The latter finding was also noted by Skilton et al. in time-lapse video microscopy analyses of the developmental cycle and recovery from penicillin treatment (15). The seminal study of Matsumoto and Manire (14) laid the foundation for future work examining chlamydial cell division as noted below.

Given that penicillin affects *Chlamydia* and that the mechanism of action of this beta-lactam is through its inhibitory effects on penicillin-binding proteins (PBPs), the obvious question became, “Does *Chlamydia* express PBPs?” This question was answered in a study from the Caldwell group in 1982 (16). The authors demonstrated that *Chlamydia trachomatis* expresses three PBPs: two high-molecular-weight variants and one low-molecular-weight variant. The relative levels of the PBPs were higher in RBs than EBs, consistent with a role for the PBPs in division. As PBPs function to cross-link peptidoglycan (PG) monomers, the authors attempted to detect PG through muramic acid measurements. They also noted, as did Tamura and Manire (10), that muramic acid was not detectable when the threshold for detection was lowered to 0.02% by weight.

At this stage, several key facts were known concerning the effects of penicillin on *Chlamydia*: (i) it blocks progression through the developmental cycle, (ii) it induces aberrantly enlarged RBs by blocking division, and (iii) it binds three PBPs in *Chlamydia*. However, no study had been able to demonstrate the presence of PG or muramic acid in these organisms. This led Moulder in 1993 to propose the chlamydial anomaly, wherein he codified these observations and their incongruence with the lack of detection of PG (17).

Part 1: the substance (also known as peptidoglycan in *Chlamydia*). Moulder’s anomaly was partially solved in 1998 with the publication of the first *Chlamydia* genome sequence (3). A nearly complete pathway for the synthesis of PG was revealed, including the cytoplasmic *murA* to *F* genes (and others), and genes encoding enzymes

associated with the lipid carrier (lipid II) of the PG monomer, *murG* and *mraY*. The prior observations and these new genome sequence data suggested that chlamydiae made PG strictly for cell division purposes. A further tantalizing clue in support of this hypothesis came from members of the Rockey lab in 2000, who observed the ringlike pattern of an antigen that was strictly localized at apparent division sites (18). Ironically, their antibody was prepared from an adjuvant control derived from mycobacterial cell wall extracts, and the authors postulated that their antibody reacted with a nonproteinaceous, possibly PG-like substance present in the adjuvant. Indeed, the localization of their "SEP" antigen was redistributed in penicillin-treated organisms in a pattern consistent with a disruption in PG synthesis. These studies provided an impetus to determine how and why *Chlamydia* makes PG.

Several labs sought to leverage the genome sequence data to determine whether various chlamydial components were functionally active. Work from the Maurelli lab in 2003 demonstrated that a chlamydial homolog of MurA, the first committed step in PG synthesis, was functional using a combination of *in vitro* biochemical assays and *in vivo* complementation assays of a *murA* mutant in *E. coli* (19). Interestingly, *Chlamydia* is naturally resistant to fosfomycin because it contains a resistant allele of *murA*, and this resistance was conferred to the *E. coli* strain expressing chlamydial MurA. Hesse et al., also in 2003, and later McCoy et al. in 2005, similarly showed functional activity for each component of the chlamydial MurC-Ddl fusion protein (20, 21). This was followed by studies examining MurE (22) and MurF (23). Concurrent with these studies examining a role for the cytoplasmic components of PG synthesis were studies attempting to determine the functionality of lipid I and II synthesis in *Chlamydia*. Henrichfreise et al. provided biochemical evidence for the functionality of chlamydial MraY in synthesizing lipid I and MurG in synthesizing lipid II (24). These initial studies showed in all cases that the chlamydial homologs were functional and, when tested, complemented their respective *E. coli* conditional mutants, thus strengthening the likelihood that *Chlamydia* generates a canonical PG monomer.

Recently, newly synthesized PG has been visualized in bacteria using novel fluorescent tools developed to label PG precursors *in vivo* (25, 26). This technique allows for metabolic labeling of live bacteria with D-amino acid precursors that are incorporated into the PG monomer in the terminal positions of the pentapeptide sidechain. This tool was leveraged to demonstrate in 2014 that chlamydial PG could be labeled and that its localization was specific to the division septum (27), consistent with earlier hypotheses and the results obtained with the antibody against the SEP antigen (18). More importantly, its presence is necessarily transient as it is detected only in division sites, which helped explain why detection of chlamydial PG had not been successful using other methodologies. A follow-up study confirmed the composition of muropeptides and also demonstrated evidence of both transpeptidated and transglycosylated PG fragments (28). Therefore, the combination of characterization of individual PG genes, the use of the novel fluorescent tool, and detailed mass spectrometry studies have confirmed that *Chlamydia* generates a canonical PG and that its role is apparently restricted to cell division.

Part 2: the players (also known as divisome components). The assumption, and later confirmation, of the role of PG in chlamydial cell division revealed surprisingly little about the mechanism of division in these unique bacteria. It is the divisome components or machinery that orchestrates and regulates the division process. Arguably, the function of the divisome is to specifically recruit the PG synthesis machinery (i.e., FtsI/FtsW) to the division site. There are two primary modes of PG synthesis in bacteria: one associated with sidewall synthesis (i.e., cell wall growth in rod-shaped or ovicoccoid bacteria) and one associated with cell division (see reference 29 for a review). The first growth mode is regulated by MreB, an actin-like homolog found in organisms such as *E. coli* and *B. subtilis* (30–32). The second growth mode associated with cell division is regulated by FtsZ, a tubulin-like homolog found in almost all eubacteria (4, 33). *Chlamydia* is among the rare eubacteria that lack FtsZ (3). However, it does possess an

MreB ortholog (34). This is surprising because *Chlamydia* is a coccoid bacterium, and MreB is classified as a rod shape-determining protein, as it imparts a rod or ovoid shape to bacteria that express it; thus, *mreB* is generally missing from coccoid bacteria. When MreB activity is blocked or conditionally deleted in rod- or ovoid-shaped bacteria, the resulting cells become round (35). Yet, *Chlamydia* retains a coccoid shape even though it expresses MreB.

These observations and the presumed function of PG in division led Ouellette et al. in 2012 to hypothesize that MreB may functionally substitute for FtsZ in *Chlamydia* by recruiting cell division machinery to the divisome (34). These authors demonstrated that inhibition of MreB activity with MreB-specific antibiotics blocked chlamydial division at an early step. Morphologic characterization of MreB-inhibited bacteria revealed slightly enlarged RBs that otherwise appeared normal. These slightly enlarged RBs may imply that cell envelope growth or synthesis is also linked to MreB—an area of active investigation in our laboratories. This phenotype was very distinct from the grossly enlarged penicillin-treated organisms, indicating that cell envelope growth continues in the absence of peptidoglycan transpeptidation activity. Importantly, when infected cells were treated with both MreB-specific antibiotics and beta-lactams, the MreB-inhibited phenotype was observed. This indicated that MreB functions upstream of PBPs, which catalyze the terminal steps in PG synthesis. Finally, MreB was shown to interact with the chlamydial homolog of FtsK, an ATPase involved in chromosome segregation. Subsequent work from Kemege et al. localized MreB to puncta at apparent division sites (36). By transforming *C. trachomatis* serovar L2 with an MreB-6×H expressing construct, we recently observed, using superresolution microscopy, MreB rings at the site of division (37). Thus, these studies support a function for MreB in chlamydial cell division.

In addition to MreB, *Chlamydia* possesses additional homologs of rod shape-determining proteins. These include the high molecular weight PBP2, a monofunctional transpeptidase, and RodA, a putative chaperone for PBP2 and predicted transglycosylase (3). In the previously described study, Ouellette et al. also examined the role of PBP2 using the PBP2-specific antibiotic, amdinocillin, in comparison to the PBP3/FtsI-specific antibiotic, piperacillin (34). Although long-term treatment with both beta-lactam derivatives induced grossly enlarged RBs and blocked division, each induced subtle differences in morphology. Amdinocillin induced fragmented forms reminiscent of the internal membrane structures described by Matsumoto and Manire (14). How exactly these internal membrane structures form is not currently understood. Piperacillin treatment, in contrast, was more commonly associated with elongated forms and membrane blebbing, reminiscent of the effects of beta-lactams on *E. coli* (i.e., filaments). Each PBP interacted homotypically and heterotypically, and each interacted with FtsK by two-hybrid assays (34). Interestingly, each PBP localized to puncta at the membrane of RBs (34), similar to what was later observed for MreB (36). These data indicate a link between the rod shape-determining proteins of *Chlamydia* and the cell division apparatus, potentially explaining their conservation in these bacteria.

Another chlamydial rod shape-determining protein homolog has recently been identified and characterized. Although unannotated, the chlamydial RodZ showed some homology to the cytoplasmic domain of *E. coli* RodZ (38). RodZ is a cytoskeletal element that interacts with MreB at the inner leaflet of the cytoplasmic membrane in other organisms. The interaction of chlamydial MreB with its cognate RodZ was confirmed by bacterial two-hybrid assays. Kemege et al. made similar observations and further extended them to observe that RodZ localized at chlamydial membranes in an apparently uniform distribution, suggesting RodZ may perform additional functions in *Chlamydia* (36), though this supposition remains to be investigated. Interestingly, in *Waddlia*, an organism related to *Chlamydia*, RodZ localizes to division sites prior to MreB, suggesting it may be necessary to recruit MreB to the divisome (39). Collectively, these data on rod shape-determining proteins suggest that *Chlamydia* has retained them and specifically adapted their function to division, as opposed to cell wall growth.

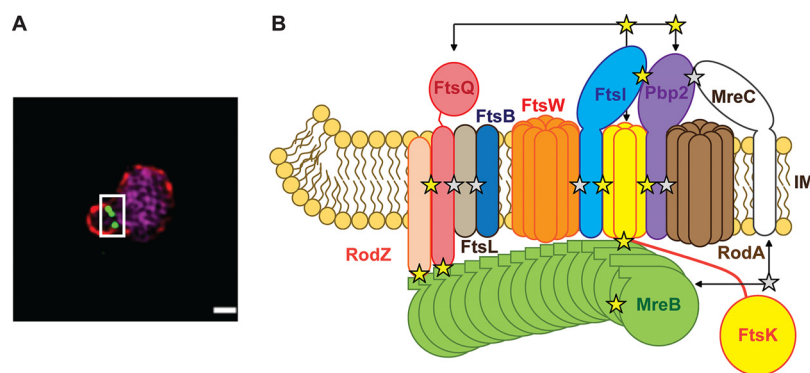


FIG 1 (A) Structured illumination microscopy image of 10.5-h postinfection *C. trachomatis* expressing MreB-6×H. Major outer membrane protein (MOMP) (red), Hsp60 (purple), and chlamydial MreB (green) are shown. Scale bar = 0.5 μ m. The boxed region is illustrated in panel B. (B) Schematic illustrating the known and putative chlamydial inner membrane divisome components. Known interactions are shown with a yellow star, whereas putative interactions based on orthologous systems are shown with a gray star.

Although clear homologs are present for FtsK, FtsI/PBP3, and FtsW, the original annotation of the chlamydial genome lacked homologs to most other divisome components. Recently, Ouellette et al. helped identify some of these missing pieces by characterizing chlamydial FtsL and FtsQ homologs (40). Indeed, this was the first study to ectopically express a genetically tagged GFP division protein in *Chlamydia* using recently developed genetic tools. The authors localized GFP-FtsQ to puncta, similar to what has been observed for the PBPs and MreB (see above), and more specifically demonstrated its localization at clear division sites. Importantly, chlamydial FtsQ interacted with a variety of chlamydial division proteins by two-hybrid analysis.

All of the divisome components described to this point reflect the machinery necessary to direct division of the inner membrane of Gram-negative organisms. However, a different set of components, the Tol-Pal system, is required to effectively direct division of the outer membrane (41). Recently, work in *Waddlia* has characterized a role for the homologous Tol-Pal system in division in this organism (42). The *Waddlia* Pal protein was localized to division sites and was also shown to bind peptidoglycan. As *Chlamydia* possesses homologs of this system, it is likely that these proteins will perform similar functions in these bacteria, although this remains to be formally demonstrated.

The current chlamydial inner membrane cell divisome components are depicted in Fig. 1. Interestingly, none of the divisome components tested to date (FtsQ, MreB, and RodZ) have been demonstrated to efficiently complement *E. coli* conditional mutants of the equivalent homologous proteins (36, 38, 40). These data indicate, not surprisingly, that the chlamydial divisome is likely highly specialized. Because of the highly integrated cell division machinery (43), every potential interaction between divisome proteins would need to be recapitulated to allow for complementation. Given that *Chlamydia* lacks the early components of a canonical FtsZ-dependent binary fission pathway (i.e., FtsZ, FtsA, etc.), critical interactions with these components would not be predicted to occur with the chlamydial homologs of the divisome. That being said, a recent study from Ranjit et al. suggested that chlamydial MreB and RodZ may allow, at least transiently, for cell division in the absence of FtsZ in *E. coli* (44), perhaps indicating that multiple chlamydial divisome components must be included to allow for efficient complementation. Notably, the *E. coli* Δ mreB mutant strain did not revert to a rod shape when chlamydial MreB-RodZ proteins were coexpressed, thus reinforcing that the chlamydial orthologs are unable to complement the *E. coli* function of these proteins. Overall, these results may suggest that *Chlamydia* uses its machinery in a completely different division mechanism/strategy, as described below, which is incompatible with binary fission mechanisms.

Part 3: the process (also known as division without fission). The canonical binary fission process used by most bacteria (those that have been studied in detail) relies on the central coordinator protein, FtsZ (see reference 45 for a review). FtsZ is a tubulin-like homolog possessing GTPase activity that can polymerize to drive peptidoglycan synthesis in the septum (46–49). The cytosolic FtsZ recruits additional proteins in both a hierarchical and temporal manner (i.e., early versus late) to the division septum that forms in the middle of the cell. Early proteins recruited by FtsZ in *E. coli* include FtsA and ZipA, which tether the Z-ring to the membrane (50, 51). Proteins recruited later include FtsK, an ATPase that drives chromosome segregation, the FtsLBQ complex, thought to stabilize the septal ring, FtsI and FtsW, PG synthetases that transpeptidate and transglycosylate PG monomers in the septum, and AmiC, a PG hydrolase that separates daughter cells (52–55). A separate machinery involving the Tol-Pal system is used to drive membrane invagination of the outer membrane in Gram-negative organisms (41). During binary fission, cell size increases before a septum forms in the middle of the cell to produce two equal daughter cells. At all times, the cell size of the two nascent daughter cells remains nearly equal. It is worth noting that other types of FtsZ-dependent division mechanisms have been characterized and that not all organisms possessing FtsZ homologs divide by binary fission (see reference 56 for review).

We propose that MreB and its associated proteins RodZ, MreC, PBP2, and RodA form the core of an apparatus that substitutes for the early divisome components FtsZ and its associated proteins (e.g., FtsA, ZapA, etc.). It is notable that the late divisome components have mostly been conserved in *Chlamydia*. Why *Chlamydia* has substituted the MreB system for the FtsZ system is unknown and may have been an accident of evolution, since chlamydiae routinely pass through a bottleneck from one developmental cycle and host to another. Alternatively, it may represent the most thermodynamically energy-efficient means for dividing a bacterium lacking a PG-based cell wall. Regardless, we now know that *Chlamydia* uses PG solely for cell division and have identified the key divisome components. However, none of this information directly informs the process of cell division in these bacteria.

Given that *Chlamydia* lacks FtsZ, it is, *a priori*, reasonable to hypothesize that this unusual organism does not undergo a binary fission process. However, a simple PubMed search for “chlamydia and binary fission” reveals the earliest mention of binary fission by Schechter in 1966 (57). Schechter did not directly assess chlamydial division mechanisms but based her conclusion on rates of macromolecule synthesis and incorporation. An earlier study by Gaylord in 1954, when the designation for the *Chlamydia* strain tested was meningopneumonitis virus, also inferred from electron micrographs that *Chlamydia* divides by binary fission despite observing “buds” in some images of RBs (58). Nevertheless, it appears that the assumption that *Chlamydia* divides by binary fission has been generally accepted and propagated by those in and out of the field. Ironically, it was Matsumoto and Manire who later hinted at another possible mechanism of chlamydial division when they reported on the presence of budding forms during recovery from penicillin treatment (14). However, this observation was not pursued until recently.

In investigating early steps in the differentiation of an EB to an RB, our labs reported in 2016 the novel finding that chlamydial organisms are highly polarized (59). The major outer membrane protein (MOMP) was localized as a cap on one side of the organism, whereas the cytosolic marker heat shock protein 60 (Hsp60) was localized at the opposite side interior to the lipopolysaccharide (LPS) outer membrane marker. Intriguingly, as the RB initiates its first division, we observed the clear formation and development of an asymmetric bud from the MOMP-enriched pole of the cell. As the nascent daughter cell grows, a new MOMP pole forms elsewhere on the progenitor mother cell membrane. The daughter cell eventually grows to equal size as the mother cell before scission occurs. At this stage, it resembles binary fission. Both live cell imaging and fixed cell imaging were performed to verify these findings. Importantly, in live cells our GFP-FtsQ marker localized to the pole that will give rise to the daughter cell before the

budding process began, localized as two spots on either side of the budding site for most of the process, and eventually coalesced to a single point during the late stage before scission. Short treatments with penicillin G caused bud formation to stall, suggesting that PG synthesis is important for bud outgrowth, potentially by allowing the recruitment of cell envelope machinery (e.g., phospholipid synthases) or by stabilizing the mother-bud structure so new phospholipids can be added to the bud. Indeed, we have recently noted specific functions for PBP2 and PBP3 in distinct steps of PG synthesis during the budding process (J. V. Cox, Y. M. AbdelRahman, and S. P. Ouellette, submitted for publication). Conversely, MreB inhibitors caused the budding process to collapse, again supporting the hypothesis that MreB acts as a central coordinator for division and PG synthesis in *Chlamydia* (34). Based on the findings of Abdelrahman et al., we have proposed that chlamydiae divide by an MreB-dependent polarized budding mechanism (59).

One of the key methodological approaches that allowed the visualization and capture of budding forms in fixed cells was the use of “gentler” fixation methods employing aldehyde cross-linkers, as opposed to the commonly used methanol fixation, which severely dehydrates and depolarizes cell membranes. Not surprisingly, there exists in the literature a variety of support for the budding model of chlamydial division from transmission electron micrographs (EM). EM samples are typically prepared with aldehyde fixation, and this preserves the budding forms. As mentioned previously, Matsumoto and Manire made note of a budding process during recovery from penicillin treatment, and a careful analysis of other images (e.g., their Fig. 1A) in their seminal report reveals budding forms during the normal developmental cycle (14). A careful examination of various reports throughout the decades of chlamydial research also shows evidence of budding forms (e.g., see references 11 and 59 to 64). Indeed, Gaylord also referenced buds but did not consider this when concluding that division occurs by binary fission (for example, see Fig. 9 in reference 58). Because the budding process eventually leads to a stage where the daughter cell has equal volume to the mother cell and resembles binary fission, it is easy to understand how budding forms were (and continue to be [see below]) inadvertently overlooked. However, our approaches relied on a combination of strategies, including live cell imaging with a GFP-tagged division protein, which cannot be discounted as a fixation artifact (59).

A recent article from the Tan lab has suggested that *Chlamydia* divides by binary fission (64). That *Chlamydia* might utilize two separate division mechanisms is seemingly incongruent with the reduced genome these bacteria possess. This would suggest that *Chlamydia* either possesses two sets of division apparatus or utilizes the same set in two different ways. The parsimonious hypothesis would favor one mode of division with one apparatus throughout their developmental cycle. The study from the Tan lab was based solely on EM analysis, where the authors measured RB volumes of the mother and daughter cell and concluded that their ratios were equal throughout the division process. However, budding forms were readily detectable in the images they presented (see, for example, Fig. 1a of reference 64), suggesting their analysis may have been skewed to favor the quantification of forms that resemble binary fission. This is not inconsistent with the budding process, as we noted that the end of the budding process produces forms that appear to result from binary fission. Recently, we performed the same type of analysis as Lee et al. on all RBs we imaged from several time points postinfection and observed budding cells that initially have small daughter and large mother cell volumes that give rise to forms resembling organisms undergoing binary fission (Cox et al., submitted). We would again stress that our approaches characterizing polarized budding relied on a variety of live and fixed cell imaging, including the use of genetically tagged cell division markers (59), whereas Lee et al. based their conclusions on a single assay while potentially discounting budding forms as artifacts (64).

Other support for a budding mechanism of division in *Chlamydia* comes from related organisms that also lack FtsZ, such as the *Planctomycetes*. *Gemmata obscuriglobus* is a planctomycete whose division process has been characterized. The Fuerst lab

showed clear live and fixed cell microscopy evidence that this bacterium buds as it produces a daughter cell (65). Importantly, at the terminal stages of the process, forms that resemble a binary fission mechanism can be visualized, and this is consistent with what we have observed for *Chlamydia* (59). Therefore, a budding process may be widespread among organisms that have evolved to rely on FtsZ-independent division mechanisms.

Conclusions. Chlamydial cell division remains an intriguing microbiological question. Recent advances in the development of tools for genetic manipulation of *Chlamydia* will allow the field to move beyond the basic observational studies of localization to mechanistic investigations of the division components and process. Outstanding questions remain. How is polarity established in the EB/RB? How is the division site selected? What are the temporal and hierarchical steps required to initiate budding, expand the daughter cell, and effect scission? How is the chlamydial membrane, devoid of PG, deposited into the daughter cell and how is force generated for membrane expansion into the daughter cell? How does MreB substitute for FtsZ when it is a highly conserved protein? In regards to the latter question, we recently demonstrated a unique N-terminal domain of chlamydial MreB that may allow it to associate with membranes with higher affinity (37). Future work in these areas will continue to be enlightening and further our knowledge of how FtsZ-independent polarized division occurs in these unique bacteria.

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