The Bacterial Actin Cytoskeleton

Actin-like proteins in bacteria form a cytoskeleton that helps to determine cell shape, much as happens in eukaryotic cells

Jeff Errington

Microbial shape, long used as a taxonomic criterion, is genetically determined. But how DNA sequence information is translated into morphology is a seminal question still without a complete answer for any microorganism. Recently, however, when investigators discovered that actin-like proteins are involved in determining bacterial cell shape, they revolutionized the field. This insight provides a simple and potentially unifying view: actin cytoskeletons shape bacterial cells, just as in eukaryotic cells.

If bacteria had no control over their own shape, they would all grow into simple spheres. In reality, bacteria grow into a wide range of shapes, including elongated rods or filaments, spirals, and subtler curves, while many cells also contain exotic appendages that may localize at the poles or in the middle of the cell (Fig. 1). Even roundness among cocci differs, with streptococci typically being more elongated than staphylococci.

The classic view is that the rigid external cell wall determines the shape of bacterial cells. Two lines of evidence strongly support this view. First, treatments that strip the intact cell wall from a bacterium produce an emptied sacculus that retains its shape, indicating that the wall itself contains information related to cell shape. Second, several genes implicated in controlling bacterial cell shape encode proteins that are involved in cell wall synthesis. However, attempts to identify chemical differences in walls from differently shaped bacterial cells have failed. Also, the synthetic enzymes responsible for wall synthesis are highly conserved, leaving it difficult to explain how this machinery by itself could generate so many different morphologies.

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Identifying Proteins That Determine Bacterial Cell Shape

Peptidoglycan (PG), the major component of eubacterial cell walls, is composed of long glycan strands that are cross-linked by short peptides containing amino acids in both the D isoform and the L isoform typically found in proteins. PG appears to be a single molecule covering the whole surface of the bacterial cell, and thus needs to be enlarged continuously as the cell grows. However, it also must retain its continuity at all times, to avoid lysis due to the high internal osmolarity of the bacterial cytosol. Thus, the hydrolysis of bonds to allow insertion of new material needs to be carefully managed.

The precursors for PG synthesis comprise a well-conserved family of disaccharide pentapeptides. These are made in the cytosol and then flipped to the outer leaflet of the lipid bilayer, where they are added to the PG meshwork by two distinct enzyme-catalyzed reactions. One of them, called transglycosylation, adds disaccharide units to extend the linear gly-

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can strand. In the other, transpeptidation, interstrand cross links form between juxtaposed peptide side chains.

These two processes are carried out by redundant enzymes, some of which possess both catalytic activities combined in a single protein. Transpeptidation is very important because it is the target of the β-lactam antibiotics. Indeed, the transpeptidases were first identified by their ability to bind penicillin and, hence, are frequently referred to as "penicillin-binding proteins" (PBPs). Most bacteria contain multiple PBPs. For example, according to their respective genome sequences, *Escherichia coli* has about 10 PBPs and *Bacillus subtilis*, about 16.

In general, the roles of specific PBPs are not well defined. However, a clue that particular PBPs could help to determine cell shape came from the finding that certain β-lactam antibiotics, such as mecillinam, can generate round cells. This and similar antibiotics have a high affinity for a particular PBP of *E. coli*, called PBP 2. Moreover, some mutations conferring increased resistance to mecillinam lie in the gene encoding PBP 2 (*pbpA*). These observations suggest that PBP 2 has a specialized role in determining the cylindrical shape of the cell.

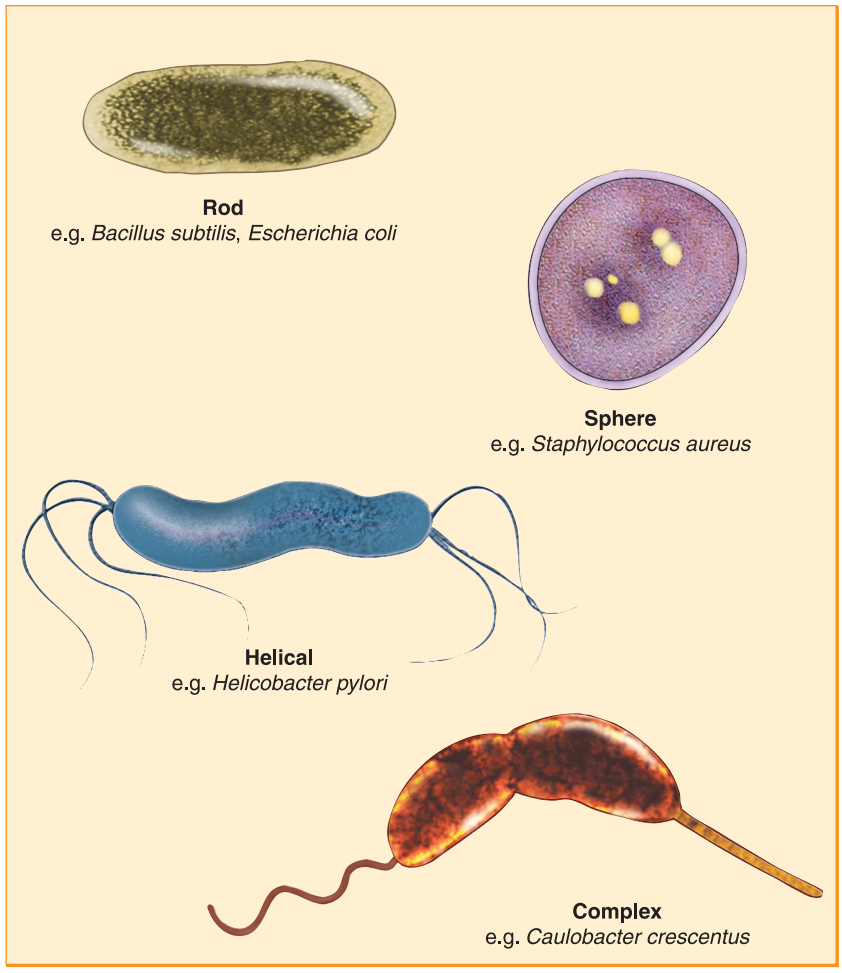
Other mutations affecting cell shape were identified directly by the loss of the normal rod shape (*rod* mutations). One such mutation, defining a gene called *rodA*, lies in a gene that is immediately adjacent to *pbpA* in *E. coli* (and also in many other microorganisms). RodA is a multiple transmembrane protein, whose function is almost certainly tied to that of PBP 2.

Meanwhile, *E. coli* as well as other kinds of bacteria encodes a closely related pair of specialized proteins, PBP 3 and FtsW, that function in cell division. Mutants in which PBP 3 or FtsW is altered, or cells that are treated with β-lactams that bind specifically to PBP 3, remain rod-shaped but fail to divide, producing elongated, filamentous cells.

In *E. coli*, the activities of these two dual-gene systems appear to alternate, giving rise to periods of growth when the RodA/PBP 2 system dominates, punctuated by cell division when the second FtsW/PBP 3 system comes into play. How the switch between these two dual-protein systems is made is not yet understood, but a possible regulator of the switch is encoded by the *bolA* gene.

Gram-positive bacteria contain another major polymer in addition to PG in their cell walls, called teichoic acid. This set of anionic polymers is essential for gram-positive bacterial cell viability, but their precise function is unknown. At least one gene, *tagF*, encoding a key enzyme in teichoic acid synthesis in *B. subtilis*, was identified by a *rod* mutation, *rodC*. The function of the teichoic acids will need to be understood at some level before their role in cell shape can be

FIGURE 1



Diversity of bacterial cell shapes.

Broad Interests—from Bird Plumage to Soccer, Singing, and Sporulation

In the mid-1990s, the drive in pharmaceutical companies to develop new antibiotics led many researchers in microbiology to begin looking for new bacterial targets and good assays for evaluating them. Jeff Errington jumped right in, filing several patents that cover methods to screen for molecules that interfere with the bacterial cell cycle.

Frustrated when major drug companies did not license those inventions, Errington started his own drug development company. Today Errington, who remains a professor at the Sir William Dunn School of Pathology at the University of Oxford in the United Kingdom, is proud of that company, which continues to grow. "Five years down the line, Prolysis Ltd, based just outside Oxford, is a thriving biotech company with several antibiotic programs and a bright future," he says. "It provides me with a ready outlet for ideas for new targets and approaches, and a fascinating interface with the world of commercial science."

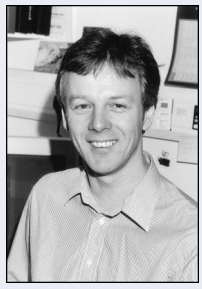
Not bad for this 47-year-old microbiologist, whose early interests in biology revolved around bird plumage. "I used to breed budgerigars, and they come in a range of color variants, most of which are inherited by straight Mendelian rules," he says. "A few of them are more complicated and exhibit features like incomplete dominance. They also highlight the fascinating problem of patterning in development. These [interests] got me reading science texts in my early teens, and are probably the main reason why I wanted a career in science research, and why genetics, development, and the cell cycle have been my main interests."

Besides an early love for exotic birds, Errington also developed a keen interest for soccer. "I have played soccer since early childhood," he says. "I probably was good enough to play semipro, but science got the priority. I still manage to play five-a-side once a week at a reasonable standard. I follow my hometown team, Newcastle United, for better or for worse— usually the latter."

Errington's father worked as a carpenter, and his mother was a homemaker who sang in the church choir and managed a trio of women who sang at local community centers and other small venues. "My poorly honed but enthusiastic musical skills are down to her—I enjoy karaoke when in the right mood and surroundings," he says.

Errington also likes to ski and snorkel–whenever a scientific meeting comes up in the right location—and uses house renovation to escape occasionally from doing experiments. His wife is an environmental health officer by training with expertise in microbiology and pollution, especially noise and air. She has devoted recent years to raising their two daughters, now 12 and 15, but recently began retraining to teach high school biology. Although Errington would like to see his daughters pursue careers in science, he says, "They're bright girls and we're inclined to trust them to find their own way. . . . My major regret is that they don't share my passion for soccer.

"I have a brother, 10 years older than me, who was an incredible mentor, with a wide range of self-taught scientific interests on both the physical and biological sciences," Errington continues. "He got me interested in math and taught me to play chess. He was a big influence and partly responsible for my getting the grades I needed to get to university."



After Errington earned his undergraduate degree in genetics and zoology, and his Ph.D. in microbial genetics, he began studying sporulation in *Bacillus subtilis* with Joel Mandelstam at Oxford during the early 1980s. "Understanding the molecular basis for spatial and temporal control of gene expression during development was a long-term project," he says, referring to bacterial sporulation. Some 15 years later, he and other researchers had identified more than 200 genes involved in this process and understand its main features—"at least in outline," he says.

"I turned my attention to other problems of central importance to biology, particularly the cell cycle and cell morphogenesis," Errington continues. "I realized that *B. subtilis* was an excellent experimental model for studying these processes because of its general experimental tractability and . . . sporulation. Moreover, powerful new cell biological methods needed to shed light on these problems had emerged."

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assessed properly, but for the moment, most activity is focused on the PG.

MreB as a Bacterial Homologue of Actin

Masaaki Wachi and his collaborators at the Department of Bioengineering, Tokyo Institute of Technology, Japan, identified *mreB* in *E. coli* as a target for mutations that confer resistance to mecillinam. Searches comparing the sequences of *mreB* genes show that they are conserved widely throughout the eubacterial lineage, although they are not ubiquitous. Nonetheless, among the 100 or so eubacteria whose genomes are sequenced, there is a striking correlation between nonspherical shape and the presence of at least one *mreB* homologue.

Bacteria with sequenced genomes can be divided into three broad taxonomic groups, with organisms from all three groups possessing *mreB* (Table 1). Presumably this gene appeared early during evolution and was present in a distant common ancestor of all bacteria. However, in various independent lineages, the gene has apparently been lost. In particular, in cocci, which apparently arose at least three times independently, the gene is always absent. In rod-shaped or spiral organisms, there is usually at least one *mreB* gene. And a few bacteria have more than one *mreB* homologue—for example, *B. subtilis* has three.

As is usually the case in biological systems, there are interesting exceptions. Thus, two distinct groups of rod-shaped bacteria, gram-negative organisms of the *Agrobacterium/Rhizobium* group and gram-positive bacteria of the *Mycobacterium/Corynebacterium* group, are rod shaped but do not contain *mreB* homologues (Table 1). Nevertheless, aside from these exceptions, this correlation points to an important role for MreB proteins in controlling bacterial cell shape. Sequence alignments of these proteins demonstrate that MreB is a highly conserved family and that the proteins are very uniform in size and probably in secondary structure. Several residues are absolutely conserved in the alignment.

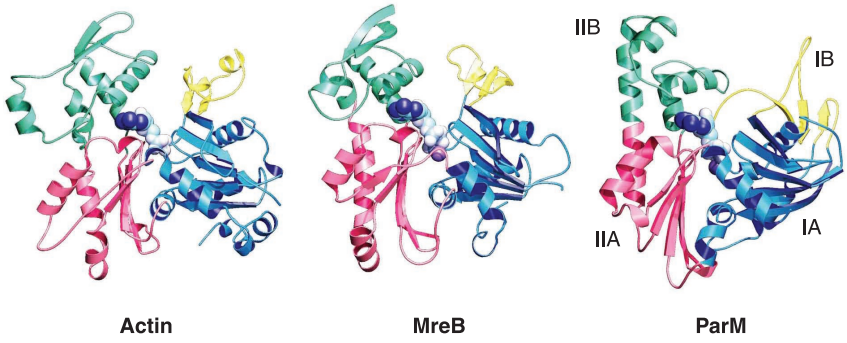
MreB and bacterial cell shape

Shape	MreB present (blue shading) or absent (no shading)		
	Gram positive (low G + C)	Gram negative	Other eubacteria
Rods	*Bacillus subtilis* *Clostridium actetobutylicum* *Listeria monocytogenes* *Streptomyces coelicolor* *Mycobacterium tuberculosis* *Corynebacterium glutamicum*	*Caulobacter crescentus* *Agrobacterium tumefaciens* *Sinorhizobium meliloti* *Escherichia coli* *Salmonella typhimurium* *Vibrio cholerae* *Haemophilus influenzae* *Pseudomonas aeruginosa*	*Thermotoga maritima* *Synechocystis* sp.
Spirals		*Campylobacter jejuni* *Helicobacter pylori*	*Borrelia burgdorferi* *Leptospira interrogans* *Treponema pallidum*
Cocci	*Lactococcus lactis* *Staphylococcus aureus* *Streptococcus pneumoniae*	*Neisseria meningitidis*	*Deinococcus radiodurans*
Other/unclear		*Rickettsia conorii*	*Chlamydia trachomatis* *Mycoplasma genitalium*

About 10 years ago Pier Bork and colleagues at EMBL in Heidelberg, Germany, drew attention to the fact that several of the conserved residues in actin are also present in MreB proteins, as well as in other members of the "actin superfamily." Two other actin superfamily members, DnaK and FtsA, are widespread in bacteria. However, these proteins are not direct functional equivalents of actin because they contain prominent domains that are not present in actin.

In contrast, MreB and another protein, Stb (now designated ParM), are similar in length to actin, and key conserved residues across the protein sequences can be aligned without introducing any major insertions or gaps. The small gaps that are present in the bacterial sequences tend to lie in surface-exposed loops and could probably be accommodated without significant changes in overall structure.

FIGURE 2



Comparison of the crystal structures of eukaryotic actin, and bacterial MreB and ParM proteins. The four subdomains of actin are shown in different colors and the same colors are used for the equivalent domains of the bacterial proteins. The conventional subdomain labels of actin are indicated against the ParM structure.

The question of structural similarity was settled in 2001 when Jan Löwe and colleagues at the MRC Laboratory of Molecular Biology in Cambridge, United Kingdom, solved the crystal structure of an MreB family member from the thermophilic eubacterium *Thermotoga maritima* and showed that it is congruent with that of eukaryotic actins (Fig. 2). Their structure contains an added bonus in that it provides some insights into the likely quaternary structure of actin, which had not been evident in the crystal structures of eukaryotic actins. Löwe and colleagues later solved the crystal structure of the ParM protein in both its ATP- and ADP-bound forms. These structures reveal a massive conformational change in the protein that could be very important in understanding the role of nucleotide hydrolysis in regulating polymerization, which may have implications for other actins.

The Biochemistry and Cell Biology of MreB Proteins

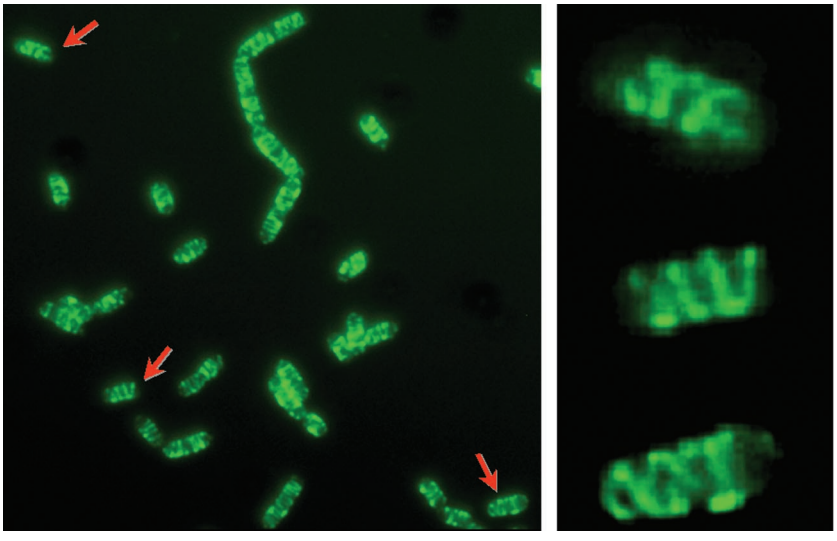
The biochemical properties of several MreB family members are being studied. These bacterial proteins appear not to require the complex chaperones that eukaryotic actins need for folding and activity. This requirement has seriously hampered studies of eukaryotic actins because it is difficult if not impossible to isolate the protein in an active state after it is produced in bacterial cells. The bacterial actins turn out not to suffer from this disadvantage and behave well in biochemical assays after being produced and purified as recombinant proteins from *E. coli*.

Although much remains to be done to characterize bacterial actin homologues, Löwe and his colleagues continue to study the *Thermotoga* MreB protein, while Kenn Gerdes and collaborators at Odense University in Denmark are studying the plasmid-encoded ParM, and my colleagues and I are studying the *B. subtilis* Mbl protein. Together, we find that these proteins carry out nucleotide binding and hydrolysis, and that they can polymerize into linear protofilaments, similar to eukaryotic actins.

In the case of ParM, however, the protein is not involved in controlling cell shape but rather in segregating plasmids. This finding raises the exciting possibility that ParM filaments form a track along which plasmid molecules segregate.

Fluorescence imaging of the MreB and Mbl proteins in *B. subtilis* and ParM in *E. coli* has shown that the proteins assemble into filamentous structures equivalent to actin filaments

FIGURE 3

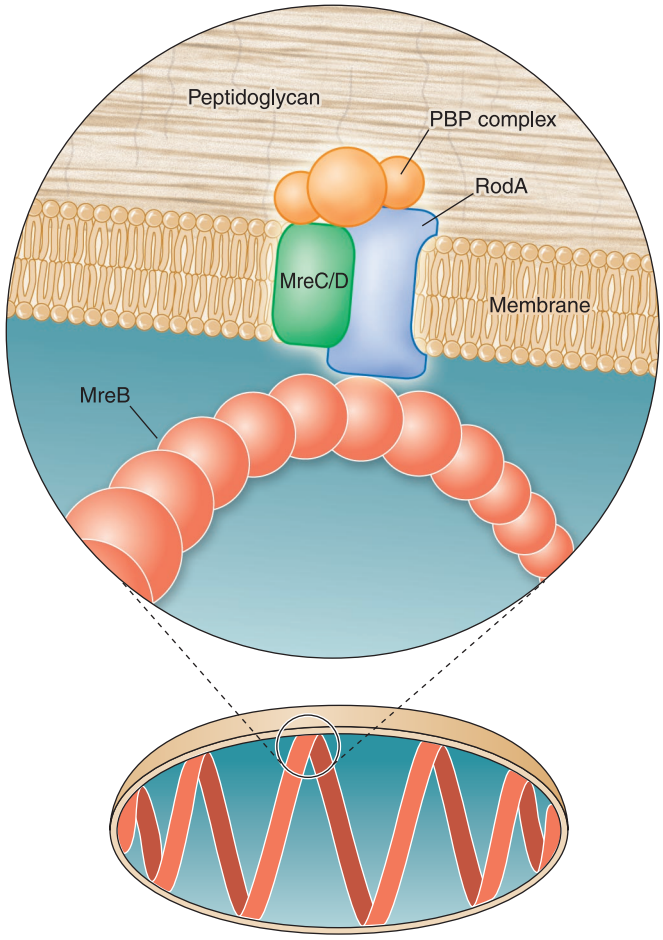


Helical cytoskeletal "cables" visualized by fluorescence microscopy of live cells of *B. subtilis* expressing a GFP fusion to the MreB homologue, Mbl. Three cells from the field (arrowed) are shown enlarged to the right.

in vivo. *B. subtilis* Mbl protein retains this function when fused to the green fluorescent protein (GFP), which provides a means of visualizing the behavior of Mbl in living cells. Mbl forms linear helical structures that run around the periphery of the cell, apparently just under the cell membrane (Fig. 3). These structures probably correspond to bundles of protofilaments and have been called "cables" by analogy to the actin cables of yeast cells.

Studies with the GFP-Mbl fusion protein show that these cables are dynamic structures that elongate and reconfigure continuously during the cell growth and division cycle. Fluorescence recovery after photobleaching (FRAP) studies reveal that Mbl cables continuously remodel, turning over in about 7 minutes. This matches the turnover rates measured for actin in various eukaryotic cell types, reinforcing the concept that the bacterial proteins are functional homologues of eukaryotic actins. The cables appear not to be highly rigid because they can be severed when the division septum forms. Moreover, when the cell wall is removed, they lose their helical shape.

FIGURE 4



Schematic view of how cytoskeletal filaments formed by MreB proteins could direct cell shape by localizing proteins in a "peptidoglycan factory."

Taken together, these results suggest that the helical configuration of Mbl cables results from an intrinsic curvature that is modulated by restraining forces exerted by the cell cylinder in which the helix develops.

How Do MreB Proteins Determine Cell Shape?

By analogy to models for the role of actin in yeast, MreB proteins likely play a direct role in controlling bacterial cell shape by influencing the pattern of cell wall synthesis. In principle, there are several ways in which control might be exerted.First, Mbl cables could form a rigid cage specifying the shape of the cell membrane from inside, enabling PBPs in the cell wall to make new PG according to this specified shape. This mechanism would seem unlikely if the cables form ephemeral structures and are being continuously remodelled.

Alternatively, cables could specify where cell wall synthesis takes place—in effect, localizing either the enzymes (such as the PBPs), critical synthetic steps, or the delivery of necessary precursors. If correct, Mbl cables would dictate the pattern of cell wall synthesis. Various studies on microorganisms such as *E. coli* and *B. subtilis* indicate that new material is incorporated relatively diffusely along nearly the entire cylindrical part of the cell wall. However, with novel staining methods that are based on use of a

fluorescent derivative of the antibiotic vancomycin, Richard Daniel in my lab found that *B. subtilis* cells incorporate new material in a helical pattern into their walls. Moreover, Mbl proteins determine this pattern.

MreB proteins appear not to have hydrophobic segments or amphipathic helices that would allow them to be inserted into membranes, although the cables appear to be located close to the cytoplasmic membrane of bacterial cells. Because PG synthesis occurs outside this membrane, other proteins probably are needed to coordinate or couple the cables to the membrane.

Right now the best candidates for such proteins seem to be encoded by *mreC* and *mreD*, which lie immediately downstream from *mreB* in many bacteria. MreC is predicted to have a single membrane span, with its major C-terminal domain located outside the cytoplasmic membrane. MreD is predicted to have multiple transmembrane spans. In *B. subtilis* (and probably also *E. coli*), mutations in *mreC* and *mreD* lead to a loss of rod shape, but how these proteins contribute to that change of shape is not known. In principle, PBPs could interact with MreB/Mbl, because they have a small N-terminal cytoplasmic domain. Moreover, RodA, whose function is not known, is also a candidate "coupling" protein.

A complex containing the MreB/Mbl proteins might determine the shape of bacterial cells during wall synthesis (see model, Fig. 4). Joachim Höltje and his collaborators at the Max-Planck Institute in Tubingen, Germany, find good evidence for the existence of such a multiprotein complex involving several PBPs that insert new material into the *E. coli* cell wall. According to our speculative model, an extracellular multi-PBP complex could act with the RodA, MreC, and MreD proteins, with this whole assembly spatially controlled by the MreB cables in the cytosol.

Possibly other proteins, such as those carrying out the later steps of PG precursor synthesis, are organized into a more complex "peptidoglycan factory"—which, by analogy to DNA replication and transcription, could contain a dozen or more protein components. Systematic protein localization studies now under way in several labs are beginning to provide support for this general concept.

ACKNOWLEDGMENTS

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