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Review Article

The evolution of spherical cell shape; progress and perspective

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Bacterial cell shape is a key trait governing the extracellular and intracellular factors of bacterial life. Rod-like cell shape appears to be original which implies that the cell wall, division, and rod-like shape came together in ancient bacteria and that the myriad of shapes observed in extant bacteria have evolved from this ancestral shape. In order to understand its evolution, we must first understand how this trait is actively maintained through the construction and maintenance of the peptidoglycan cell wall. The proteins that are primarily responsible for cell shape are therefore the elements of the bacterial cytoskeleton, principally FtsZ, MreB, and the penicillin-binding proteins. MreB is particularly relevant in the transition between rod-like and spherical cell shape as it is often (but not always) lost early in the process. Here we will highlight what is known of this particular transition in cell shape and how it affects fitness before giving a brief perspective on what will be required in order to progress the field of cell shape evolution from a purely mechanistic discipline to one that has the perspective to both propose and to test reasonable hypotheses regarding the ecological drivers of cell shape change.

Introduction

> "To be brutally honest, few people care that bacteria have different shapes. Which is a shame, because the bacteria seem to care very much." - Young, K.D. [1]

The shape of a bacterial cell is defined by the physical limits of the cell, but cell shape likewise imposes limits on many crucial bacterial processes. Motility, DNA segregation, nutrient acquisition, waste disposal, surface attachment, size, predation, and parasitism — these are all aspects of microbial life that are significantly affected by cell shape. It is remarkable then that after many hundreds of years of classifying and characterising bacteria according to shape, we have just started to approach the study of how and why cell shape evolves. If bacteria can be said to have something like a shared last common ancestor, the shape of that ancestral bacteria was most likely to have been a rod. We have hints, based on phylogenetic and phenotypic work on extant bacteria, as to how these rod-like predecessors might have become spherical, and we can put a lower limit on the number of times that this has happened independently. Here, we will summarise some recent findings on the evolution of spherical cell shape focusing on the molecular players and their roles. The key to understanding the evolution of spherical cell shape is an understanding of the bacterial cell envelope and the bacterial cytoskeleton, particularly FtsZ, the division determinant of both spherical and rod-like cells, and MreB which is the main cytoskeletal determinant of the rod-like shape in bacteria. We will also give a perspective on the study of cell shape going forward including experimental methods and ecological considerations required to move towards understanding not just how but why these significant transitions take place.

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Bacterial cell shapes today and in the ancient past

Bacteria are commonly classified as rods, curved cells, or spheres (cocci). Many of the best-known bacteria fall within these simple categories: *Escherichia coli* and *Bacillus subtilis* are simple rods; *Helicobacter pylori* is a curved cell; and *Staphylococcus aureus* is a typical coccus. Although this simple approach is a convenient way to describe commonly studied species, it is far from an accurate account of the true diversity of bacterial cell shapes. Bacteria can be oblongs or spheroids (*Pneumococcus*), star-shaped (*Stella*), or short curved rods (*Caulobacter* and *Vibrio*) [2]. Furthermore, bacteria can modify their shapes by making specialised structures, such as extensions that act as appendages in *Caulobacter* [3]; or by switching between different forms in response to changing environments as seen in *Mycoplasma* [4]. If we also consider variations in cell size, then the range of bacterial morphologies becomes even more staggering.

Insights from the early 20th century into the metabolic diversity among prokaryotes [5,6] laid the ground work for the use of genetic sequences for determining evolutionary relationships among bacteria [7,8]. Comparative analysis of genetic sequences was bolstered by the championing of the 16S rRNA, ideal for establishing phylogeny as it is widely distributed among different species, readily isolated in bacteria, and relatively resistant to mutation [9]. The use of 16s rRNA for establishing phylogeny led Woese & Fox [10] to the classification of all organisms within three domains: bacteria, archaea, and eukarya, now widely accepted as the basis for the three-domain system.

It was Fox and Siefert who showed that the deepest branches of a 16s rRNA phylogenetic tree of 180 bacterial species were made up of exclusively rod-shaped or filamentous species [11]. In this early work the authors noted that once a branch has adopted a spherical shape, it did not revert to a rod-like shape. This led Siefert and Fox [11] to propose the idea that the common ancestor of all bacteria were most likely rod-shaped, and that spherical cells evolved from these rods as an end-state morphology. Similar observations were presented earlier by Stackebrandt and Woese [12] and by Woese et al. [13]. Further evidence supporting the notion of a rod-shaped ancestor for bacteria is presented in Figure 1, which presents a new phylogenetic meta-analysis using the 16srRNA tree of 253 bacterial species, correlated with cell shape and the presence of *mreB* (by Blast AA ID of 48% and above and suitable length). The full table describing the names of these species is provided as Supplementary Table S1. Two salient points can be gleaned from such an analysis. The first of these is that according to this tree, of the 27 instances in which rod-like cells have become spherical (Figure 1 Red circles), 17 of these, or 63% correspond to a loss of *mreB* (summarised in Supplementary Table S2). This is likely to be an underestimate due to some uncertainty (Figure 1. Triangle). Secondly, this analysis confirms that spherical cells, once evolved from a rod-shaped ancestor, generally retain this shape as an end-state morphology as suggested by Siefert and Fox in 1988 [11]. One possible exception to this is shown in the *Deinococcus* lineage for which the majority of extant members are coccoid but in which *D. deserti* (Figure 1. Black branch and purple star) has regained rod-like shape. This reversion to ancestral shape is coincidental with the apparent gain of a single copy of the *mreB* gene into *D. deserti.* This raises the possibility that a gene transfer event has restored the lost copy to this lineage. It should be noted that a separate analysis which included additional extant *Deinococcus* species suggested an alternative hypothesis that loss had occurred multiple times independently, but failed to rule out a more parsimonious hypothesis involving gene gain in ancestral branches [14]. The dearth of successful transfer of *mreB* genes may reflect the difficulty in acquiring genes that are homologous enough to the ancestral MreB that was lost to perform in protein complexes that are critical to cell survival.

Rod-like shape appears to be ancestral to modern bacteria, but this need not imply that the primitive progenitors of bacteria were always rod-like. Errington and colleagues have suggested that cell wall-free, irregularly shaped cells called L-forms may have pre-dated rod-like cells [15]. One intriguing suggestion is that the first bacteria may have developed the rod-like shape in close coordination with the development of the peptidoglycan cell wall [15]. This would not necessitate that all bacteria sprung from this original rod-like progenitor, rather, that this combination of potent, fitness improving traits may have been transferred together amongst ancient prokaryotes.

Bacteria actively maintain cell shape through the construction of their peptidoglycan cell wall

The bacterial envelope is made up of three main parts: the cell membrane, the peptidoglycan layer, and an outer membrane in the case of Gram-negative organisms. Gram-positive cell walls are thick layers of peptidoglycan that contain teichoic and lipoteichoic acid components; whereas Gram-negative cell walls are made up

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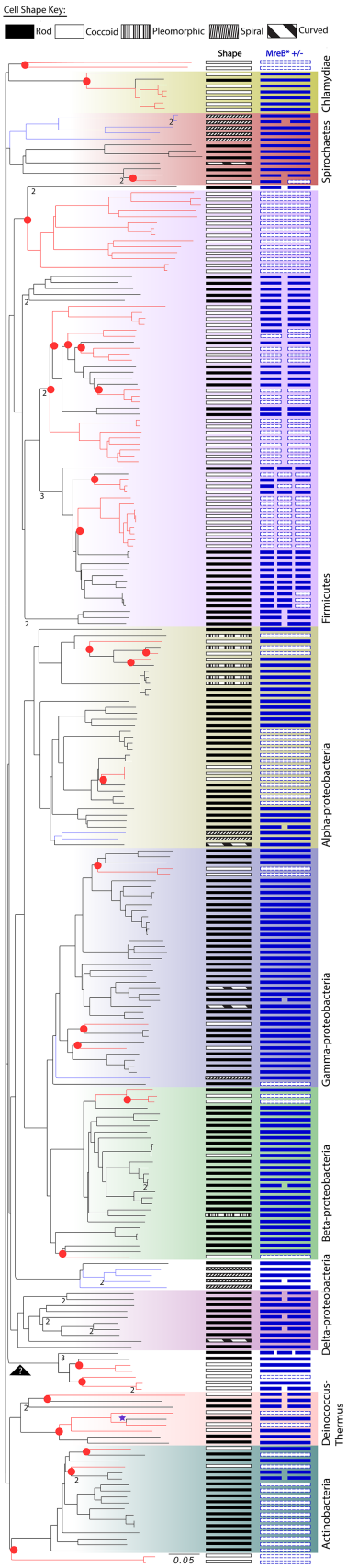

Figure 1. Cell shape change mapped onto a 16s rRNA tree for 253 completely sequenced bacterial species.

Cell shape is represented as follows: rod-like cell shapes as black bars; coccoid cell shapes as white bars with black borders; pleomorphic shapes as white bars with black vertical lines; spiral shapes as white bars with thin diagonal lines; and curved shapes as white bars with thick diagonal lines. Inferred MreB copy numbers are represented by blue bars, and MreB-loss/ absence is represented by white bars with blue broken lines. The branch points at which major changes in cell shape are inferred to have taken place are marked with red circles. Black branches are indicated probable rod-like progenitors, red branches indicate spherical progenitors; and blue branches indicate helical ancestral types. In the case of *Deinoccocus deserti*, a transition from a spherical to a rod-like shape is noted by the change in branch colour from red to black (described in text). This change in shape is coincidental with the presence of MreB, probably due to a gene transfer event, which is represented by a purple star. Points at which the copy number of MreB likely increased based on a parsimony analysis of the extant lineages are shown as small black numbers in the phylogenetic tree. The authors are agnostic as to whether these events were duplications or gene gain events. The authors have used parsimony to infer MreB genes that are absent (white boxes) in cases where the shared common ancestor of a set of closely related species is inferred to have had multiple copies of MreB.

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of a thin layer of peptidoglycan inserted between an outer and inner membrane. In either case, it is the peptidoglycan layer which provides the mechanical basis of cell shape.

Bacterial cell shape is a characteristic attributed to the structure of peptidoglycan, and to the action of the enzymes that continually build and remodel it [16]. Most bacteria have a peptidoglycan-based cell wall that provides shape and protection against osmotic pressure. This integral structure is composed of glycan chains linked together by peptide bridges, which together make up peptidoglycan. The basic glycan chains are composed of two precursors: N-acetylglucosamine (GlcNAc) and N-acetylmuramic acid (MurNAc). These are connected by a β-1,4 glycosidic bond which form long alternating strands of GlcNAc and MurNAc (Figure 2A). Each MurNAc is connected to a short peptide chain that extends from it at a right angle. These peptide chains are cross-linked to other peptide chains from flanking glycan strands through the action of transpeptidases [17] (Figure 2B). Together, these components form a strong, often multi-layered mesh that provides structure and protects against osmotic lysis and demarcates the boundary of the cell [18].

The peptidoglycan cell wall can be removed from cells and yet retain the native shape of the living cell [19,20]. The peptidoglycan in a growing cell is flexible and is dynamically changing over time. Using low-angle laser light scattering, the cell wall of *E. coli* was observed to have the ability to expand up to 300% when affected by physical stress [21]. This observation is supported by Yao et al. [22] who used atomic force microscopy to provide direct physical evidence of peptidoglycan elasticity; and by Boulbitch, Quinn and Pink [23] who derived equations that theoretically demonstrate the elasticity of peptidoglycan. In *E. coli* and *B. subtilis*, cell wall disruption and loss in the presence of certain osmoprotectants result in the formation of spheroplasts — round, osmotically sensitive cells [24]. Some bacteria also produce, or can be induced to produce, natural spheroplast variants which are also sensitive to osmotic effects and can provide resistance to antibiotics that

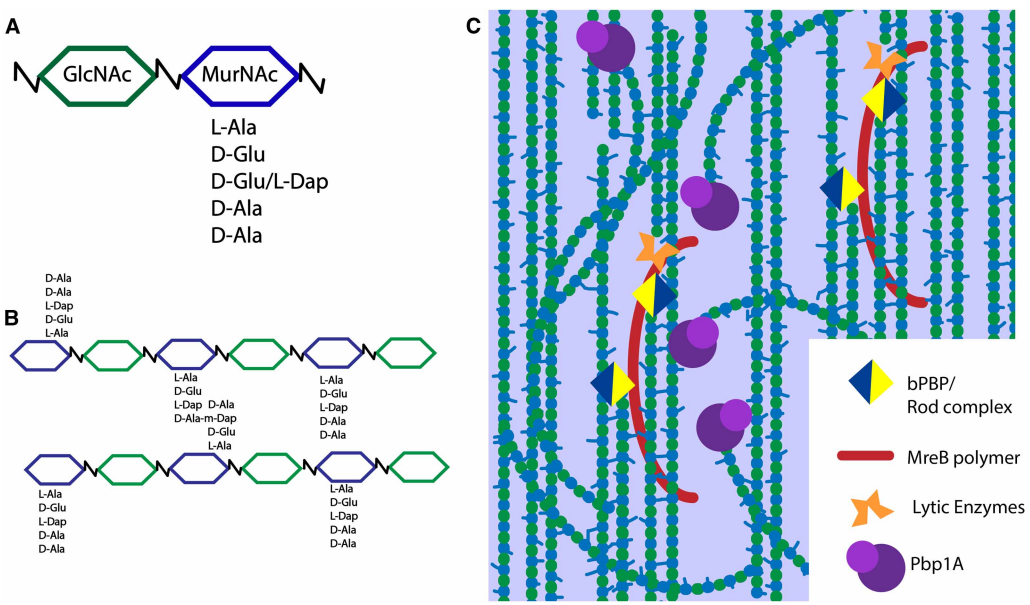

Figure 2. Peptidoglycan and the construction of the bacterial cell wall.

(**A**) The basic peptidoglycan subunits GlcNAc and MurNAc. (**B**) A representation of a single layer of the peptidoglycan mesh showing alternating GlcNAc and MurNAc as the base unit of the glycan chains linked by pentapeptide cross-bridges (adapted from Cava and Pedro [27]). (**C**) The 'Break before Make' model of peptidoglycan synthesis. MreB (curved red bars) associated endopeptidases and lytic enzymes (orange stars), cleave the cross-links in mature PG. The Rod complex generates a PG template that is cross-linked to the cell wall by Class B PBPs (blue and yellow box) making ordered, circumferential glycan strands. Additional, disordered strands are generated by Class A PBPs (purple circles) cross-link new peptidoglycan on one side with mature peptidoglycan on the other (disordered glycan strands are shown as curved as by Dion et al. [28]). The interaction of Class A PBPs with the Rod/MreB complex is currently undefined. Cross-linked pentapeptides are formed when a new peptidoglycan strand containing a pentapeptide is cross-linked with another one (blue branches).

target the cell wall [25]. These show that the cell wall is flexible but strong, and can withstand internal pressures to maintain cell shape whilst conferring a protective function against osmotic stress [26].

Peptidoglycan synthesis

The major players involved in cell wall construction have been established from studies using the model organisms *E. coli* and *B. subtilis*. Peptidoglycan construction begins with the synthesis of the precursor UDP-NAM-pentapeptide by the Mur proteins in the cytoplasm [29]. This is ligated to a carrier lipid by the membrane-associated enzyme MraY, generating lipid I, which is then ligated to a GlcNAc residue generating lipid II [30]. Following this, lipid II is translocated or flipped from the cytoplasm to the outer face of the membrane for integration into the existing peptidoglycan network [29]. Upon translocation, lipid II is polymerised into glycan strands via a transglycosylation reaction, which are subsequently cross-linked to other glycan strands via a transpeptidation reaction [31].

The final steps of assembly and modification are performed by enzymes called Penicillin Binding Proteins (PBPs). PBPs derive their name from their affinity to penicillins or beta-lactam antibiotics [32,33]. PBPs are classified into two categories: high molecular mass (HMM) PBPs, and the low molecular mass (LMM) PBPs. HMM PBPs have two major domains that are responsible for transpeptidation and transglycosylation activities that contribute to the addition of new peptidoglycan strands into the pre-existing cell wall [34–37]. HMM PBPs can be further classified as either Class A or Class B PBPs, depending on the structure and function of their N-terminal domain. The N-terminal domain of Class A PBPs have glycosyltransferase activity, elongating uncross-linked glycan chains. In Class B PBPs, the N-terminal domain plays a role in interacting with other proteins involved in the cell cycle [38–40]. In both types of PBPs, the C-terminal penicillin-binding domain performs transpeptidation, which cross-links adjacent glycan chains.

Monofunctional enzymes (MGTs) that have glycosyltransferase domains similar to those in class A PBPs have also been identified, but their exact function is unknown [41]. LMM PBPs (also known as Class C PBPs) are MGTs involved in a range of important functions including cell separation, peptidoglycan cross-linking, peptidoglycan maturation and recycling. For example, LMM PBPs in *E. coli* such as PBP5, PBP6, and PBP6b make stem peptides unavailable for cross-linking by cleaving the last D-alanine of stem pentapeptides [42,43]. Other examples include PBPs such as PBP4 and PBP7 that perform endopeptidase activities by cleaving cross-links between PG strands [44,45].

Recent advances have prompted a rethinking of the behaviour of some of the major enzymes involved in peptidoglycan synthesis. Transglycosylation reactions were historically attributed to the function of only Class A PBPs [41]. However, this view was challenged by experiments in *B. subtilis* and *Enterococcus* spp. which showed that cells can grow (although poorly) in the absence of all Class A PBPs, suggesting the existence of other enzymes that perform transglycosylation reactions [46–48]. For example, it is now known that RodA has transglycosylation activity [49], and that growth defects of a *B. subtilis* strain lacking all Class A PBPs can be rescued by the overexpression of RodA [49,50]. RodA was also demonstrated to have transglycosylation activity in *E. coli* [51]. FtsW, was also observed to have transglycosylation activity when in complex with its cognate Class B PBP [52].

Until recently, the most widely accepted model of cell wall construction posited that MreB guides the transglycosylation reactions of Class A PBPs, as well as the transpeptidation reactions of both Class A and B PBPs, directing the pattern of peptidoglycan polymerisation and assembly [43]. MreB is the ancient actin structural homologue in prokaryotes. It is a cytoskeletal element found in most rod-shaped bacteria, which performs a critical role in directional peptidoglycan assembly during cell elongation [53]. However, Cho et al. [51] recently discovered that MreB and Class A PBPs operate in independent complexes and do not form distinct assemblies as previously believed. It was discovered that Class A PBPs had both a fast and diffusive motion, as well as a much slower movement speed [54,55]. This was interpreted by Zhao et al. [54] as short periods of fast diffusion spaced apart by temporary pauses. In addition, MreB strongly interacts with Rod proteins in *E. coli*, which in turn interacts with PBP2, a Class B PBP [56]. This complex (MreB-RodAZ-bPBP) is thus referred to as the 'Rod complex' [54] (Figure 2C Blue and Yellow Box, Red bar).

These recent discoveries led Zhao et al. [54] to propose a new scheme of peptidoglycan synthesis called the 'Break before Make' model (Figure 2C). In this model, endopeptidases or lytic enzymes first cleave cross-links in mature peptidoglycan. From this break, the Rod complex generates a new peptidoglycan template which is cross-linked to the existing cell wall through the action of Class B PBPs. Class A PBPs generate additional strands that are cross-linked with the existing cell wall on one side, and with the peptidoglycan strand generated

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by the Rod-complex on the other. More recently, the interaction between Class A PBPs and the Rod-complex has been proposed to be balancing the tendency to reduce width in the case of the Rod-complex, and to increase width through the less-ordered addition of glycan strands by Class A PBPs (curved glycan strands Figure 2C). The data supporting the latter were established in *B. subtilis*, but many of the results for cell shape in simple rods appear to be generalisable [28]. This model reconciles previous models of peptidoglycan synthesis with current discoveries and gives us a better understanding of the general pattern of cell wall construction in bacteria.

The bacterial cytoskeleton

To spatially organise and direct key cellular processes such as cell division, chromosome segregation, and intracellular transport, eukaryotic cells use cytoskeletal elements composed of three main structures: tubulin microfilaments, intermediate filaments (IF), and actin microfilaments. Until the 1990s, the cytoskeleton was believed to have existed only in eukaryotes. Bacteria were simply thought to be unorganised bags with components randomly moving around inside [57]. However, we now know that bacteria do indeed form highly organised internal structures that are co-ordinated by the action of bacterial cytoskeletal elements [57,58]. Bacterial homologues of the three eukaryotic cytoskeletal elements are now known to exist in bacteria [59], and understanding how these function independently is important for understanding how they might function when one is disrupted.

Tubulin is a dynamic, GTP-dependent microfilament that function as a track for motor proteins in eukaryotes [60]. The most well-known homologue of tubulin in bacteria is FtsZ, a highly conserved protein in free-living bacteria. FtsZ forms a contractile structure called the Z-ring that is composed of long filaments that use GTP hydrolysis to bend and pull the membrane inwards for septum formation [61,62]. Importantly, it also acts as a scaffold for other proteins that are necessary for cell division [63]. Other tubulin homologues in bacteria include TubZ in *Bacillus thuringiensis* [64], and RepX in *B. subtilis* which may have plasmid-partitioning functions [59]; and BtubA and BtubB in *Prosthecobacter*, whose functions are still unknown [57].

IF proteins form strong rods or fibres of dimeric α-helical coils that resist mechanical stresses in eukaryotic cells [65]. In bacteria, probably the best example of an IF-like protein is crescentin. Similar to eukaryotic IFs, crescentin has a coiled-coil structure and self-assembles in a nucleotide-independent manner [60]. It is responsible for the bent-shape of *Caulobacter crescentus*. The deletion of crescentin causes *C. crescentus* to lose its characteristic shape and become a straight rod [57]. Other examples of IF-like proteins have been found in bacteria, but their functions are not well understood. Examples include RsmP from *Corynebacterium glutamicum* and Ccrp from *Bdellovibrio bacteriovorus* [57]. Similar to crescentin, FilP from *Streptomyces coelicolor* also has a coiled-coil structure that has been suggested to play a role in hyphal formation [59].

Actin-like proteins are present in all domains of life [66]. Actin-like proteins have a characteristic structure of four distinct domains stabilised by an ADP molecule. These proteins polymerise in the presence of ATP, and form either globular (G-actin) or filamentous (F-actin) structures [57]. In eukaryotes, actin is known to polymerise and undergo a treadmilling action wherein monomers are added to one end of the filament and removed from the opposite end [67]. Similar characteristics are seen in bacterial actin homologues like MamK, ParM, and other plasmid-segregating homologues [68]. MamK forms filaments that organise magnetic vesicles, or magnetosomes, in *Magnetospirillum magneticum* [69,70]. ParM is a plasmid-encoded actin-like protein involved in plasmid partitioning [59].

In contrast, MreB, the major actin homologue responsible for determining rod-like shape in bacteria, has been demonstrated to have no intrinsic polarity and does not undergo treadmilling [71–73], forming instead antiparallel double filaments. MreB is a highly conserved protein among most rod-shaped bacteria [74] and has a very similar structure to eukaryotic actin [75]. Using a theoretical model, Lan et al. [76] proposed that MreB modifies newly synthesised PG strands by pre-stretching them prior to cell wall insertion. Evidence showing that MreB has a direct influence on cell integrity was later provided by S. Wang et al. [77] who showed using an optical trap experiment that in *E. coli*, ∼50% of cell rigidity comes from MreB itself, showing that this actin-like protein contributes as much to mechanical integrity as the cell wall. In contrast with its role in providing bending stiffness, MreB does not provide longitudinal stiffness to cells [78].

MreB contributes to rod-like cell shape

The formation and maintenance of the rod shape is conferred by the Mre proteins, MreB, MreC, and MreD [79]; RodZ [80,81]; and the RodA-PBP2 pair [51]. This complex, collectively called the Rod complex (or

elongasome) is well-conserved in rod-shaped bacteria. The complete Rod complex is typically not found in cocci, though some members such as MreCD, RodZ or RodA/bPBP may be present [80,82]. In addition, disruption of MreB polymerisation by the chemical agent A22 leads to spherical cell shape. For this reason, the Rod complex is considered as the major determinant of the rod-like shape in bacteria [74,83]. The spatial coordination of the Rod complex is conferred by MreB [74,75]. The loss or depolymerisation of MreB causes deformities in rod-shaped cells which ultimately grow as spheres in its absence [74,81,84].

Recent studies suggest that MreB forms short filaments that move beneath the inner membrane and travel around the rod-like cell [71,85–87]. These data were used by Errington [53] to propose a revised model for MreB under the assumption that MreB does indeed form filaments that migrate with peptidoglycan strand insertion, but with greater emphasis on the orientation of the filaments relative to cell shape. In this model, MreB filaments co-ordinate PG synthesis in a snake-like manner therefore driving the elongation of the smooth cylinder.

More recently, the laboratory of Ethan Garner presented an updated view of how MreB filaments orient to form and maintain the rod shape in bacteria [88]. Using total internal reflection microscopy, Hussain et al. [88] demonstrated that MreB filaments are able to sense the shape of bacteria, orienting along surfaces with the greatest negative curvature (Figure 3 Pink arrows). This allows MreB filaments to find the correct orientation and move around the circumference of rod-shaped cells. In contrast, these MreB filaments move in all directions in bacteria that were made spherical. The group concludes that MreB thus creates the rod shape by directing cell wall synthesis by sensing and reinforcing differences in cell curvature.

Aside from coordinating cell elongation, MreB is also known to interact with FtsZ for correct septum synthesis, thereby linking it to the cell division machinery [89] (Figure 3.3 grey arrows). To understand this relationship, it is necessary to first understand the cell division machinery, which is discussed below.

Cell division in model organisms such as *E. coli* and *B. subtilis* begins with the polymerisation of FtsZ, forming a contractile Z-ring at the centre of the cell via a treadmilling-action [72]. There, it co-ordinates with other proteins to initiate and guide cell division [90–92]. Z-ring formation is a tightly-co-ordinated process and ∼70% of FtsZ localises into helical patterns at any given time [93,94] as a result of restricted diffusion [95]. These patches are dynamic, migrating continuously throughout the cell, searching for new septation sites [96]. Aside from its role as a cell division coordinator, FtsZ also provides a contractile force that bends the inner membrane [61]. This bending is initially resisted by the rigid cell wall but is eventually overcome by the recruitment of proteins that remodel the cell wall [61].

In line with this, Fenton and Gerdes [89] demonstrated that MreB is recruited to the Z-ring and interacts with FtsZ in *E. coli* for the transfer of cell-wall biosynthetic enzymes from lateral growth activity to septal peptidoglycan synthesis. Interestingly, they showed that the recruitment of a mutated form of MreB into the Z-ring results in elongated cells that are unable to divide, having Z-rings that have no PG synthesis activity. Furthermore, they observed that PBP2 and PBP1B that are normally present in the septum of dividing WT *E. coli* cells (in addition to their localisation to MreB) are not seen in cells that had the mutated form of MreB. In their model, Fenton and Gerdes [89] propose that the recruitment of PBP2 and PBP1B from the elongasome to the divisome, through the action of MreB, plays an essential role in septal and/ or pre-septal PG synthesis. Thus, this shows that in rod-shaped bacteria, MreB is not only important to cell elongation, but to cell division as well.

Loss of MreB or MreB-like proteins lead to shape defects and cell death

Although MreB is regarded as the main rod-shape determining gene, it is not the only protein performing this function. In *B. subtilis*, two additional MreB homologues have been found — these are MreBH and Mbl. The deletion of each of these proteins produces slightly different effects, but in general, these lead to deleterious cell shape defects that are only viable upon the addition of supplemental magnesium [97–99]. In *Spiroplasma* and *Haloplasma*, even more (five to seven) MreB homologues have been found. The exact function of these homologues is unknown, but phylogenetic evidence led Ku et al. [100] to conclude that these different MreB homologues are the result of independent ancient duplications. In bacteria that have only one MreB homologue, such as *E. coli* [101] and *C. crescentus* [102], MreB is essential. The loss of MreB results in cell death in rod-shaped model bacteria such as *E. coli* [103], *C. crescentus* [104], and *Pseudomonas aeruginosa* [105], that are grown in standard culture media. The loss or deletion of MreB in these organisms causes a loss in shape, ultimately leading to cell death.

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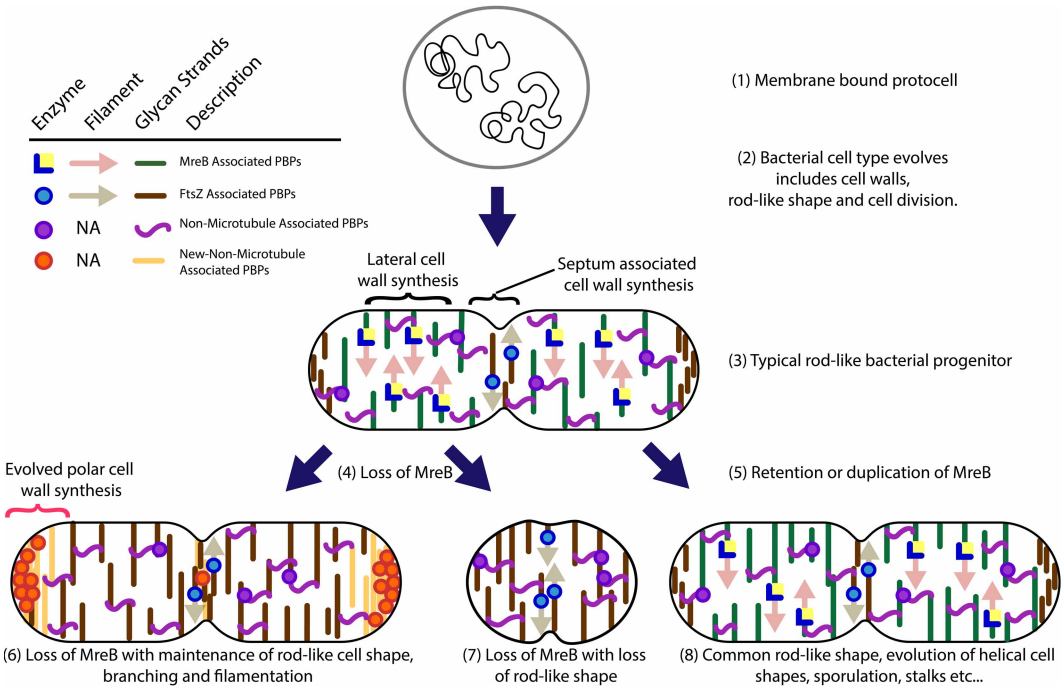

Figure 3. A rough model of the evolution of filament guided and non-filament guided PBPs that shape the cell wall.
(1) Before the cell wall was an evolved feature of bacterial cells, bacterial progenitors may have existed as amorphous, membrane-bound DNA bearing protocells. (2) The bacterial cell, as we know it, comes into existence through the combination of organised cell division, the cell wall and rod-like shape in some progenitor cell. (3) The crystallisation of the typical bacterial cell includes the ancient actin homologue, MreB (pink arrows) the tubulin homologue, FtsZ (grey arrows) and their associated glycan strands (green and brown) along with their associated PBPs (blue and purple circles, blue and yellow squares). (4) In some lineages MreB is lost. The majority of these lineages become spherical and cell wall synthesis at the septum dominates in these cells (7). Some lineages regain or retain rod-like shape through the evolution of polar cell wall synthesis mediated by newly evolved polar-PBPs (orange circles). (8) Rod-like lineages can also evolve new cell morphologies including helical shapes, sporulation, stalks etc.

MreB loss appears to be an early step to evolving spherical cell shape

Phylogenetic analysis has shown that spherical-shaped bacteria arose from rod-shaped precursors multiple times during the course of evolution, probably due to a loss of genes [11]. Consistent with this, rod-shaped bacteria can be made to have a spherical morphology through the loss or perturbation of MreBCD [106]. All three Mre proteins are important for maintaining the rod-shape in bacteria — loss of which leads to the formation of a spherical cell shape [107]. However, MreB is particularly interesting and important because aside from maintaining the rod-like shape, it is also absent from most spherical bacteria, unlike MreC and MreD which are present in extant spherical species such as *Streptococcus pneumoniae* and *Staphylococcus aureus* [108,109].

In Neisseria, loss of the protein YacF is similarly associated with the transition in shape from being rod-like to spherical. The loss of YacF, a coordinator of cell elongation and division that is present in many bacteria, was shown to precede MreB-loss in this group of bacteria [110]. Together with MreB, Veyrier et al. [110] identify YacF to be another important protein involved in the evolution of spherical bacteria.

In the long-term evolution experiment conducted in *E. coli* by Lenski and Travisano many of the cells have become larger by increasing their cell width and reducing their cell length, giving them a more 'round' appearance, over the course of tens of thousands of generations [111]. Ultimately, amino acid changing mutations in the MreB gene that is essential in *E. coli*, were demonstrated to be responsible for these cell shape changes in this experiment, reinforcing the central importance of the MreB gene in cell morphology change during evolution [112].

Notable exceptions to the loss of MreB leading to a spherical cell shape is found in Actinobacteria (*Streptomycetes* and the *Mycobacteria*) and Rhizobiacae (*Bradyrhizobium* and *Agrobacterium*). These lineages

lost MreB very early on but have maintained a rod-like shape [113]. Using fluorescent cell wall-specific antibiotics and D-amino acids, it was found that these bacteria evolved the ability to retain their rod-like shape by generating new cell wall material specifically at the cell poles, instead of following the ancestral pattern of lateral cell wall growth [114,115].

Following the loss of important shape-determining genes such as MreB, bacteria are able to persist and grow as spheres by directing cell wall synthesis and therefore growth at or near the septum [116]. Spherical bacteria were previously believed to grow either as a perfect sphere via exclusive septal growth, as in the case of *S. aureus*, or as an elongated ellipsoid (ovococcus) through slight elongation around the septal region, as in the case of *S. pneumoniae* [116]. However, it is now known that even *S. aureus* still undergoes some elongation through the action RodA–PBP3, which are elongasome-associated proteins. Reichmann et al. [117] demonstrated that the loss of RodA–PBP3 results in even more spherical *S. aureus* cells.

In the case of spherical bacteria, it is noteworthy that some species can temporarily revert to a rod-like shape in response to specific environments. As an example, the ovococcus species *Lactococcus lactis* and *Streptococcus salivarius* are able to form rod-like cells in synthetic media by modifying their septation activity [118]. Pérez-Núñez et al. [118] propose that these are survival strategies common to ovococci.

What has shape got to do with bacterial fitness?

The maintenance and modulation of characteristic cell shapes in bacteria imply that it is an actively regulated physical property that has selective value. Young [119] argues that bacteria use cell shape to improve survival in the face of different selection pressures, the most basic of which being nutrient acquisition. Although variables like nutrient gradients and the ability to move towards food sources can influence a cell's ability to find nutrients, it is ultimately diffusion that delivers these nutrients from the external environment into the cell [120]. This imposes a limit on how large a bacterial cell can become since it would need a large surface-to-volume ratio to support its needs. Cell shape affects diffusion because a spherical shape would have the lowest surface-to-volume ratio compared with other non-spherical cell shapes. This is the reason why many bacteria are believed to have rod-shaped, filamentous, or curved cells [120].

Another factor that can be strongly influenced by shape is motility. Mitchell [121] demonstrated that energy costs for active movement can vary by as much as 1×10^5 ergs (a measure of energy) in cells that change in shape or size at sub-micron scales. The effect of shape on motility is further illustrated in *E. coli* cells that move more quickly compared with a filamenting phenotype [122] or swim in a different direction when shape is mechanically altered [123]. Different shapes have also been hypothesised to allow bacteria to swim better in various environments. An interesting example is *Helicobacter pylori*, a human pathogen with a characteristic corkscrew appearance. It has been shown that *H. pylori* uses its shape to travel through the thick mucus layer of the stomach epithelium to allow colonisation, and that loss of this shape actually reduces their ability to colonise the stomach [124]. In contrast with these findings, shape and motility may not always be coupled. El Baidouri et al. [125] showed that cell shape and motility do not necessarily evolve together in all bacteria. In a comparative study of 325 Firmicute species, the authors found no association between shape and motility, proposing that the independent evolution of shape and motility might allow greater evolutionary flexibility. The relationship between shape and motility may not be universal, but there is clearly a correlation between these characteristics in many bacteria.

Cell shape can also influence survival by affecting other selective pressures such as predation, surface attachment, and passive dispersal [1,3]. In aqueous environments, bacterial predators such as heterotrophic nanoflagellates are estimated to graze between 25% to 100% of phytoplankton including bacteria in a single day [126,127], imposing a substantial selective pressure on bacteria. It is estimated that ~50% of bacterial mortality in open oceans can be attributed to grazing by protists [128]. To survive, bacteria exhibit morphological plasticity that helps them evade predation [129]. Bacterial capture can be affected by irregularities in size and shape — filamentous bacteria can be too large for ingestion, exceptionally tiny cells may escape capture more easily, and the formation of strong surface attachments and biofilms may help reduce predation pressure [129,130]. In deep aquifers, most bacteria that are recovered are cocci or coccoid rods [131]. Using artificial geological media and gravity filtration, Weiss et al. [131] demonstrated that smaller, coccoid cells are able to move more rapidly through geological strata than rod-shaped cells, suggesting a potential benefit to rod-shaped cells that evolve to become spherical. See the review by Yang et al. [3] for a more extensive discussion of this topic.

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An intriguing model was recently proposed by Smith et al. [132]. Modelling of bacteria in simple biofilms suggested that rod-like cells were better at colonising solid interfaces and that in such circumstances, spherical cells would be propelled to the top of biofilms. This was borne out in the laboratory on solid media. Perhaps cell shape evolution has been affected by the competition to be at the upper surface of biofilms in some circumstances [132]. Further experimental approaches into the adaptive or non-adaptive nature of this evolutionary transition in the future will help us to understand an aspect of bacterial evolution that has occurred many times independently and in a range of microbial environments.

Perspectives

- **Highlight the importance of the field:** Exploring the molecular and evolutionary underpinnings of cell shape is key to progressing both the fundamental and the applied disciplines of microbiology. Although shape was among the first observations we made regarding these invisible 'animalcules' it is still not completely understood. Shape is a critical characteristic that determines many facets of bacterial existence and as such, the enzymes or scaffolding proteins that dictate shape are excellent targets for selecting and designing antimicrobials.

- **Summarise current thinking:** Cell shape is a highly adaptive trait of bacteria that is actively maintained and tuned throughout the cell cycle and over evolutionary time. Phylogenetic parsimony suggests that ancient bacteria were rod-like, and that spherical cell shape is the result of gene loss events. Shape evolves either through the loss, duplication, or gain of cytoskeleton proteins or PBPs that build the peptidoglycan cell wall. The tools for analysing how cell shape changes over evolutionary time are well in hand; comparative genomics, fluorescent antibiotics, and fluorescent D-amino acids along with a host of well-honed microbial cell biology tools have brought us to the point of understanding the complex inner workings of bacterial cells.

- **Comment on future directions:** In order to dissect why cell shape changes over evolutionary time scales we will need to explore the environmental and ecological pressures that bacteria experience. This is extremely challenging and requires interdisciplinary approaches that bridge the fields of cell biology, chemistry, ecology and evolution. Ultimately in order to understand a topic such as the evolution of cell shape, novel methods for measuring fitness in ecologically relevant frameworks will have to be developed. A natural history of cell shape requires experimentalists to bring the aforementioned disciplines to bear on expanding and testing our hypotheses regarding the causes and consequences of cellular morphology.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

Abbreviations

HHH, high molecular mass; LMM, low molecular mass; MGTs, Monofunctional enzymes; PBPs, penicillin binding proteins.

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