[Frontiers in Bioscience S4, 1582-1606, June 1, 2012]

The actin-like MreB proteins in Bacillus subtilis: a new turn

Arnaud Chastanet[1,2], Rut Carballido-Lopez[1,2]

[1]INRA, UMR1319 Micalis, F-78352 Jouy-en-Josas, France, [2]AgroParisTech, UMR Micalis, F-78350 Jouy-en-Josas, France

TABLE OF CONTENTS

1. ABSTRACT

A decade ago, two breakthrough descriptions were reported: 1) the first helix-like protein localization pattern of MreB and its paralog Mbl in *Bacillus subtilis* and 2) the crystal structure of *Thermotoga maritima* MreB1, which was remarkably similar to that of actin. These discoveries strongly stimulated the field of bacterial development, leading to the identification of many new cytoskeletal proteins (1) and the publication of many studies describing the helical patterns of protein, DNA and even lipid domains. However, today, new breakthroughs are shaking up what had become a dogma. Instead of helical structures, MreBs appear to form discrete patches

that move circumferentially around the cell, questioning the idea of MreB cables forming an actin-like cytoskeleton. Furthermore, increasing evidence of biochemical properties that are unlike the properties of actin suggest that the molecular behavior of MreB proteins may be different. The aim of this review is to summarize the current knowledge of the so-called "actin-like" MreB cytoskeleton through a discussion of the model Gram-positive bacterium *B. subtilis* and the most recent findings in this rapidly evolving research field.

2. A FEW GENERALITIES

MreB proteins are widely conserved in the bacterial kingdom (2) and were identified a quarter of a century ago. Associated to the acquisition of bacterial rod shape (3-5), these proteins were consequently named "Mre" for "Murein Region E" (murein being the main component of the cell wall; see below for further details and annex 4 for definitions). Evidence accumulated in the 1990s suggested that these cytoskeletal proteins, once thought to be unique to eukaryotic cells, were also present in bacteria (6-8). However, 2001 was the turning point. The subcellular localization and the role in cell morphogenesis of MreB and Mbl (MreB-like) of *B. subtilis* were described (9), and the 3D structure and polymerization properties of MreB1 of *T. maritima* were elucidated (8). This *in vitro* work showed that actin and MreB monomers are structural homologs and that MreB1 forms polymers under conditions similar to those allowing actin polymerization. Taken together, these findings opened a dynamic field of study for the following decade, which has been strongly influenced by the known mechanisms and functions of eukaryotic actin. In eukaryotic cells, actin is involved in a large variety of functions (see annex 1, "Actin in brief"). Actin makes filaments through a dynamic polymerization process (known as treadmilling –see annex 4) that can be used as tracks for intracellular trafficking. The meshwork of actin filaments also forms a structurally resistant scaffold that directly maintains cell shape. In bacteria, MreBs were known to be involved in cell morphogenesis (3-5). Thus, when they were also identified as structural and presumably functional (9) homologs of actin, an actin-like role in other cellular processes, including chromosome segregation, cell polarity, structural resistance and intracellular trafficking, was postulated. An abundance of literature was published concerning, in particular, its potential (and still largely controversial) role in the segregation of chromosomes in *B. subtilis*, *Escherichia coli* and *Caulobacter crescentus,* the three major models used to study the bacterial cytoskeleton (see section 5 and annexes 1, 2 and 4). Similarly, models were generated postulating the existence of either the structural MreB scaffold or MreB treadmilling-based dynamic tracks that directed the localization of macromolecular complexes in cells, both illustrating the mode of action of filamentous actin (F-actin). Finally, the so-far sparse biochemical work on MreB proteins has focused on demonstrating their actin-like polymerization properties. Yet, as we will describe in this review, the broad picture emerging from recent studies is that MreB proteins significantly differ from actin at both the biochemical level and in biological functions, and the MreB "actin-like cytoskeleton" designation is probably less obvious than ever.

The primary function of MreB proteins was and remains the control of the cylindrical cell shape. The link between MreB and cell elongation is further supported by the fact that MreB is widespread in bacteria with complex (non-spherical) shapes but absent from bacteria displaying coccoid (spherical) morphologies (for a survey see (2)). This assertion admits several exceptions because some cocci, such as *Methylococcus capsulatus*, *Synechococcus sp*. or *Anaplasma marginale*, possess *mreB* genes (suspected to have lost their function) while some rod-shaped bacteria, such as *Francisella tularensis*, *Agrobacterium tumefaciens* and *Mycobacterium tuberculosis*, do not, suggesting that an alternative, MreB-independent system of elongated growth has evolved (10). Indeed, in the rod-shaped actinomycetes (i.e., *Streptomyces coelicolor*) the elongation function is not dependent on MreB despite its presence (10). Despite these exceptions, most rod-shaped bacteria possess MreB homologs; thus, it is currently believed that an MreB-dependent mechanism of cell shape control is conserved. In this view, an important difference between *B. subtilis* and Gram-negative models is the existence of three paralogs, MreB, MreBH (MreB homolog) and Mbl (MreB-like), which display partial functional redundancy (see section 7). The existence of several paralogs, in particular in Gram-positive but also in some Gram-negative bacteria, may illustrate the specialization in functions or sub-functions (see section 7).

The mechanism underlying the control of shape by MreB proteins is, however, not yet clearly established. Whereas F-actin plays a scaffolding role in eukaryotic cells, the shape of the bacteria does not seem to be directly dependent on the MreB scaffold. In most bacterial cells, the cell wall (CW), a macromolecular meshwork composed primarily of the polymer peptidoglycan (PG - see annex 4), acts as an exoskeleton (for details see (11) and section 5). PG forms a single giant molecule called the sacculus (see annex 4), which envelopes the entire cell and provides it with physical integrity and mechanical strength, protecting it from turgor pressure. Consistently, PG removal leads to swelling (rounding of cells) and lysis whereas isolated sacculi of cells devoid of their cytoplasmic content typically retain the shape of the original cell. This finding indicates that the CW is generally necessary and sufficient to maintain cell shape. Although recent work in *E. coli* and *B. subtilis* suggests that a mechanical function of MreB filaments may also contribute to this process (12, 13), the prevailing models postulate that MreB proteins control cell shape by organizing PG synthesis.

In this review we aim to 1) summarize the current knowledge on MreB in light of the rich literature released this year and 2) sort facts established specifically for *B. subtilis*, *C. crescentus* and *E. coli*, the main models in which MreB is studied. We primarily focus on the Gram-positive bacterium, but we provide quick overviews of the Gram-negative models (annexes 2 and 3) and a table summarizing the data on MreBs in the rest of the bacterial field (Table 1). We first discuss the discovery of the

Table 1. MreB studied in other bacteria

Species	Gram	Mutant phenotypes (or A22*-treated cells)	Localization	Suspected function	Reference
Salmonella typhimurium	-	spherical cells (instead of rod)	-	shape control	(128)
Azospirillum brasilense	-	- round cell (instead of spiral) - capsule thicker than in wild type and of different composition	-	shape cyst differentiation	(129)
Anabaena sp. PCC7120	-	- increased size (growth rate, division and filamentation unchanged) - no chromosome segregation defect - swollen cells if overexpressed	polar	unclear, probably shape	(130)
Vibrio parahaemolyticus	-	-	patches and bands (perpendicular to long axis of the cells)	unclear	(131)
Streptomyces coelicolor	+	- normal during vegetative growth - swelling and lysing during sporulation	- diffuse in vegetative cells - septal, then bipolar, then diffuse in membrane in sporulating hyphae	spore CW synthesis	(132)
Bdellovibrio bacteriovorus	-	- MreB1 probably essential; blocked in development or become spheroblast - MreB2 abnormal shape (round, elongated, branched) during attack-phase	unclear because fusion is not functional unclear because fusion is not functional	shape, development	(133)
Rhodobacter sphaeroides	-	probably essential (abnormal shape with partially functional tagged MreB)	some foci and transverse band at mid-cell, ring-like	shape	(134)
Helicobacter pylori	-	- reduced growth rate severely (spiral shape and diameter unaffected) - affects nucleoid positioning and size (but no anucleated cells observed) - reduced urease activity but not at the expression or protein level	-	shape (long axis) chromosome segregation regulation of urease activity	(135)
Pseudomonas aeruginosa	-	Polar localization of PilT, thus affecting pilus localization	-	pilus positioning	(136)
Myxococcus xanthus	-	- probably essential; round cells in the presence of A22 - mislocalized FrzS and AglZ, proteins required for 2 motility complexes	patches and "helical-like" bands	Shape control required for S and A motility	(137)
Vibrio cholerae	-	- essential; depletion leads to spherical cells, enlarging and lysing - A22 affects number of Chromo I and II ori, and increased anucleated cell number - nucleoid more compact and absent from pole	dynamic patches	Shape Nucleoid compaction Chromo segregation and ori positioning	(138)
Leptospira biflexa	-	probably essential; localized increased width, but general spiraled shape maintained	-	shape	(45)

*A22 : a drug that depolymerizes MreB in Gram-negative bacteria by interacting with nucleotide binding pocket (139-141)

specific pattern of localization of MreB proteins (section 3). Then, we discuss their biological functions, including what seems to be their primary role in CW morphogenesis (section 4), and additional suspected or established functions (section 5). We next describe the current knowledge regarding the biochemical properties of MreB and how they compare to the properties of actin (section 6). Finally, we discuss the established models linking biochemistry and biology and provide an overview of future perspectives for the field.

3. THE LOCALIZATION PATTERN OF MreB: A REVOLUTION

A striking feature of MreB proteins is without a doubt their subcellular localization. A decade ago, the first description of their subcellular localization accompanied the emergence of fluorescence microscopy techniques in bacterial cell biology and has had a lasting impact. Today, improvements in fluorescence labeling techniques and the advent of sophisticated live cell light microscopy technologies have allowed substantial improvements in the temporal and spatial resolution that are now breaking through the diffraction limit (14, 15). Together with continuous developments in electron microscopy approaches, these progresses shed new light in our understanding of bacterial cell organization and function. Indeed, the dynamic revolution that proteins display inside the cells may be the beginning of a change in paradigm.

3.1. A helical world

Errington and coworkers performed the first subcellular localization study of MreB proteins in *B. subtilis* using immunofluorescence microscopy and a partially functional green fluorescent protein (GFP) fusion (9). Images of neighboring focal plans were taken through the cells containing fluorescently labeled MreB or Mbl using wide-field conventional epifluorescence microscopy. These data, together with their subsequent deconvolution to reassign the unfocused light back to its point source, were used to generate 3D reconstructions of the structures formed by MreB and Mbl. The results revealed a pattern of transversal tilted bands and arcs that were interpreted as filamentous helical structures running along the length of the cell, just underneath the cell membrane. Subsequently, both the Errington and Graumann labs confirmed this

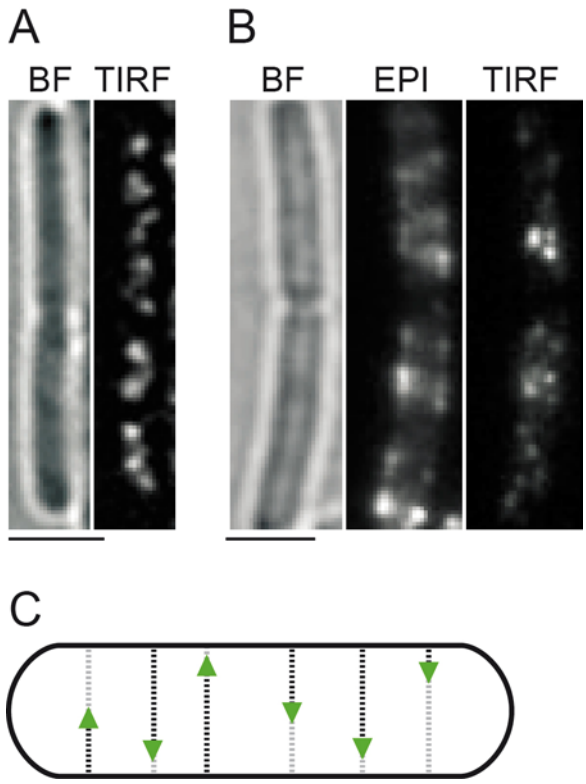


Figure 1. MreB assembles into discrete patches that move perpendicularly to the long axis of the cells. Panel A. MreB, observed by TIRFM, reveals discrete patchy localization. Panel B. Comparison of fluorescent signal obtained with "classical" epifluorescence (Epi) and TIRFM. In Epi, fluorescence from the median cellular plan is observed, revealing bright dots along the sidewall and weaker transversal bands. In TIRF, only the reflected light (evanescent waves) from the first 1/3 of the cell cylinder is observed, revealing discrete dots. Panel C. Cartoon depicting MreB (arrowhead) movements along parallel tracks (dotted lines), as revealed using time-lapse TIRF imaging. Note that MreB can travel in both directions. BF stands for bright field illumination, TIRF for total internal reflection fluorescence. Scale bars: 2 µm.

localization pattern using functional GFP fusions to both MreB and Mbl (16, 17) and later MreBH (10, 17). Simultaneously, similar observations were reported for MreB in *E. coli* (18) and *C. crescentus* (19). In addition to this original (at the time) 3D helical pattern, time-lapse microscopy and fluorescence recovery after photobleaching (FRAP -see annex 4) experiments revealed that MreB-like structures were not static but highly dynamic, suggesting that they undergo dynamic changes and are continuously remodeled during cell elongation (10, 16) and that individual filaments moved through the cell along helical tracks on a scale of seconds (10, 16, 17).

3.2. A revolutionary dynamics: the end of a helical world?

The dynamic, helix-like pattern of MreB localization has led to a multitude of reports describing the helical-like distribution of proteins (20-28) and, more recently, of DNA and even lipid domains (20, 29-31) in bacteria. Among these studies, an increasing number of reports have indiscriminately described as 'helical' any discrete localization of a few dots along the membrane. Recently, two independent breakthrough studies revisited MreB localization and dynamics during *B. subtilis* exponential growth. GFP fusions to MreB, Mbl and MreBH were imaged using confocal microscopy combined with high-precision particle tracking and internal reflection fluorescence microscopy (TIRFM - see annex 4) (32, 33). These advanced fluorescence microscopy techniques improve temporal resolution and, *in fine*, quantitative analysis of the dynamics of membrane-associated proteins. Two unexpected observations were made in these studies:1) MreB, Mbl and MreBH do not actually form extended helical structures. Instead, they assemble into discrete patches along the sidewalls (Figure 1A-B) and move processively around the cell cylinder. In earlier reports, MreB and Mbl dots and patches moving through the cell had been observed, but these were thought to move along helical tracks (17). However, both Dominguez-Escobar *et al.* and Garner *et al.* showed that all three MreB paralogs formed discrete structures moving independently and did not observe underlying tracks or filaments. Consistent with these findings, another recent report using electron cryotomography (ECT) showed that no long helical filamentous structures could be observed in *B. subtilis* or in five other Gram-negative species (34).2) The movement of MreBs followed trajectories perpendicular to the long axis of the cell (i.e., circumferential) (Figure 1C) and was not, as one would have expected, tilted to follow a more helical pattern.

Similar MreB patch-like localization patterns and dynamics were observed using TIRF or epifluorescence for the Gram-negative *E. coli* (32, 35) and *C. crescentus* (32), suggesting that these features are widely conserved in bacteria. How do we reconcile these findings with the extended helical structures previously described for MreB proteins? This problem can actually be divided into two different questions: does MreB form patches or filaments, and are they helical? The answer to the first question seems to be both, and the differences lie in the experimental conditions. Indeed, MreB proteins form elongated filamentous structures when over-expressed or when observed in late phases of growth. In virtually all older reports describing MreB localization in *B. subtilis,* inducible (i.e., mostly overexpressed) GFP fusions were used, and observations were often made during the late exponential stage (e.g., (16, 36)), when the structures were easier to visualize. In contrast, with the improved techniques used in recent reports, weaker signals could now be observed, and shorter exposures were needed, allowing observations during exponential growth, which revealed small patches. Consistently, when cells entering the stationary phase were imaged using either conventional microscopy or TIRFM, MreB was localized to transverse (not helical) bands (32). Thus, old reports were biased in that patches were not visualized because of technical limitations.

The more complicated question is whether these structures were helical. In retrospect, when reading back the publications over the last decade, few pictures actually show real, convincing helices. The pattern undoubtedly appeared helical only in the earlier studies when images where heavily processed and treated (often randomly) using deconvolution tools. Conversely, in more recent publications, less processed images repeatedly yielded patchy patterns made up of discrete foci and/or short bands. The spacing between dots seemed compatible with a large-scale helix spanning the cell, and the structures were consequently described as helical. When Domínguez-Escobar *et al.* simultaneously imaged cells using TIRFM and conventional epifluorescence in a single cell, GFP fusions to the MreBs form motile discrete patches when visualized using TIRFM and display a pattern reminiscent of other recently published 'helical' images when visualized using epifluorescence (Figure 1B) (32). Van Teeffelen and coworkers made similar observations in *E. coli* cells of moving patches along the bottom plane and a pattern of discretely spaced MreB spots along the side wall when the focal plane was close to mid cell (35).

Thus, a tempting hypothesis is that MreBs form small discrete patches that are organized along an underlying helix or that the spacing between these circumferentially moving dots creates a pattern *grosso modo* mimicking a helical pattern. The absence of a correlation between the moving patches argues, however, against a continuous MreB helix in vegetatively growing cells (32). At this point, it is unclear whether the overuse of the post-acquisition treatment of images, possibly amplified by experimental conditions, favored the earlier observation of helices. Considering the images acquired in the last five years of studies concerning MreBs, in light of the most recent finding, the question remains whether MreB assembles into helices at all. (This point is discussed in section 7.1.)

3.3. Colocalization of MreB isoforms

The earliest reports showed that MreB, Mbl and MreBH formed helical-like filamentous structures, but the pattern appeared distinct for each isoform (slightly different extensions of the helical structures along the sidewall and relative to the cell poles, slightly different helical pitches, etc) (9, 17). However, when pair-wise combinations of YFP/CFP and GFP/RFP fusions were expressed in the same cell and imaged using conventional epifluorescence and TIRFM respectively, all three MreBs colocalized in live *B. subtilis* cells (10, 32). Defeu-Soufo and Graumann addressed the same question using a combination of fluorescence resonance energy transfer (FRET - see annex 4) and bimolecular fluorescence complementation (BiFC - see annex 4) microscopy (37). These studies conclusively showed that all three MreBs of *B. subtilis* belong to the same structures in the cell. The discrepancy relative to the original reports may almost certainly be due to the imaging conditions; in the original localization experiments, the MreB isoforms had been visualized in separate cell populations, with different genotypes or treated for IFM with different antibodies whereas simultaneous, same-cell imaging was used in the dual-labeling experiments. However, it is important to consider that the different fluorophore tags used in the colocalization experiments (even if the fusions were functional in complementation assays) and the expression levels of the fusion proteins (almost all expressed from inducible promoters) may have impact the interaction and/or localization pattern of these proteins. Indeed, an overriding concern that all three isoforms colocalize upon fluorescent tagging and/or overexpression cannot be excluded at this point. This idea is indirectly supported by a recent study where MreB, Mbl and MreBH had different filament architectures when individually expressed in *E. coli* cells but colocalized in a single filamentous structure when co-expressed (12). Indeed, when the three isoforms were co-expressed in different pair-wise combinations or all together in *E. coli,* they influenced each other's filament architecture and colocalized in a single structure. Thus, it is still unknown whether the three paralogs co-exist in mixed bundles of single filaments (of individual protein) or whether they co-polymerize to form mixed filaments (comprising a mixture of all three proteins).

4. MreB PROTEINS CONTROL CELL MORPHOGENESIS

4.1. Inactivation of *mreBs* reveals their role in shape control

Although the precise roles and underlying mechanistic details of MreBs are still not understood, their primary function in cell shape determination is well established and was always associated with cell wall homeostasis. In *B. subtilis*, as in most bacteria studied so far (38), *mreB* and *mbl* are essential under standard laboratory conditions (9, 39) while *mreBH* is essential only

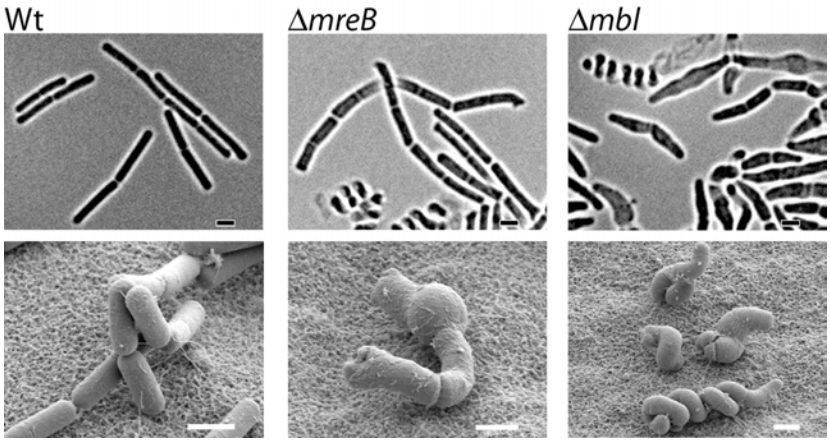


Figure 2. MreB and Mbl mutants present specific morphological defects. Here are presented (from left to right) a wild-type *B. subtilis*, an *mreB* mutant and an *mbl* mutant during exponential growth, using bright field (upper row) and scanning electron (lower row) microscopy (see in text for details). Black scale bars: 2 μm; White scale bars: 1 μm.

under certain growth conditions (10, 40) (see below). Mutants of each single paralog exhibit characteristic shape defects. For *mreB*, a complication comes from the co-transcription with the downstream *mreC* and *mreD* genes (36), which are also essential and involved in the same morphogenetic pathway (41) (see below). Indeed, the phenotype originally described for *mreB* mutants (rounded and inflated cell morphologies) was actually that of *mreBCD* mutants (9, 42). Subsequent construction of *mreB* mutants allowing the independent expression of *mreC* and *mreD* (mutant strains either carrying a marker-less deletion of *mreB* or an ectopically expressed inducible *mreCD* in a null *mreBCD* background (36, 43)) and the generation of single mutants of *mreC* or *mreD* (41) revealed the respective (yet similar) phenotype of each mutant. Cells lacking MreB stop growing, become wider near the division site, display characteristic bulging poles (Figure 2) and ultimately lyse (36, 43).

The *mbl* mutants also display highly distorted cell morphologies with characteristic bends and twists and frequent "comma-shapes" (Figure 2; (9, 39, 44)), which indicate that Mbl is important in maintaining the linearity of the longitudinal axis of growth (i.e., sidewall elongation). These mutants additionally have a mild chaining phenotype that, together with cell bending/twisting, leads to a more or less important swirling aspect, with the formation of typical coiled chains of cells that resemble a telephone cord (Figure 2) (9, 39, 42). This regularly coiled helical shape, which strongly resembles that of *Leptospirae* (45), suggests that absence of Mbl leads to a regular alteration (rather than random modifications) in the pattern of synthesis of the sidewall PG. Coiling could also be observed, although to a much lesser frequency, in *mreB* mutants under certain conditions ((46); Figure 2). Finally, *mbl* mutant cells do not present polar bulges like *mreB* mutants, but cell width is affected in a proportion of them (39).

mreBH appears to be the least critical of all three *mreB* paralogs. Under normal growth conditions, *mreBH* mutants display normal growth and have mild cell-shape defects (altered cell width and/or length and occasional formation of curved or 'vibrio-shaped' cells) relative to wild-type cells (10, 42). However, *mreBH* becomes essential for growth at low magnesium (Mg^{2+}) concentrations (10) and under various stress conditions (40). Under these specific conditions, *mreBH* mutant cells become curved and bent at irregular angles and are clearly affected in length and width, especially during the stationary growth phase.

The deletion of *mreB* and *mbl* (but not of *mreBH*) is lethal as stated above, and the overproduction of MreB and MreBH (but not Mbl) is also lethal (40). Strains overexpressing *mreB* or *mreBH* using a strong inducible promoter displayed progressive bulging and lysis, suggesting the impairment of cell width control (40). Many of the cells were unusually long, which also suggests impairment in cell division. Only a slight increase in cell length and little or no change of cell width are observed upon *mbl* overexpression.

Together, the specific phenotypes of *mreB, mbl* and *mreBH* mutants indicate that all three genes are involved in cell shape determination but argue for specific roles for each protein; Mbl is primarily involved in straightness of cylindrical growth while MreB is involved in the control of cell width, and MreBH more as a back-up protein with a minor contributions to both processes. However, and as we will see later (section 7), this statement needs to be amended. Indeed, the three paralogs are partially redundant, and the overexpression of each can support rod-shaped growth in the absence of the other two (40).

4.1.1. Conditional lethality of *mreB* mutants: Mg^{2+} to the rescue

The shape defects of *mreB* mutants are greatly influenced by growth conditions, such as growth rate and especially medium composition. It is particularly intriguing that the presence of high levels of Mg^{2+} in the growth

medium (in the mM range) restores the viability and almost wild-type rod shape of *mreB* and *mbl* mutants (36, 39), and under these conditions, null *mreB* and *mbl* mutants and a *ΔmblΔmreBH* mutant (40) could be obtained (NB: other mutant combinations involving *mreB,* i.e., *ΔmreBΔmbl* and *ΔmreBΔmreBH,* are synthetically lethal even in the presence of high Mg^{2+}). Monovalent cations are inefficient, and no other bivalent salts (such as Mn^{2+} and Ca^{2+}) could replace Mg^{2+} with the same efficiency in part because precipitation prevented testing at concentrations > 1 mM (36). Nevertheless, the effect of magnesium is not specific to *mreB* mutants, and high Mg^{2+} also allows viability and/or rescues growth or morphology defects in mutants of several genes that are involved in CW synthesis such, as *mreC* and *mreD* (see below), *ponA*, *pgcA*, *gtaB* or *ypfP* (47).

How Mg^{2+} can complement the absence of MreBs is currently unknown and subject to speculations. Three main possibilities have been suggested: a regulatory role, a structural role or both. Many enzymes are sensitive to magnesium levels, and as detailed later, the inactivation of a single PBP (PBP1, encoded by *ponA*) (see annex 4)is sufficient to restore the viability and rod shape of an *mreB*-null mutant. Thus, it is tempting to hypothesize that high Mg^{2+} could affect and/or stabilize the activity of one or a combination of synthetic and/or lytic CW enzymes, leading to a suppressive phenotype for each single mutant. The structural hypothesis postulates a compensatory role for Mg^{2+}; it would provide resilience to the weakened CW structure of mutants affected in PG synthesis or assembly, which could otherwise not counteract osmotic forces. High levels of Mg^{2+} could stiffen the negatively charged cell envelope of Gram-positive bacteria and rescue such deleterious effects. Finally, it is plausible that a combination of both a regulatory and a structural function (leading, for example, to an increase of the level of cross-linking of the cell wall) might be required to explain the impressive reversion that Mg^{2+} exerts on *mreB*-like mutant phenotypes. Also Mg^{2+} could act directly or indirectly through the anionic polymers of the CW (i.e., teichoic and teichuronic acids - see annex 4) (36, 48), which may be required for the uptake and sequestration of cations from the medium (49).

4.1.2. Suppressor mutants of the *mreB*⁻ and *mbl*⁻ lethal phenotype

Both *mreB* and *mbl* mutants are lethal under typical lab conditions but can readily acquire a series of spontaneous intergenic suppressor mutations that restore their viability and/or morphological defects to different degrees (39, 44). Since the critical 'rescuing' role of Mg^{2+} was observed (see above), *mreB* and *mbl* mutants have been systematically isolated in the presence of high Mg^{2+}concentrations. Schirner and Errington showed that non-suppressed *mbl* mutants generated under these conditions were strictly Mg^{2+}-dependent (39). This property was used to screen Mg^{2+}-independent suppressors obtained through transposon mutagenesis (39, 46). Such suppressors are of great interest because they reveal possible targets, interactions and/or pathways related to MreBs. So far, 10 suppressor genes of either *mreB* or *mbl* have been reported: *ponA*, *pstI*, *ccpA*, *rsgI* (previously *ykrI*), *ylxA*, *yaaT*, *gltT*, *pnpA*, *yvcK* and *ltaS* (previously *yflE*), but only the four genes described hereafter (*ponA*, *rsgI*, *yvcK* and *ltaS*) have been the subject of in-depth characterization studies.

Predictably, one of these suppressors, *ltaS,* is acting through the rescuing effect of Mg^{2+} (48). LtaS is a key enzyme of the lipoteichoic acid (LTA) biosynthetic pathway (50). The anionic LTAs may constitute a buffering zone by scavenging and allowing control of divalent cations, i.e., beneficial Mg^{2+} and deleterious Mn^{2+}, on the cell surface (49). Consistently, in the absence of LtaS (LTA synthase), and consequently of LTA, *B. subtilis* requires lower amounts of Mg^{2+} (but is unusually sensitive to Mn^{2+}) presumably because of a more immediate access of these ions to the cell surface and their yet unknown targets (48). As a consequence, this mutation also allowed the restoration of the growth and morphology of both *mbl* and *mreB* mutants under standard medium conditions (48).

ponA (encoding PBP1, a high molecular weight penicillin-binding protein (PBP) with transglycosylase and transpeptidase activity - see annex 4) suppresses the *mreB* mutant through an effect on CW homeostasis (46). This effect may seem surprising because PBP1 is an important enzyme involved in CW synthesis. However, in an *mreB* mutant, PBP1 re-localizes from the sidewall to the poles, leading to an accumulation of PG and the subsequent characteristic polar bulging of *mreB* mutants (46). Consequently, the deletion of *ponA* eliminates hump formation and suppresses deleterious effects, allowing the *mreB* mutant to sustain more robust growth. Thus, the absence of PBP1 is less deleterious than its uncontrolled activity. Surprisingly, the inactivation of *ponA* also suppresses the lethality of an *mbl* mutant, suggesting that Mbl could also contribute to PBP1 control (40) although the localization of PBP1 seems unaffected in the absence of *mbl* (46).

Independently, the Galinier lab found that *yvcK* mutants growing under gluconeogenic conditions display shape defects that are similar to *mreB* mutants because of the mislocalization of PBP1 and that deletion of *ponA* was sufficient to restore growth (23, 51). Surprisingly, the over-expression of *yvcK* in turn suppressed the requirement for MreB in rich medium by re-localizing PBP1 through an unknown mechanism (23). This suggests that PBP1 could be independently localized along the sidewall through two independent mechanisms: *via* MreB or *via* YvcK. Interestingly, YvcK-GFP formed impressive helical-like structures that were independent of and did not colocalize with MreB structures (23), suggesting the existence of true helical localization pathways in bacteria (independent of MreBs, whose helical localization is now subject to caution - see the discussion above, section 3.2.).

Finally, *rsgI* was isolated from a screen performed on a newly isolated Mg^{2+}-dependent *mbl* mutant (39). The *ΔrsgIΔmbl* mutant displays a wild-type growth rate and cell width but retains a light twist (39). RsgI is an anti-sigma factor that represses the alternative sigma factor

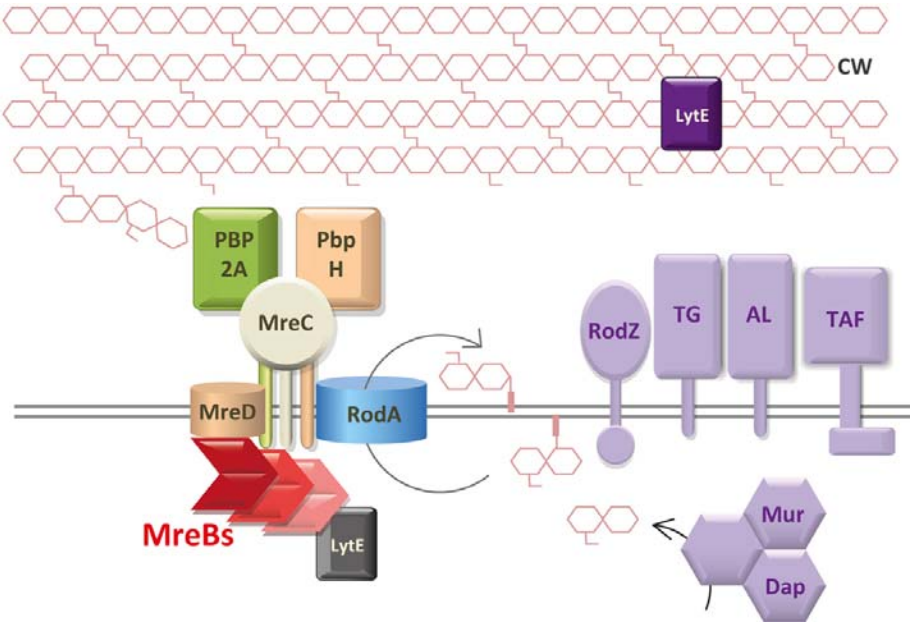


Figure 3. Model for CW elongation complex in *B. subtilis*. The putative CW elongation complex comprises the transpeptidases PBP2a and PbpH, the morphogenetic proteins MreC and MreD, the putative LipidII-flippase RodA and the cytosolic membrane-associated MreB (dark red), Mbl (light red) and MreBH (pink). LytE interacts with MreBH before it is exported to the CW. Several other protein or complexes (purple) are expected to be part of the machinery, including transglycosylases (TG), autolysins (AL), teichoic acid factories (TAFs), the cytosolic enzymes synthesizing the PG precursors (Mur/Dap) and RodZ.

σ^I (a stress response regulator involved in resistance to high temperature (52)), and as such, its deletion leads to increased σ^I activity. Although σ^I seems to control *mreBH* (53), the suppressing effect of *rsgI* on *mbl* does not rely on *mreBH* regulation or the Mg^{2+} effect (39), and its mechanism remains unclear. Recently, Tseng *et al.* showed that σ^I also controls the expression of *lytE* (54), which encodes a CW hydrolase that directly interacts with MreBH and is required for rod-shaped growth under low Mg^{2+} conditions (10) (see below). Unfortunately, the suppressive effect of LytE on *mreB* mutants has not been investigated yet, but it is hard to imagine that this nonessential autolysin could be the only mediator of the σ^I-mediated suppression. Indeed, σ^I has an unmatched capability in suppressing *mreBs* mutants: its over-expression is to date the only way to simultaneously delete all three *mreB* paralogs. Notably, even though the resulting *ΔmreBΔmblΔmreBHΔrsgI* mutant has a spherical morphology and depends on Mg^{2+} for viability, the CW still retains some resilience, and chromosome partitioning and cell division occur in the absence of all three *mreB* paralogs, a point we shall return to (see section 5.1.) (39).

4.2. Role of MreBs in control of bacterial cell wall biosynthesis

The cytoskeletal-like structures formed by MreB proteins and the phenotype of *mreB* mutants have always indicated a function in the control of cell wall synthesis. This function was confirmed through investigation, using fluorescent probes, of the pattern formed upon the insertion of nascent PG and how this pattern is affected in cells lacking MreBs (40, 55, 56). Recently, more direct evidence has revealed a link between PG synthetic enzymes and the MreB cytoskeleton, reinforcing a model in which MreBs organize CW-synthesizing machineries along the sidewalls of rod-shaped bacteria (Figure 3-4). Before going any further into this model, we will take a quick overview of CW synthesis, particularly the primary factors involved in PG elongation in *B. subtilis*.

4.2.1. Cell wall synthesis in *Bacillus subtilis*

In Gram-positive bacteria, such as *B. subtilis*, the CW is a thick external structure mainly composed of PG and anionic polymers (mainly teichoic acids, TA - see annex 4), which are both present in roughly equal proportions. TAs fall into two classes synthesized by distinct metabolic pathways: the major wall-TA (WTA), a phosphate-rich polymer bound to the PG, and the minor lipo-TA (LTA), anchored to the membrane (49, 57). Their functions are not completely understood, but it was recently shown that WTA and LTA are synthetically essential (48), with distinct roles in cell morphogenesis: WTA is important during the elongation process (58) while LTA is more involved in cell division (48). Moreover, the LTAs participate in the control of divalent cation homeostasis, as discussed above (48).

The PG forms the sacculus, which is made of long sugar chains that are highly cross-linked by peptide bridges, conferring physical properties to the CW. PG synthesis starts with the formation of the PG precursor, a disaccharide of *N*-acetylglucosamine and *N*-acetylmuramic acid (the latter containing a linear stem pentapeptide), in the cytosol; this precursor is flipped across the membrane, possibly *via* RodA (see section 4.3.2). Next, the precursors

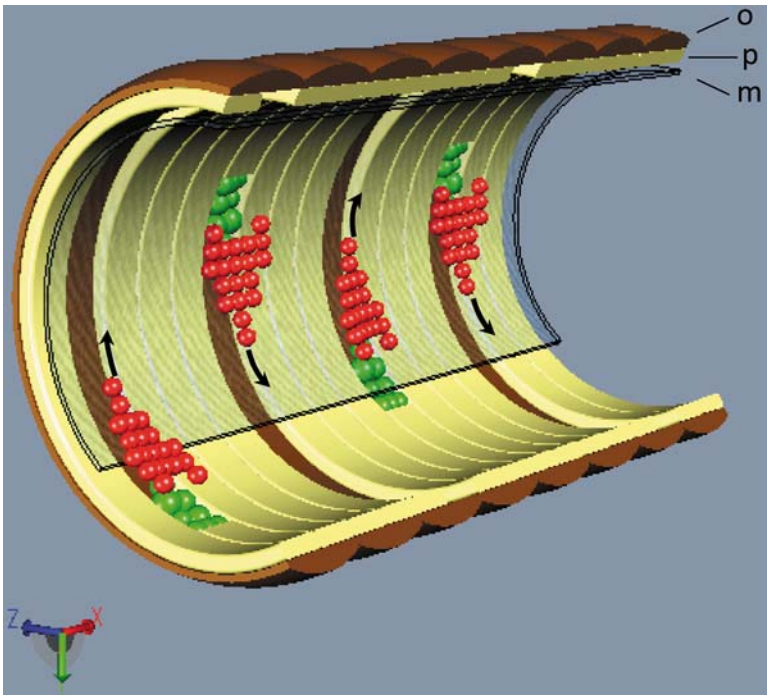


Figure 4. Model for sidewall biosynthesis in *B. subtilis*. Cytosolic MreBs filaments (*red)* and associated PG biosynthetic complexes (*green*) involved in sidewall elongation in the membrane (m) move processively along peripheral tracks perpendicular to the long axis of the cell. These elongation complexes move in both directions (arrows), inserting discrete circumferential bands of new PG (p, *yellow*) on the outside of the membrane under the overlying existing old PG strands (o, *brown)* used as guiding scaffolds.

are incorporated into the existing PG mesh-layer outside of the cell by a subset of PBPs with transglycosylase and/or transpeptidase activities. PBPs are usually classified into 3 categories depending on their size and enzymatic activities: Class A PBPs (bifunctional transglycosylases/transpeptidases), Class B PBPs (transpeptidases) and Class C PBPs (low-molecular weight carboxypeptidases and endopeptidases). These proteins share the specificity of bearing names that often vary without any obvious logics relative to their respective gene names and between orthologs (for a review see (59)). Although their biochemical activity is fairly established, the precise function of each PBP is still elusive and hampered by redundancy (for a review see (26)). Furthermore, the viability of a *B. subtilis* mutant depleted of the four known Class A PBPs, which produces a PG wall with only small structural differences from that of the wild-type strain, indicates the existence of still uncharacterized enzymes with glycosyltransferase activity (60). In addition, efficient PG synthesis involves the concomitant cleavage of existing CW bonds by lytic enzymes (also called autolysins; for a review see (61)) to allow coordinated expansion of the sacculus during growth without compromising CW integrity (61-64). Increasing evidence suggests that large multi-enzyme complexes (CW-synthesizing machineries) that contain both PG synthases and hydrolases are present in the cell ((65); Figure 3). Although their existence as discrete factories has not been conclusively shown yet and their precise composition remains unknown, CW division-specific machineries and CW elongation-specific machineries are hypothesized to exist. The latter would also contain MreBs, MreC, MreD, RodA and RodZ (66) (see below). TA-synthesizing machineries may also exist and be coupled to the PG-synthesizing holoenzyme (58), and growing evidence indicates that cytosolic enzymes required for PG precursor synthesis may also be physically associated with these CW machineries (see also annex 2) (Figure 3).

4.2.2. MreBs and sidewall elongation complexes

A brilliant trick allowing the direct visualization of the incorporation of PG precursors in the CW was described a few years ago by Daniel and Errington (55). The antibiotic vancomycin (Van) recognizes and tightly binds to the last two peptides of the PG precursor (namely, D-Ala-D-Ala) that have been externalized (membranes are not permeable to Van) but not yet cross-linked by transpeptidases (67). Using a fluorescently labeled vancomycin derivative (Van-FL), Daniel and Errington could visualize the topology of insertion of the nascent PG in live *B. subtilis* cells. They observed 1) intense staining at division sites (as expected because cell division requires the synthesis of the thick septal wall to separate daughter cells); 2) little or no staining at the cell poles, in agreement with previous studies suggesting that there is low CW turnover at these places; and 3) discrete dots and bands perpendicular to the long axis of the cells. This last observation suggested that incorporation of PG occurs in a discrete, banded pattern along the cylindrical part of the cell, which they described as “reminiscent of the helical

pattern." The similarity of the 'helical-like' pattern displayed by the Van FL staining and the MreB/Mbl structures suggest that either or both of these proteins could spatially direct PG synthesis along the sidewalls.

The deletion of each *mreB* paralog (or of *mbl* and *mreBH* simultaneously) does not eliminate sidewall PG insertion, even in the absence of Mg^{2+} (36, 40, 56), but the depletion of MreB in an *mbl* mutant leads to the loss of lateral Van-FL labeling (40), indicating that lateral incorporation of PG requires both MreB and Mbl in *B. subtilis*. This finding has important implications concerning the partial redundancy of function of MreBs (see below) and indicates that in *mreB* or *mbl* mutants, the remaining MreBs could support lateral PG insertion. This idea is also supported by the fact that the over-expression of any of the three MreB isoforms in the absence of the other two is sufficient to maintain rod-shaped growth (40).

These findings must be considered in parallel with two other series of data. The first is the observation that PBP1 localization occurs along the cylindrical sidewalls, which is required for its proper biological function and depends on the presence of MreB, as previously mentioned (46). In the same study, a direct interaction between PBP1 and MreB was shown in pulldown assays using a histidine-tagged MreB and bacterial two-hybrid assays in *E. coli* host cells. Additionally, several other PBPs, detected with the fluorescent penicillin analog Bocillin FL and presumably corresponding to PBP2a, 2b, 2H, 2c, 4 and 5, were pulled-down by MreB, and bacterial two-hybrid analysis detected direct interactions between MreB and PBP2a, 2b, 2c, 2d, 3, 4, H and I (but not PBP4*, 4a, 5, 5* and X) (46). Furthermore, the profile of the PBPs obtained using His-tagged Mbl was similar to that obtained with His-tagged MreB, even in the absence of MreB and MreBH (40). Taken together, these findings provided further support for the existence of a tight link between the partially redundant MreB/Mbl and the PG synthetic machinery.

The second line of evidence for the existence of MreB-containing CW elongation complexes comes from two recent studies revisiting the localization and dynamics of MreBs using TIRFM and high-precision particle tracking (32, 33). These studies showed that the monofunctional transpeptidases PbpH and PBP2a, previously shown to play redundant roles in sidewall synthesis (68, 69), and the morphogenetic membrane proteins MreC, MreD and RodA, also previously linked to sidewall elongation (see below), moved similarly and colocalized with MreB/Mbl/MreBH motile patches, suggesting an association in multiprotein complexes. In addition, the quantitative analysis of the lateral Van-FL pattern visualized using TIRFM (which was similar to the pattern previously reported using conventional epifluorescence microscopy and described as "helical") revealed that Van-FL bands were actually oriented approximately 90° relative to the long axis of the cells, similar to the patch trajectories of MreBs (and PbpH, PBP2a, MreCD and RodA) (32). Finally, when PG synthesis was inhibited using antibiotics (phosphomycin, vancomycin, ampicillin or amdinocillin) or through genetic methods (deletion of *pbpH* or *pbpA* or depletion of RodA, RodZ or Pbp2A), the movement of MreB patches was blocked, suggesting that the movement was powered by PG synthesis itself (32, 33). Taken together, these results strongly support a model in which MreBs are part of sidewall-synthesizing machineries containing at least some PBPs and several morphogenetic proteins (Figure 3) that are mobilized by PG synthesis (Figure 4).

4.3. MreBs-associated morphogenetic factors in *Bacillus subtilis*

The association of MreBs with sidewall-synthesizing complexes raises several questions: Do MreBs interact with PBPs only through the small cytoplasmic domains of these mainly extracellular proteins? Do integral membrane proteins bridge PBPs and/or anchor MreBs to the membrane? What additional functions (players) does the CW elongation machinery bear? What other proteins interact with MreBs, and what are their purposes? In the following section, we will describe the morphogenetic factors that directly or indirectly interact with MreB.

4.3.1. MreCD

The precise role of the essential transmembrane proteins MreC and MreD is still unclear, but these proteins are clearly associated with MreB and sidewall elongation. The depletion of either MreC or MreD confers similar gross changes in cell morphology to those changes observed with the depletion of MreB (short, rounded cells) (41, 70). As for *mreB,* an in-frame deletion of *mreC* could be obtained in the presence of high levels of Mg^{2+}. However, unlike the *mreB* mutant, the *mreC* mutant was unstable and difficult to propagate and displayed a spherical shape, indicating that Mg^{2+} was unable to restore a normal rod-shaped morphology (41). Attempts to isolate a *ΔmreD* mutant have been unsuccessful so far, suggesting a more critical function for MreD than for MreC and MreB (41). MreC- and MreD-depleted mutants display spheroidal shapes and intense bands of Van-FL staining at mid-cell but no spotty/banded (helical-like) pattern along the sidewalls, indicating that growth is only supported by septal PG synthesis (41). The reported subcellular localization pattern of MreC and MreD has evolved considerably over time with the improvement of cytology approaches, from only septal (70) to homogeneously distributed in the membrane (43), then septal and displaying a helical-like configuration along the sidewalls (37, 41) and finally in MreB-associated patches moving in circular trajectories under the cell cylinder (32, 33).

MreD is a small integral membrane protein with virtually no residues outside the membrane (it is predicted to have 4 to 6 transmembrane spans and both N- and C-termini in the cytoplasm), whereas MreC is predicted to have a single transmembrane domain, with a short N-terminal cytoplasmic tail and a large C-terminal domain outside the cytoplasmic membrane and was shown to dimerize (41, 71). For some time, these proteins have been considered to link the extracellular CW synthetic machinery and the cytoplasmic MreBs (41, 72). The evidence in *B. subtilis* is scarce, but this idea is reinforced

through data in other bacteria (see annexes 2 and 3). So far, in *B. subtilis*, it has been shown that MreC directly interacts with itself, several PBPs (PBP1, 2a, 2b, 2c, 2d, 3, 4, 4a, 4b and PbpH but NOT with PBP4*, 5*, 5 and PbpX) and MreD in bacterial double hybrid assays (71, 73) and with Mbl using BiFC and FRET (37).

Upon crystallization of MreC in *Listeria monocytogenes*, van den Ent *et al.* proposed that MreC could have a more structural (rather than enzymatic) purpose and could organize PBPs while forming a scaffold with MreD to couple them to the cytoplasmic MreBs (71). Although this hypothesis is totally plausible, there is a considerable lack of information concerning these two proteins and their possible biochemical activity. Note that both MreC and D are also present in bacteria devoid of MreB (74), indicating that if they act as a linker with MreB, then scaffolding is not their sole purpose (see section 6).

4.3.2. RodA

Little information is available for RodA, an essential integral membrane protein from the SEDS (shape, elongation, division and sporulation) family that has been linked to CW elongation and PbpH and PBP2a (69, 75). However, two recent findings highlight RodA as an important component of the CW-elongation machinery. First, another SEDS member, the integral membrane protein FtsW (an essential protein of the bacterial division machinery), has been recently identified as a flippase of lipid-linked PG precursors in *E. coli* (76). This result strongly suggests that its three homologs in *B. subtilis*, RodA, FtsW and SpoVE, may also be flippases that are associated with specific PG synthesis complexes involved in sidewall synthesis (elongation), septum formation (division) and sporulation, respectively. Second, RodA formed discrete motile patches that behaved like MreB patches (and MreC, MreD, PBP2a and PbpH patches) in speed and orientation and colocalized with MreB and Mbl over time in two-color TIRFM imaging experiments (32).

4.3.3. LytE

LytE is an extracellular CW hydrolase (77) comprising a signal peptide region, a cell-wall binding domain and a putative catalytic PG hydrolase domain (10). Surprisingly, LytE interacts specifically with MreBH through its catalytic extracytoplasmic domain (10), which means that this interaction may be transient and take place before LytE export. A functional LytE-GFP fusion localized in a punctuated pattern along the cylindrical part of the cell surface, and this pattern was dependent on the presence of MreBH (and not of MreB or Mbl) (10). Because the absence of *lytE* and of *mreBH* had similar consequences on shape, it was concluded that control of the hydrolysis (that is maturation and/or degradation) of the CW is an integral function of the MreBs and that they achieve this control by directing the export of autolysins at their specific sites of action (Figure 3). This function was somehow expected because all models attempting to explain CW growth require the coordinated action of PG hydrolases along with PG synthases as stated above. MreBH controls an early step in the process of LytE action, namely, the spatial positioning of the protein, but a tight control of its autolytic activity is also required to prevent cell lysis and allow coordination with PG synthesis to mediate controlled elongation. Whether MreBs also have a regulatory function over CW enzymes (see section 7) or whether elongation is mediated by other factors is an important question to be addressed by current research.

4.3.4. EF-Tu

The second morphogenetic factor that physically interacts with an MreB isoform is the bacterial translation elongation factor EF-Tu (22). Defeu-Soufo and coworkers uncovered an interaction between MreB and EF-Tu in pull-down experiments and confirmed this interaction using recombinant proteins purified from *E. coli*, which showed a 1:1 stoichiometry. The localization of EF-Tu revealed a patchy pattern along the sidewalls (again interpreted as extended 'helical' structures) that colocalized with and depended specifically on MreB. Surprisingly, the 'helical structures' formed by EF-Tu were rather static in contrast to the highly dynamic MreB 'helices,' in apparent contradiction to the reported colocalization. Reducing EF-Tu expression by ~60% did not have a detectable effect on translation but induced defects in cell shape (which were not rescued with high Mg^{2+}) and in the localization pattern of MreB. How EF-Tu and MreB affect each other's localization remains mysterious, but the authors proposed that EF-Tu plays a dual role in translation and cell morphogenesis in *B. subtilis*. For cell morphogenesis, the authors proposed that this factor could be an integral part of the cytoskeleton, possibly forming tracks for MreB filaments. A large conceptual gap exists between the observed discrete dots and a postulated static track. However, one could also imagine that localization of EF-Tu at the membrane is required to optimize the coupling of translation with the export of some component(s) of the CW machinery that are associated with MreB.

5. ADDITIONAL CELLULAR FUNCTIONS OF MreB HOMOLOGS IN *B. Subtilis*

In addition to its largely conserved role in cell shape maintenance, MreB is involved in other cellular processes, including motility in *Myxococcus xanthus*, spore formation in *Streptomyces* and chromosome segregation and cell polarity in several bacteria. In *B. subtilis,* two additional functions have been documented, chromosome segregation and localization of viral DNA and replication proteins, with the former being the subject of conflicting reports.

5.1. Role in chromosome segregation

Arguably, the most controversial aspect of the function of MreB-like proteins is their potential role in chromosome segregation. In fact, the same controversy currently exists in *E. coli*, *B. subtilis* and *C. crescentus* (see annexes about *E. coli* and *C. crescentus*). In *B. subtilis*, the first evidence for a link between MreB and DNA segregation were reported by Defeu-Soufo *et al.*, who showed that depleting the cells of MreB leads to the appearance of 25% anucleated cells (while the depletion of Mbl or MreBH leads to a milder, yet considerable, defect of up to 5% and 1% anucleated cells, respectively) (42). The

same group later showed that the origins of replication are randomly distributed in MreBCD-depleted cells (instead of displaying a regular bipolar distribution similar to wild-type cells) and that the termini also lose their specific subcellular localization (42). Similarly, in the absence of *mreB*, an abnormal random distribution of replication foci was observed (via a DnaX-GFP fusion protein) (43). Another series of observations suggested that the state of the chromosome influences the formation of normal MreB filaments. Diffuse cytosolic signals were observed for GFP-MreB in both *parE*$^-$ and *smc*$^-$ anucleated cells (17, 43), which was interpreted as evidence that MreB requires the presence of DNA to assemble into membrane-associated filaments. However, the delocalization of MreB in these mutants may be an indirect consequence of the absence of DNA (e.g., because of the depletion of another short-lived factor). More intriguingly, static filaments of GFP-MreB were observed in *parE*$^-$ cells containing DNA (there were no filaments in cells without DNA). This observation was of particular interest because of the absence of shape defects in the *parE* mutant (78), which suggested that the dynamic localization of MreB is not required for shape control in this context. However, this observation is not consistent with another published result from the same group showing that a point mutation in the phosphate 2 motif of MreB (MreB^{D158A}), which presumably reduces its ATPase activity by analogy to eukaryotic actin, resulted in the formation of static MreB filaments and subsequently to important cell morphology and chromosome segregation defects (see below, phenotype of mutant MreB^{D158A}; (37)). No hypotheses were proposed to explain these phenotypes and discrepancies, but these studies implicate a role for MreB (and Mbl) in chromosome segregation in *B. subtilis*. However, when Formstone and coworkers reported the construction of an in-frame *mreB* mutant that avoids polar effects on the expression of the downstream genes, there was no detectable effect on chromosome segregation (36). Moreover, the chemically or genetically generated anucleated cells presented a wild-type pattern of localization of MreB, and no obvious link between the MreB structures and the nucleoids could be detected (36). Finally, no defects in chromosome segregation were observed in cells lacking all three MreB isoforms. This observation excludes a functional complementation between the 3 isoforms regarding this function (39, 40), conclusively ruling out a significant (if any) role of MreB proteins in chromosome segregation in *B. subtilis.*

What could account for the discrepancies between the Graumann and Errington groups concerning this issue? One of the differences between these two laboratories is the use of two different wild-type strain backgrounds, namely, PY79 (used by Graumann *et al.*) and 168 (used by Errington *et al.*). Although these two strains are parents to each other (in short, PY79 is a prototrophic revertant of 168), they present a significant number of differences (see (79)). For example, strain PY79 possesses a total deletion of 180 kb in its genome relative to strain 168, along with numerous SNPs and a 33 kb region originating from a third *B. subtilis* wild-type strain, leaving room for different or missing genes (e.g., we noticed that PY79 was missing a gene encoding a homolog of topoisomerase III). However, when *mreBs* deletions were transferred from the 168 to the PY79 background, no anucleated cells were observed in the resulting strain (A.C., unpublished data).

5.2. Role in organization of viral DNA replication

For many years, evidence has shown that the replication of prokaryotic genomes occurs at or involves the cytoplasmic membrane (80, 81). Recently, Muñoz-Espín *et al.* (24, 82) proposed that MreB is a primary organizer of phage replication. The replication efficiency of phages SPP1 and ϕ29 of *B. subtilis* and that of phage PRD1 of *E. coli* was severely reduced in *mreB*-like mutants (24). Several components of the ϕ 29 replication machinery, along with ϕ 29 double-stranded DNA, localized in a punctuated helix-like pattern and colocalized with MreB. Furthermore, a direct interaction between MreB and the ϕ 29 membrane-protein p16.7 (required for optimal *in vivo* ϕ 29 DNA replication) was shown using pull-down and bacterial two-hybrid analyses (24). Interestingly, the helical-like localization of p16.7 and the ϕ 29 polymerase was lost and became diffuse in the absence of anyone of the three MreB isoforms. Consistently, replication efficiency was affected in each individual mutant, suggesting that proper localization is mandatory for this function. So far, these are the only proteins that require all three paralogs for proper localization and function and, thus, the first example where functional redundancy may not occur.

6. STRUCTURE AND BIOCHEMICAL PROPERTIES OF MreB PROTEINS

So far, the big picture emerging from the compiled data suggests a major role for MreB proteins in elongation (lateral CW synthesis) through interactions with the CW-synthesizing machinery. However, a question remains: what is their precise function in this process? Is it structural? Regulatory? Enzymatic? One way to address this question is to try to understand the biochemical functions of MreBs. However, except for a few studies on MreB1 from the thermophile *T. maritima* and one recent report on the assembly properties of *B. subtilis* MreB, difficulties in the expression and purification of the MreB proteins have precluded their biochemical characterization. Thus, although some information has been gathered from MreB1 regarding biochemical properties and structure, little data are currently available for *B. subtilis* MreB, and there are no data at all for Mbl and MreBH (or MreBs from other species).

6.1. Biochemistry and structure

When the subcellular localization of MreB and Mbl was reported (9), the crystal structure of MreB1 of *T. maritima* (hereafter referred to as MreB^{Tm}) was almost simultaneously resolved (8), which allowed for several *in vitro* studies and biochemical analysis on MreB^{Tm}. However, after a decade of intense study of MreB proteins in several organisms, in particular *E. coli*, *B. subtilis* and *C. crescentus*, only a single biochemical study on the assembly properties of MreB of *B. subtilis* (hereafter referred to as MreB^{Bs}) has been reported (83). Because *T. maritima* is an extremophile, its lifestyle imposes physical

constrains that could significantly impact both the biochemical properties and/or the functions of MreB. Besides, *T. maritima* is one of the few examples of bacteria that appear devoid of *mreC* despite the presence of *mreB* in their genome and, among these bacteria, the only rod-shaped bacterium (2). Thus, one could wonder to what extent we can extrapolate the data collected from this organism to other species. Moreover, interspecies differences in MreBs may exist at the biochemical level and induce differences in their biological functions. However, information about $MreB^{Bs}$ is so scarce that it is necessary to present it in light of what is known for $MreB^{Tm}$.

6.1.1. MreB1 of *Thermotoga maritima*

Van den Ent *et al.* elucidated the 3D structure of $MreB^{Tm}$ (8) and revealed its remarkable similarity to actin. First, $MreB^{Tm}$ and actin monomers present a complete conservation of their topology, in terms of both the domains that are conserved in all actin superfamily proteins (6) and the variable domains; this conservation is in sharp contrast to other members of the actin superfamily, such as FtsA, Hsp70 and sugar kinases (8). The conservation of secondary and tertiary structures raised the possibility of conserved mechanisms and/or functions. Second, $MreB^{Tm}$ monomers, like actin monomers, are assembled into double-stranded protofilaments. One-dimensional protofilaments of $MreB^{Tm}$ were present in the crystals, and both the orientation of the monomers and the general structure of the filaments were highly similar to those of actin filaments. In this study, $MreB^{Tm}$ filaments in the crystals were straight (8), in contrast to F-actin, which is made of double helical protofilaments. However, recent work from Popp *et al.* showed that both linear and helical $MreB^{Tm}$ protofilaments exist (84). Electron microscopy (EM) revealed frequent $MreB^{Tm}$ bundles (8, 84-86) and also higher-order structures, such as sheets (8, 84) and multilayered sheets of interwoven protofilaments (84). These structures may confer significant mechanical resistance and stiffness if they form in cells. Another stimulating EM observation is that of curled bundles and sometimes closed rings of filaments (8, 85, 86). Although the curvature radius of these structures was much smaller than the cell diameter, these data suggest that $MreB^{Tm}$ protofilaments are not necessarily straight and can spontaneously adopt a curved structure in certain conditions.

Whereas the general ability to polymerize seems to be conserved between F-actin and $MreB^{Tm}$, several differences exist between their biochemical properties. First, $MreB^{Tm}$ presents a critical concentration that is approximately 2 orders of magnitude lower than that of its eukaryotic counterpart (85). This lower critical concentration could be an adaptation to the much lower intracellular concentration of MreB1 protein in *T. maritima* than that of actin in eukaryotic cells (85), but it could also indicate the existence of completely different functions and/or mechanisms between them. Second, unlike F-actin, $MreB^{Tm}$ filaments are unlikely to exhibit dynamical instability (see annex 4)(87). Third, there is no apparent nucleation in the $MreB^{Tm}$ polymerization reaction, which seems to occur through monomer addition only (rather than end-to-end annealing of protofilaments) (85). Fourth, $MreB^{Tm}$ polymerizes equally well in the presence of ATP or GTP (8, 86) whereas F-actin assembly is favored by ATP (88). $MreB^{Tm}$ hydrolyses the two nucleotides at similar rates, and no significant morphological difference exists between ATP- or GTP-induced filaments. The curled filament structures observed by EM (8, 85, 86) may reflect the different states of the monomers inside the protofilament in relation to their nucleotide-bound form. Indeed, although $MreB^{Tm}$ polymerization requires ATP or GTP, the observation that phosphate release is almost simultaneous to polymer assembly and the absence of dynamic instability strongly suggests that ADP- and/or GDP-bound MreB exists in the filament. In a similar manner, the linear or helical protofilaments observed for $MreB^{Tm}$ by Popp *et al.* seem to reflect the bound state of nucleotides: ATP/GTP for helical filament versus ADP/GDP for linear protofilaments (84). Because MreB bound to ATP or GTP displays slightly different properties, one tempting hypothesis is that differential nucleotide binding and hydrolysis could be used to modulate MreB activity for different biological functions.

Finally, the difference between the recent (84, 87) and previous (8, 85, 86) results (i.e., non-linear vs. linear protofilaments) may be due to the use of native instead of tagged recombinant $MreB^{Tm}$ protein, which may retain different specificities relative to the *in vivo* counterpart. Consequently, if such discrepancies are obtained with the same protein from a single species, then relevant interspecies differences in the biochemical properties of MreB proteins likely exist.

6.1.2. MreB of *B. subtilis*

Like $MreB^{Tm}$, $MreB^{Bs}$ polymerized in the presence of divalent cations in a protein concentration-, temperature- and pH-dependent manner, although its sensitivity to divalent cations and temperature was different (83). Also like $MreB^{Tm}$, $MreB^{Bs}$ could bind and hydrolyze both ATP and GTP, but $MreB^{Bs}$ polymerization occurred regardless of the type of bound nucleotide and, surprisingly (unlike for $MreB^{Tm}$ and eukaryotic actin), even in the absence of nucleotide (83). This result is indeed disturbing because nucleotide binding and hydrolysis is an integral part of the actin polymerization process. Although there is no proof that aggregation of $MreB^{Bs}$ (instead of polymerization) did not occur in these experiments, the data suggest that a different mechanism may underlie the polymerization of $MreB^{Bs}$ and raise the question of the purpose of its hydrolase activity (see section 7). Another intriguing conclusion from this study is that, according to the critical concentration reported for $MreB^{Bs}$ and to the properties of the cytoplasm, polymerization should not be favored *in vivo* (83). Thus, only transient and/or localized assembly of MreB may occur *in vivo* in *B. subtilis* cells, potentially depending on the crowding effect and/or on MreB-binding proteins promoting polymerization. Although these findings are extremely interesting, this is the only study on an MreB other than $MreB^{Tm}$ reported so far, and the authors relied almost exclusively on light scattering experiments. Additional studies and other

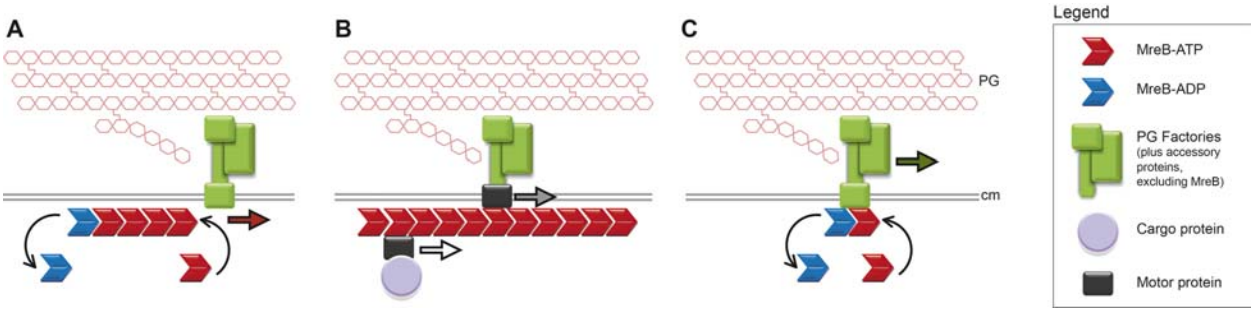


Figure 5. Three ways to explain the MreB-controlled CW synthesis. Until recently, MreBs were proposed to control and motor peptidoglycan (PG) synthesis. Considering the known mechanisms on MreB eukaryotic homolog actin, the polymerization of MreB (through treadmilling) was hypothesized to propel the machinery (Panel A), or CW machinery was hypothesized to use actin filaments as a track using uncharacterized motor proteins (Panel B). Instead, recent results suggest that PG factories are energizing and thus dragging MreBs (Panel C). In this model, several alternatives exist: 1) MreB acts as a platform for PG machinery assembly and/or for recruitment of precursors or 2) MreB is an anchor that restrains the speed and direction of PG factories, allowing the efficient synthesis of parallel strands (these two hypotheses are non-exclusive). Note that the function of nucleotide hydrolysis is presently unclear.

biochemical approaches are needed to convincingly and fully characterize *B. subtilis* MreB (and Mbl and MreBH) at the biochemical level.

6.2. Treadmilling

The structural polarity (asymmetry) of actin filaments allows a directionality of polymerization/depolymerization known as treadmilling (see annex 4). During 'steady-state treadmilling,' actin subunits (globular actin, G-actin) constantly assemble at one end of the filaments (in their ATP-G-actin form) and simultaneously disassemble at the other end (in their ADP-G-actin form). This process results in the directional translocation of the filaments, which is the basis of actin-driven movement in the cell, while the total F-actin cellular content and length of the filaments remains constant. Treadmilling was proposed to explain the continuous movement of bundles of individual MreB and Mbl filaments through *B. subtilis* cells (17) and the dynamics of MreB-YFP in a quantitative single-molecule study in *C. crescentus* (89) (Figure 5A). Unfortunately, the biochemistry has not yet tackled the question of whether some asymmetry exists between the extremities of the MreB filaments. However, we recently used cytological approaches (partial and inverse FRAP- see annex 4) to test the turnover within the MreB/Mbl motile patches observed using TIRFM (see above) to investigate whether treadmilling was the mechanism underlying their movement as expected. Surprisingly, we showed that the processive movement of MreB and Mbl patches is not treadmilling-driven but instead powered *via* cell wall synthesis (32). Because the bright patches observed with TIRFM are probably short filaments or bundles of filaments, these experiments do not formally exclude the possibility that treadmilling exists within individual MreBs filaments inside the patches. However, if such turnover occurs, its timescale must be different (much slower) than that of the movement of MreB patches across the cell, ruling out treadmilling as the primary mechanism driving their motion.

In toto, information from biochemical and cytological studies has had a limited impact on our understanding of the biochemical properties of MreB proteins. In addition, MreBs from *B. subtilis* and/or other bacteria may behave differently from that of *T. maritima*, which is thus far the main model for MreB biochemistry. Furthermore, in light of the most recent findings (32, 33, 35, 83), the similarity to eukaryotic actin and the relevance of polymerization and nucleotide hydrolysis for the function of MreB are questionable.

7. OPEN QUESTIONS AND CONCLUDING REMARKS

7.1. To be or not to be helical: that is the question

As we discussed earlier, the recent results describing MreBs dynamics and localization came as a surprise because the helical pattern has been an accepted fact for a full decade. In retrospect, the past years have produced its share of data that, without disproving the existence of a helix, would perfectly fit a model without. This does not mean that there are no helices but that the evidence in support of helices is decreasing, at least in *B. subtilis*.

Considering the two recent reports using high-resolution techniques (32, 33), a provocative hypothesis is that the increased depth of field attained using conventional epifluorescence microscopy produces misleading images that could be amplified by post-processing treatments that had been so far (mis)interpreted as 'helical' but that MreBs actually localize only as discrete peripheral patches moving independently from each other. Therefore, the spacing between the moving patches may create a pattern that gives the illusion of a helix in motion. Another possibility is that the discrete MreB patches have a higher level of organization, forming a large-scale helix. This organization would require the coordinated movement of the patches via the existence of an underlying, possibly helical, structure. The nature of this structure is for the moment elusive, but some proteins, such as EF-Tu, could be considered as a natural candidate. Preliminary work does not support such

a model (our unpublished data), but this point certainly requires deeper study.

Circumferentially moving patches of MreB have been now observed in vegetatively growing cells of *E. coli* and *C. crescentus* (32, 35) as well, suggesting that the MreBs of Gram-positive and Gram-negative bacteria share a common behavior. However, in *E. coli*, these observations are in sharp contrast to the striking images (unmatched in *B. subtilis* or other bacteria) of long helices spanning the length of the cells that were first reported by the Rothfield group and more recently by Strahl *et al.* (90-93). These impressive helical patterns provide evidence that, at least in *E. coli* and maybe under specific conditions, long helical MreB filaments may exist.

7.2. Mechanism underlying the control of CW synthesis by MreBs

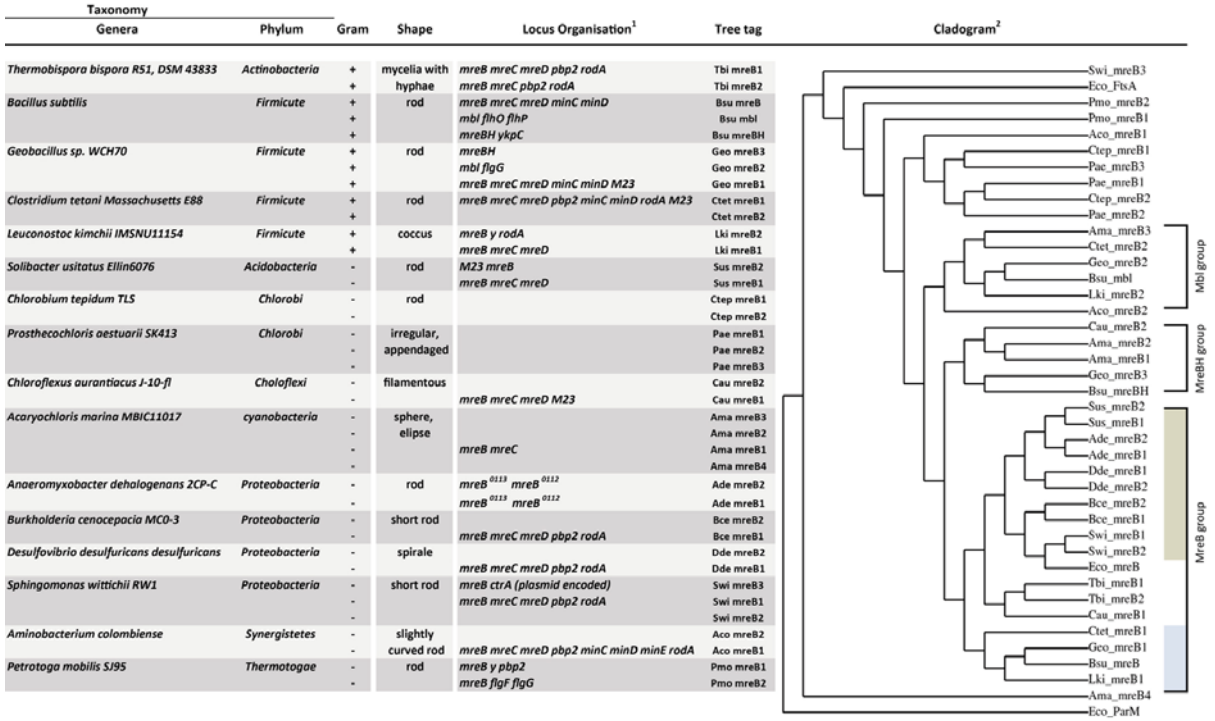
The observation that treadmilling (polymerization) is not the motive force for MreB filaments changes the prevailing view of the possible mechanism underlying the role of MreBs in the control of CW morphogenesis. MreBs do not form a force-generating "cytoskeleton" that pushes the CW elongation machinery or a track that the machines would follow (propelled by putative bacterial motor proteins analogous to eukaryotic myosin) (Figure 5A and B, respectively). Instead, the predominant driving force for the processive movement of MreB presumably comes from the incorporation of PG precursors into the existing sacculus. These findings force us to reconsider the biological function of the essential MreB proteins. We showed that PbpH and PBP2a displayed a directional, processive movement in the presence of MreB but moved randomly and much faster (forming rapidly diffusing patches) in its absence (32). Thus, MreB filaments may serve as mechanical clamps that restrict the mobility of the elongation complexes along the membrane and give them directionality, possibly by preventing the backward movement in a Brownian ratchet-like process (32) (Figure 4-5C). This movement would be reminiscent of plant cell morphogenesis, where cortical microtubule arrays direct cell morphogenesis by organizing cellulose synthase complexes. In the current plant cell model, microtubules serve as passive constraints, forming channels that confine the lateral movement of cellulose synthases (94). Alternatively, or rather coincidently, MreB filaments may serve as passive platforms in the cytoplasm for the recruitment and export of PG precursors, coordinating them with the CW-synthesizing machines. Indeed, in *C. crescentus,* MreB directs the localization of the membrane-associated protein MurG, which catalyzes the final step in the synthesis of the PG precursor molecules in the cytoplasm and of several other cytosolic murein biosynthetic enzymes, such as MraY, MurB, MurC, MurE and MurF (95, 96). Consistently, we have recently found that MreB-associated complexes involved in the synthesis of PG precursors also exist in *B. subtilis* (manuscript in preparation). Another appealing hypothesis is that MreB polymers could have active regulatory function(s), e.g., they could regulate the delivery of the PG precursors to sites of active PG synthesis. Finally, the activity of PBPs must be tightly controlled in space and time, depending on the growing conditions and the resources available. Interestingly, two point mutations thought to (although not proved to) reduce the ATPase activity of MreB did not affect MreB dynamics but induced shape defects in *B. subtilis* ((33, 37); Dominguez unpublished), consistent with an inhibition of MreB function. These observations are consistent with biochemical data that suggest that *B. subtilis* MreB polymerization is disconnected from its hydrolase activity (83). Thus, a tempting hypothesis is that the activity of PBPs is somehow dependent on the ATPase (or GTPase) activity of MreBs. The elucidation of the current controversial role of MreB polymerization and its ATPase/GTPase activity (32, 33, 37, 97) seems critical.

7.3. Why have several *mreB* paralogs?

A puzzling question that remains is why *B. subtilis* and most Gram-positive organisms possess several *mreB* paralogs that act redundantly (or functionally converge) whereas *E. coli*, *C. crescentus* and most Gram-negative bacteria have only one copy of *mreB* (2, 9, 55). The hypotheses generally proposed for the interspecies variability in number of paralogs are 1) that Gram-positive bacteria have a substantially thicker and more complex cell wall than Gram-negative bacteria, which requires the joint action of several MreBs and/or 2) that each *mreB* paralog may have specialized functions or interacting partners.

In contrast to the sacculus of Gram-negative bacteria, which is composed of a single layer of PG chains (for review, see (11, 98)), Gram-positive sacculi are made up of several PG layers and additionally contain wall teichoic acids (WTAs) in roughly equal proportions to the PGs, which are also essential for cell shape (see section 4.2.). The localization pattern of proteins involved in several steps of WTA synthesis and export (Tag proteins) is similar to that of MreBCD proteins and to the pattern of insertion of new PGs reported using Van-FL staining. In addition, two hybrid experiments revealed direct interactions between several Tag proteins and MreCD (58), which also interact with several PBPs (71). These findings suggested that, in *B. subtilis* at least, WTA-synthesizing multi-enzyme complexes may exist and be associated with MreB-associated-PG-synthesizing complexes at the membrane (58). The coordinated action of several MreBs may be necessary to organize such complexes and couple their activities for balanced CW composition and growth.

The alternative (although not mutually exclusive) possibility that each paralog bears specialized functions was supported by observations that in *B. subtilis mreB, mbl* and *mreBH* mutants behave differently and display specific cell-shape defects and that some combination of mutants could be constructed (*ΔmblΔmreBH*) while others (*ΔmreBΔmbl* and *ΔmreBΔmreBH*) could not. The experiments of Kawai and coworkers have nevertheless been critical regarding this issue. Normal rod-shaped growth and viability could be supported by any of the three paralogs in the absence of the other two when expressed at the appropriate level, indicating that functional redundancy exists and that any individual MreB is sufficient to accomplish the primary task of cell-shape control in *B. subtilis* (40). So why would each mutant have a specific



Taxonomy						Cladogram[2]
Genera	Phylum	Gram	Shape	Locus Organisation[1]	Tree tag	
Thermobispora bispora R51, DSM 43833	*Actinobacteria*	+	mycelia with hyphae	*mreB mreC mreD pbp2 rodA*	Tbi mreB1	Swi_mreB3
		+		*mreB mreC pbp2 rodA*	Tbi mreB2	Eco_FtsA
Bacillus subtilis	*Firmicute*	+	rod	*mreB mreC mreD minC minD*	Bsu mreB	Pmo_mreB2
		+		*mbl flhO flhP*	Bsu mbl	Pmo_mreB1
		+		*mreBH ykpC*	Bsu mreBH	Aco_mreB1
Geobacillus sp. WCH70	*Firmicute*	+	rod	*mreBH*	Geo mreB3	Ctep_mreB1
		+		*mbl flgG*	Geo mreB2	Pae_mreB3
		+		*mreB mreC mreD minC minD M23*	Geo mreB1	Pae_mreB1
Clostridium tetani Massachusetts E88	*Firmicute*	+	rod	*mreB mreC mreD pbp2 minC minD rodA M23*	Ctet mreB1	Ctep_mreB2
		+			Ctet mreB2	Pae_mreB2
Leuconostoc kimchii IMSNU11154	*Firmicute*	+	coccus	*mreB y rodA*	Lki mreB2	Ama_mreB3
		+		*mreB mreC mreD*	Lki mreB1	Ctet_mreB2
Solibacter usitatus Ellin6076	*Acidobacteria*	-	rod	*M23 mreB*	Sus mreB2	Geo_mreB2
		-		*mreB mreC mreD*	Sus mreB1	Bsu_mbl
Chlorobium tepidum TLS	*Chlorobi*	-	rod		Ctep mreB1	Lki_mreB2
		-			Ctep mreB2	Aco_mreB2
Prosthecochloris aestuarii SK413	*Chlorobi*	-	irregular, appendaged		Pae mreB1	Cau_mreB2
		-			Pae mreB2	Ama_mreB2
		-			Pae mreB3	Ama_mreB1
Chloroflexus aurantiacus J-10-fl	*Cholorflexi*	-	filamentous		Cau mreB2	Geo_mreB3
		-		*mreB mreC mreD M23*	Cau mreB1	Bsu_mreBH
Acaryochloris marina MBIC11017	*cyanobacteria*	-	sphere, elipse		Ama mreB3	Sus_mreB2
		-			Ama mreB2	Sus_mreB1
		-		*mreB mreC*	Ama mreB1	Ade_mreB2
		-			Ama mreB4	Ade_mreB1
Anaeromyxobacter dehalogenans 2CP-C	*Proteobacteria*	-	rod	$mreB^{0113}$ $mreB^{0112}$	Ade mreB2	Dde_mreB1
		-		$mreB^{0113}$ $mreB^{0112}$	Ade mreB1	Dde_mreB2
Burkholderia cenocepacia MC0-3	*Proteobacteria*	-	short rod		Bce mreB2	Bce_mreB2
		-		*mreB mreC mreD pbp2 rodA*	Bce mreB1	Bce_mreB1
Desulfovibrio desulfuricans desulfuricans	*Proteobacteria*	-	spirale		Dde mreB2	Swi_mreB1
		-		*mreB mreC mreD pbp2 rodA*	Dde mreB1	Swi_mreB2
Sphingomonas wittichii RW1	*Proteobacteria*	-	short rod	*mreB ctrA (plasmid encoded)*	Swi mreB3	Eco_mreB
		-		*mreB mreC mreD pbp2 rodA*	Swi mreB1	Tbi_mreB1
		-			Swi mreB2	Tbi_mreB2
Aminobacterium colombiense	*Synergistetes*	-	slightly curved rod		Aco mreB2	Cau_mreB1
		-		*mreB mreC mreD pbp2 minC minD minE rodA*	Aco mreB1	Ctet_mreB1
Petrotoga mobilis SJ95	*Thermotogae*	-	rod	*mreB y pbp2*	Pmo mreB1	Geo_mreB1
		-		*mreB flgF flgG*	Pmo mreB2	Bsu_mreB
						Lki_mreB1
						Ama_mreB4
						Eco_ParM

Mbl group
MreBH group
MreB group

Figure 6. MreB paralogs are present in many phyla. A BLAST search revealed that several species present at least two paralogs of *mreB*, not only among Gram-positive bacteria, but also in many different phyla. [1] Only genes with significant and relevant homology are indicated. Empty cells indicate that *mreB* is surrounded by "y" genes. [2] A cladogram was derived from a ClustalW alignment using the amino-acid sequences of the 38 MreB homolog listed on the left part of the figure, MreB from *E. coli* K12 and the sequence of two proteins of the actin superfamily (FtsA and ParM) from *E. coli* K12. On the right side of the cladogram, the blue bar indicates Firmicutes species, and the green bar indicates Proteobacteria species.

phenotype if any of the paralogs can do the job? Kawai *et al.* observed that any of the isoforms could complement a triple mutation (i.e., *ΔmreBΔmblΔmreBH),* but the required expression level was different for each isoform (the concentration of the inducer required was minimal for MreB and maximal for MreBH), indicating that they do not function with the same efficiency. Thus, a tempting hypothesis is that in standard growing conditions, each paralog contributes to a different fraction of the overall "shape control" function, with MreB being the major contributor, closely followed by Mbl, and MreBH plays only a minor role. This idea would be consistent with *mreB* and *mbl* being essential and their mutants easily picking up suppressors while *mreBH* mutants are viable and barely affected. It would also explain why only the Δ*mbl*Δ*mreBH* mutant is viable while the combinations involving *mreB* are not. However, each isoform may still have non-essential specific role(s) in addition to their joint contribution to CW synthesis and interact with specific partners. Indeed, MreBH specifically interacts with LytE (10), and each single isoform differentially sustained growth and morphology under various stresses (40), supporting the notion of partially redundant but specialized MreBs.

Finally, a rapid survey of annotated genomes indicates that there are also Gram-negative bacteria in almost every phylum with at least two *mreB* paralogs (with up to four in the cyanobacteria *Acaryochloris marina*), with numerous examples in the genera *Burkholderia* (β-proteobacteria) and *Desulfovibrio* (δ-proteobacteria) (Figure 6). Gram-negative species with several *mreB* paralogs may share some complex CW features to which they independently adapted by multiplying the number of *mreB* copies in their genome.

7.4. Additional functions of MreBs?

After a decade studying MreBs in *B. subtilis, E. coli* and *C. crescentus,* it is fairly established that these proteins are mainly involved in the control of CW synthesis as described above. In addition to this role, MreBs position DNA and proteins other than those required for wall homeostasis. The examples in *B. subtilis* are still scattered and thus far limited to phage ϕ29 proteins (24, 82), but proteins displaying a "helical-like" discrete pattern along the sidewalls seem to be now systematically examined for a potential localization dependency on MreB. This systematic check is revealing a growing number of examples that involve MreB in several cellular processes, such as cell motility, pilus production or cell polarity (see also Annexes on *E. coli* and *C. crescentus* and Table 1). Three additional functions have been hypothesized for MreB: the formation of tracks that could be followed by motor proteins (Figure 5B), a contribution to cell stiffness and chromosome segregation. As we described earlier, the role of MreB in chromosome segregation is rather controversial. However, it is now established that in *B.*

subtilis (as in *E. coli*), MreB is not essential for this function in standard laboratory conditions. The two other postulated roles, clearly inspired by those of F-actin in eukaryotic cells, have not been the subject of reports thus far in *B. subtilis*, although several groups have been looking for interacting partners of MreB, including potential motor proteins (so far not identified) (22, 40). The possibility that MreBs have a direct structural role and participate in the resistance of *B. subtilis* to physical constraints was suggested by the observation that MreB, Mbl and MreBH formed stable long filamentous structures that could push the membrane and deform the cells when overexpressed in *E. coli* (12). It is difficult, however, to extrapolate these results to *B. subtilis*, where no such stable structures are formed by the isoforms. A structural role was also suggested by Wang *et al.*, who reported a decreased stiffness of *E. coli* cells when (*E. coli*) MreB was depolymerized (13). A caveat to this finding lies in the use of an MreB-depolymerizing drug (namely, A22- see annex 4) that was recently shown to have several MreB-independent effects (99), weakening Wang's conclusions. In addition, the observation that MreB forms dynamic patches in *E. coli* and *C. crescentus* cells, like in *B. subtilis* (32, 33, 35), is difficult to reconcile with such a mechanical role contributing to CW stiffness. One could nevertheless imagine that under some growth conditions, MreB may switch from a highly dynamic behavior to stabilize into more static stable filaments.

7.5. Concluding remarks

Over the last decade, work on MreBs has been strongly influenced by knowledge on eukaryotic actin. Research has focused on finding similar structures (stable polymers), biochemical behavior (treadmilling), biological functions and interacting partners. However, current data suggest that, despite a conserved 3D fold and filament structure, MreB features significant differences relative to its famous sibling. The cellular functions and assembly properties of MreBs also differ significantly between bacterial species (Table 1), in contrast with the high degree of conservation of actins in eukaryotic cells.

The major challenges in the field are now to obtain a global understanding of the regulatory processes controlled by MreBs, their targets and how all these proteins interact together in functional complexes. For this, using a combination of classical approaches with novel high-resolution imaging techniques, high-throughput techniques, quantitative measurements in live cells, biophysical measurements and mathematical modeling will be needed. New hypotheses and approaches need to be adopted to tackle the challenging questions we are facing. Is nucleotide binding and hydrolysis required for MreB polymerization and/or for additional purposes? Are nucleotides and polymerization important for the biological function(s) of MreBs? What is the molecular mechanism underlying the role of MreBs in cell shape control? Are there MreB regulatory factors? Last but not least, should MreB, despite its structural similarity to actin, be considered as a "cytoskeletal" protein after all?

8. ANNEX 1: ACTIN IN BRIEF

In eukaryotes, actin is a member of a functional family of cytoskeletal proteins, along with tubulin and intermediate filaments. All three form dynamic filaments that are important for their cellular functions and/or have a clear mechanical or structural role within the cell. Actin microfilaments (filamentous actin or F-actin as opposed to globular actin or G-actin) are involved in a variety of biological processes such as cytokinesis, cell movement or organelle distribution and are consequently responsible for numerous diseases (100-102). As part of the cytoskeleton, F-actin forms a scaffold, providing a mechanical support that is involved in maintaining cell shape. Deformation of the F-actin network promotes membrane rearrangement (i.e., during phagocytosis) and cellular movement (101, 103, 104). Moreover, F-actin contributes to intracellular trafficking either by providing direct force through its polymerization or by being used as tracks for motor proteins to travel along, generating force on the microfilaments and transporting cargo such as proteins, mRNA or organelles (mitochondria, chloroplasts, etc) along the cell length (102, 105-107). The actin filament dynamics result from the ability of actin to polymerize/depolymerize into double-helical filaments. This highly regulated process depends on its biochemical ATPase activity and the action of myriad actin-binding proteins (ABPs) that nucleate, bundle, stabilize, destabilize, anchor to the membrane or link actin to other cytoskeletal filaments (108). An interesting feature of F-actin is that it is structurally, and consequently kinetically, asymmetric, and polymerization occurs at a faster rate at one extremity, resulting in a process known as actin treadmilling (108).

9. ANNEX 2: OUTLOOK OF MREB IN *E. COLI*

E. coli is arguably the most studied model for MreB after *B. subtilis,* with a rich bibliography on the link between cell shape and division and particularly on the controversial role in chromosome segregation (for recent reviews see (109, 110)). Similar to *B. subtilis*, *mreB* (and *mreC* and *mreD*, also in the same operon) is essential in this bacterium, and MreB depletion leads to round cells, ultimately followed by cell lysis (72).

9.1. MreB cellular organization

The distribution pattern reported for *E. coli* MreB mainly resembles that observed in *B. subtilis*: a punctuated pattern with pairs of dots or small bands generally described as helical (18, 72, 90, 111, 112). With the noticeable exception of studies from the Rothfield lab (90, 92, 93), in which impressive extended helices winding around the cell between the two poles were observed (using a non-functional YFP-MreB fusion though and upon massive deconvolution), all other reported images of MreB localization are consistent with the model recently established in *B. subtilis* and discussed in this review. Indeed, recent work from the Gitai group showed circumferential movement of MreB short filaments, perpendicular to the long axis of *E. coli* cells (35), consistent with our observation of *E. coli* MreB by TIRFM (32). These findings are consistent with the observation of

E. coli cells at macromolecular resolution using electron cryotomography, where no long helical filaments were detected along the cytoplasmic membrane (34). Original reports showed a diffuse membrane pattern of localization of MreB in MreC- or MreD-depleted *E. coli* cells (72), but a recent study using the functional MreB–RFP sandwich fusion revealed that MreB still localized to characteristic small discrete foci in the absence of MreC or MreD (111). A specific feature that was, however, never observed in the Gram-positive model counterpart is the relocalization of MreB into rings, often located near mid-cell (90, 92, 93). These transverse MreB ring structures have been proposed to be specialized intermediates in the segregation of MreB during the cell cycle (90, 92, 93), although the requirement for a cell cycle-dependent dynamic relocalization of MreB from "helical" to ring structures remains to be elucidated.

RodZ, a new component of the cytoskeleton with a rod shape-determining function, is widely conserved in bacteria and was reported simultaneously in *E. coli* and *C. crescentus* in three independent studies (2, 111, 113). In *E. coli*, RodZ localizes in punctuated dots along the sidewalls, colocalizing with MreB (111, 113). The formation of RodZ and MreB 'helical' structures appeared interdependent; MreB mislocalizes (making large clusters) in the absence of RodZ, and reciprocally, RodZ is widely distributed in the membrane in cells lacking MreB (while it is unaffected in cells mutant for *mreC* and *mreD* or *pbp2* and *rodA*). These findings strongly suggested the existence of a RodZ/MreB complex, which was supported by *in vitro* and *in vivo* experiments with *T. maritima* proteins (114). These authors suggested that RodZ may be an alternative candidate to MreC and MreD to bridge MreB to the CW synthetic machinery.

9.2. MreB and CW synthesis

A putative link between MreB and the CW synthetic machinery has not been investigated in this bacterium until recently (contrary to *C. crescentus*; see annex 3), although the PG synthesizing enzyme PBP2 was known to be a primary determinants of the rod shape of *E. coli* (115). The first *E. coli* report on this topic, which was published concomitantly with a similar study in *C. crescentus* (95), showed that MurG (catalyzing the last cytoplasmic step of PG precursor synthesis) is tightly associated with the membrane and localizes in MreCD-dependent foci along the lateral CW (116). MurG became cytosolic in a *ΔmreB* mutant or in the presence of A22 but not in *Δpbp2* spherical mutant cells. Reinforced by immunoprecipitation data indicating a link between MreB, MraY and MurG, these data prompted the authors to suggest that not only the CW assembling machinery in the periplasm but also the cytosolic enzymes upstream in the pathway are complexed with MreB. Earlier this year, it was shown that MreB forms patches rotating perpendicularly to the long axis of the cells (32, 35), similar to MreB motion in *B. subtilis* (32, 33). van Teefelen and coworkers also showed that depletion of diaminopimelate (or Dap, a component of PG) and the specific inhibition of MurA (essential PG-subunits synthesis enzyme) or PBP2 using antibiotics leads to an arrest of MreB dynamics. This observation suggests the existence of a coupling between MreB and the CW synthesis machinery, as proposed for *B. subtilis*.

9.3. MreB and chromosome segregation

A large fraction of the studies on *E. coli* MreB have focused on its role in chromosome segregation. As in *B. subtilis*, reports have become more and more contradictory over the years, and the proposed function remains controversial. The community currently leans towards an absence of a significant role for MreB proteins in this process (to arrive at your own opinion on the topic, see (18, 111, 117-120)).

9.4. Additional functions of MreB

Recently, it was shown that PspA and PspG (phage shock proteins induced by a loss of proton motive force - pmf) display discrete and dynamic localizations in *E. coli* (121). While some complexes stay trapped to the cell poles, others are proposed to maintain the pmf and traffic rapidly along the lateral CW between the poles. Evidence was presented that MreB may be required for both the dynamic patchy localization and the activity of PspA and PspG. Notably, however, this role of MreB was tested indirectly using A22, which unfortunately has additional MreB-independent side effects (99). Thus, although an MreB-independent localization of Psp proteins cannot be excluded at this point, this constitutes a stimulating example of MreB controlling the activity and localization of proteins apparently not involved in CW synthesis.

10. ANNEX 3: OUTLOOK OF MREB IN *C. CRESCENTUS*

C. crescentus has also been the subject of many publications in the field related to its so-called "cytoskeletal" proteins: the actin-like MreB, the tubulin-like FtsZ and specifically the intermediate filament-like protein Crescentin (for a review see (110)). MreB, MreC and MreD are also essential in *C. crescentus*, and their depletion leads to distinct shape defects: a characteristic swollen "lemon-shape" aspect for cells lacking MreB, enlargement for cells lacking MreC (also described as "lemon-shape" in some reports, suggesting the existence of intermediate phenotypes) and round for MreD-depleted cells (19, 95, 96, 122, 123).

9.1. MreB cellular organization

MreB, MreC and MreD localize in a similar manner, with bands oriented parallel to the long axis of the cell or patches along the cylinder (19, 95, 122-124). As in *E. coli,* the use of deconvolution in early studies showed structures resembling helices that, together with the description of the localization pattern of MreB in *E. coli* and *B. subtilis*, drove researchers to systematically describe the discrete punctuate localization of MreBCD proteins as "helical." Recently, we showed using TIRFM that MreB localizes to discrete mobile patches along the sidewalls of *C. crescentus* cells too. No long helices could be detected using this method or electron cryotomography (32, 34). A distinctive feature of *C. crescentus* MreB relative to MreC and MreD (and MreBs of *B. subtilis*) is that the sidewall

localization varies with cell cycle, with the formation of FtsZ-dependent MreB rings at mid-cell at the time of division (19, 123). Finally, in *C. crescentus,* MreB and MreD have an interdependent colocalization, but MreC does not localize with them; its localization is maintained in the absence of MreB or MreD, suggesting independent structures (96, 124).

9.2. MreB and CW synthesis

Most studies on *C. crescentus* MreB have focused on its link to the CW synthesis machinery. Indeed, although the first hints were revealed with the use of Van-FL in *B. subtilis*, the interaction between the CW machinery (PBP2) and the MreB "cytoskeleton" was uncovered in *C. crescentus* (19). Localization patterns similar to the patterns of MreB have since been reported for PBP2, MltA and MipA (lytic factors inserted in the outer membrane) for several proteins involved in PG precursor synthesis (MurBCDEFG, MraY), RodZ and the Van-FL staining pattern (2, 19, 95, 96). In addition, the determination of the localization dependency using genetic approaches and identification of protein-protein interactions (by yeast double hybrid and pull-down experiments) have established a model in which MreB interacts with, couples and controls the localization of two sets of proteins: 1) MreC and periplasmic CW synthesizing proteins, such as PBPs and autolysins, and 2) MreD and cytosolic enzymes involved in PG precursor synthesis (Mur proteins and MraY). In this model, the membrane proteins RodZ and RodA may couple the two complexes. In *C. crescentus,* RodA is required for shape control and PG synthesis (based on Van-FL staining) (95), where it interacts with several proteins of the "cytosolic complex" (MreB, MurG, MraY) (95, 96). However, absence of RodA affects neither MreB nor PBP2 localization (95). In *C. crescentus,* RodZ interacts with several proteins of the "cytosolic complex" (96), although its pattern of localization was significantly different (2). Furthermore, RodZ switched from polar to mid-cell localization during the cell cycle (2), suggesting a transient association with MreB. Moreover, RodZ presents a localization pattern totally different from what was described in *E. coli* (111, 113), which may question a complete conservation of functions.

9.3. MreB and chromosome segregation

As in other bacteria, a role of *C. crescentus* MreB in the initiation of chromosome segregation was proposed (123). However, the same group is now reporting that this function is important only under specific growth conditions (125) and that the Par system is actually the main contributor to this process (125, 126).

9.4. Additional functions of MreB

Work from Gitai and collaborators, which are probably the earliest studies on *C. crescentus* MreB, largely focused on its role in cell polarity. *C. crescentus* is a bacterium with a distinctive and complex cell cycle involving the differentiation of two cell types at each round of division. The resulting cells have different fates and morphological features, one developing a polar flagellum, while the other remains anchored to the surface by a polar appendage called the stalk. Thus, the polarity in *C. crescentus* cells is critical, and numerous proteins re-localize to the poles during the developmental process. MreB is critical for polar determination, and numerous developmental factors (PleC, DivJ, DivK, CckA) are mislocalized when MreB is depleted (123). Not surprisingly, stalk formation was also impaired in MreB-depleted cells, although it seems that this defect lies in CW elongation, which also requires RodA and PBP2 (127).

11. ANNEX 4: DEFINITIONS

Autolysin: extracytoplasmic enzymes with lytic activity against PG and either amidase or peptidase activity.

A22: MreB-depolymerizing drug. Co-crystallization of A22-bound MreB shows an interaction of A22 with the nucleotide binding pocket of *E. coli* MreB, suggesting an effect on MreB binding or hydrolysis of nucleotides.

BiFC: *bimolecular fluorescence complementation*. Fluorescence microscopy technique used to show direct interaction between two proteins *in vivo*, using protein fusions to two complementary fragments of a single fluorophore (usually YFP). When an interaction between the two candidate proteins occurs, the 2 halves of the fluorophore get into close proximity, which allows the reconstituted reporter to fluoresce.

Cell wall: single- (Gram-negative bacteria) or multi-layered (Gram-positive bacteria) rigid scaffold located outside of the cytoplasmic membrane of most bacteria, composed of cross-linked peptidoglycan, teichoic and lipoteichoic acids (in Gram-positive bacteria) and associated proteins.

Cytoskeleton: complex and dynamic network of filamentous, often cytoplasmic (but interacting extensively with cellular membranes) proteins that provides the cell with mechanical strength and shape and plays important roles in cell division and intracellular transport.

Dynamic instability: characteristic of some eukaryotic cytoskeletal proteins (microtubules and actin) that entails switching from polymerization to the disassembly from the same extremity of a polymer. The switch to depolymerization is a tightly controlled process also called catastrophe.

FRAP: *fluorescence recovery after photo-bleaching*. Fluorescence microscopy technique used to measure diffusion and/or active movement of fluorescent molecules, taking advantage of the photosensitivity of fluorophores (usually GFP). In this technique, a region of interest (ROI) is exposed to a very intense light that produces the irreversible loss of the fluorescent properties ("bleaching") of the fluorophores. In the absence of *de novo* synthesis of the fluorophore, the recovery of fluorescence in the ROI over time involves the transfer of fluorescent molecules from adjacent zones. Inverse FRAP (iFRAP) consists of bleaching most

of a cell, leaving only a small unbleached ROI. In this case, movement of molecules should oppositely dilute the fluorescent signal of the spared region.

FRET: *forster resonance energy transfer*. Fluorescence microscopy technique used to show the interaction between two proteins *in vivo* using protein fusions to two different fluorophores. The FRET phenomenon occurs when a light-excited fluorophore (usually CFP) transmits its energy to a second fluorophore (usually YFP), instead of emitting a photon. This transfer happens only when a very close proximity (<8-10 nm) between fluorescent molecules is achieved.

PBP: *penicillin-binding protein*. Extracytoplasmic enzymes with transglycosidase and/or transpeptidase activity involved in PG synthesis and autolytic enzymes with endopeptidase activity that are bound and inhibited by penicillin (hence their name).

Peptidoglycan (PG, also referred to as Murein): main component of the bacterial cell wall (with TA in Gram-positive bacteria). Polymer of glycan chains alternating residues of N-acetyl-muramate and N-acetylglucosamine, cross-linked by peptide bridges.

Sacculus: macromolecule of peptidoglycan that envelopes the entire bacterial cell, forming a 3D mesh-like, load-bearing scaffold that maintains cell shape and provides the cell with physical integrity and mechanical strength. The sacculus is located outside the cytoplasmic membrane, outside the cell in Gram-positive bacteria and in the periplasm in Gram-negative bacteria.

Teichoic acids (TAs, also known as anionic polymers): main components of the cell wall in Gram-positive bacteria (together with PG), accounting for half of its content. Polymers of glycerol or ribitol phosphate cross-linked by phosphodiester bonds. TAs includes wall-TA (WTA) covalently linked to PG and lipoTA (LTA) anchored to the membrane.

TIRFM: *total internal reflection fluorescence microscopy*. This advanced technique of fluorescence is based on the use of an induced evanescent wave to excite fluorophores in a thin region (100-200 nm) of the sample immediately adjacent to the glass coverslip. This technique allows reduction of the background fluorescence (which improves the signal-to-noise ratio and, consequently, spatial resolution), a better temporal resolution and, *in fine*, a strongly improved qualitative and quantitative analysis of protein dynamics within close proximity to the coverslip, i.e., close to the cell surface.

Treadmilling: process of polymerization at a filament end, while its second extremity undergoes depolymerization, resulting in a directional translocation of the polymer. This process results from the favored polymerization at one end of a filament while subunits are removed from the other end.

12. ACKNOWLEDGMENTS

Among all prokaryotic cytoskeletal proteins, the field of the actin-like MreB proteins has seen tremendous development in the last decade and has just seen important breakthroughs this year. Some publications related to MreBs could not be cited here because of length constraints. We apologize to those authors whose work contributed to the field but could not be included, or were cited only indirectly in this review. We thank L. Slamti for the critical reading of the manuscript and C. MacKichan for just-in-time proof-reading. This work was supported by the ANR (ANR-08-JCJC-0024–01) for RCL and the Marie Curie IRG (249018) for AC.

13. REFERENCES

1. M. T. Cabeen & C. Jacobs-Wagner: The bacterial cytoskeleton. *Annu Rev Genet*, 44, 365-92 (2010)

2. S. A. Alyahya, R. Alexander, T. Costa, A. O. Henriques, T. Emonet & C. Jacobs-Wagner: RodZ, a component of the bacterial core morphogenic apparatus. *Proc Natl Acad Sci U S A*, 106, 1239-44 (2009)

3. M. Doi, M. Wachi, F. Ishino, S. Tomioka, M. Ito, Y. Sakagami, A. Suzuki & M. Matsuhashi: Determinations of the DNA sequence of the *mreB* gene and of the gene products of the mre region that function in formation of the rod shape of *Escherichia coli* cells. *J Bacteriol*, 170, 4619-24 (1988)

4. P. A. Levin, P. S. Margolis, P. Setlow, R. Losick & D. Sun: Identification of *Bacillus subtilis* genes for septum placement and shape determination. *J Bacteriol*, 174, 6717-28 (1992)

5. M. Wachi, M. Doi, Y. Okada & M. Matsuhashi: New mre genes *mreC* and *mreD*, responsible for formation of the rod shape of *Escherichia coli* cells. *J Bacteriol*, 171, 6511-6 (1989)

6. P. Bork, C. Sander & A. Valencia: An ATPase domain common to prokaryotic cell cycle proteins, sugar kinases, actin, and Hsp70 heat shock proteins. *Proc Natl Acad Sci U S A*, 89, 7290-4 (1992)

7. A. Mukherjee, K. Dai & J. Lutkenhaus: *Escherichia coli* cell division protein FtsZ is a guanine nucleotide binding protein. *Proc Natl Acad Sci U S A*, 90, 1053-7 (1993)

8. F. van den Ent, L. A. Amos & J. Lowe: Prokaryotic origin of the actin cytoskeleton. *Nature*, 413, 39-44 (2001)

9. L. J. Jones, R. Carballido-López & J. Errington: Control of cell shape in bacteria: helical, actin-like filaments in *Bacillus subtilis*. *Cell*, 104, 913-22 (2001)

10. R. Carballido-López, A. Formstone, Y. Li, S. D. Ehrlich, P. Noirot & J. Errington: Actin homolog MreBH governs cell morphogenesis by localization of the cell wall hydrolase LytE. *Dev Cell*, 11, 399-409 (2006)

11. W. Vollmer & S. J. Seligman: Architecture of peptidoglycan: more data and more models. *Trends Microbiol*, 18, 59-66 (2010)

12. H. J. Defeu Soufo & P. L. Graumann: *Bacillus subtilis* MreB paralogues have different filament architectures and lead to shape remodelling of a heterologous cell system. *Mol Microbiol*, 78, 1145-58 (2010)

13. S. Wang, H. Arellano-Santoyo, P. A. Combs & J. W. Shaevitz: Actin-like cytoskeleton filaments contribute to cell mechanics in bacteria. *Proc Natl Acad Sci U S A*, 107, 9182-5 (2010)

14. L. S. Dorobantu & M. R. Gray: Application of atomic force microscopy in bacterial research. *Scanning*, 32, 74-96 (2010)

15. L. Schermelleh, R. Heintzmann & H. Leonhardt: A guide to super-resolution fluorescence microscopy. *J Cell Biol*, 190, 165-75 (2010)

16. R. Carballido-López & J. Errington: The bacterial cytoskeleton: *in vivo* dynamics of the actin-like protein Mbl of *Bacillus subtilis*. *Dev Cell*, 4, 19-28 (2003)

17. H. J. Defeu Soufo & P. L. Graumann: Dynamic movement of actin-like proteins within bacterial cells. *EMBO Rep*, 5, 789-94 (2004)

18. T. Kruse, J. Moller-Jensen, A. Lobner-Olesen & K. Gerdes: Dysfunctional MreB inhibits chromosome segregation in *Escherichia coli*. *Embo J*, 22, 5283-92 (2003)

19. R. M. Figge, A. V. Divakaruni & J. W. Gober: MreB, the cell shape-determining bacterial actin homologue, co-ordinates cell wall morphogenesis in *Caulobacter crescentus*. *Mol Microbiol*, 51, 1321-32 (2004)

20. I. Barak, K. Muchova, A. J. Wilkinson, P. J. O'Toole & N. Pavlendova: Lipid spirals in *Bacillus subtilis* and their role in cell division. *Mol Microbiol*, 68, 1315-27 (2008)

21. N. Campo, H. Tjalsma, G. Buist, D. Stepniak, M. Meijer, M. Veenhuis, M. Westermann, J. P. Muller, S. Bron, J. Kok, O. P. Kuipers & J. D. Jongbloed: Subcellular sites for bacterial protein export. *Mol Microbiol*, 53, 1583-99 (2004)

22. H. J. Defeu Soufo, C. Reimold, U. Linne, T. Knust, J. Gescher & P. L. Graumann: Bacterial translation elongation factor EF-Tu interacts and colocalizes with actin-like MreB protein. *Proc Natl Acad Sci U S A*, 107, 3163-8 (2010)

23. E. Foulquier, F. Pompeo, A. Bernadac, L. Espinosa & A. Galinier: The YvcK protein is required for morphogenesis via localization of PBP1 under gluconeogenic growth conditions in *Bacillus subtilis*. *Mol Microbiol*, 80, 309-18 (2011)

24. D. Muñoz-Espín , R. Daniel, Y. Kawai, R. Carballido-López, V. Castilla-Llorente, J. Errington, W. J. Meijer & M. Salas: The actin-like MreB cytoskeleton organizes viral DNA replication in bacteria. *Proc Natl Acad Sci U S A* (2009)

25. A. Rubio & K. Pogliano: Septal localization of forespore membrane proteins during engulfment in *Bacillus subtilis*. *Embo J*, 23, 1636-46 (2004)

26. D. J. Scheffers & M. G. Pinho: Bacterial cell wall synthesis: new insights from localization studies. *Microbiol Mol Biol Rev*, 69, 585-607 (2005)

27. P. G. Wahome, A. E. Cowan, B. Setlow & P. Setlow: Levels and localization of mechanosensitive channel proteins in *Bacillus subtilis*. *Arch Microbiol*, 191, 403-14 (2009)

28. H. Yamamoto, Y. Miyake, M. Hisaoka, S. Kurosawa & J. Sekiguchi: The major and minor wall teichoic acids prevent the sidewall localization of vegetative DL-endopeptidase LytF in *Bacillus subtilis*. *Mol Microbiol*, 70, 297-310 (2008)

29. I. A. Berlatzky, A. Rouvinski & S. Ben-Yehuda: Spatial organization of a replicating bacterial chromosome. *Proc Natl Acad Sci U S A*, 105, 14136-40 (2008)

30. C. Butan, L. M. Hartnell, A. K. Fenton, D. Bliss, R. E. Sockett, S. Subramaniam & J. L. Milne: Spiral architecture of the nucleoid in *Bdellovibrio bacteriovorus*. *J Bacteriol*, 193, 1341-50 (2011)

31. D. Lopez & R. Kolter: Functional microdomains in bacterial membranes. *Genes Dev*, 24, 1893-902 (2010)

32. J. Domínguez-Escobar, A. Chastanet, A. H. Crevenna, V. Fromion, R. Wedlich-Soldner & R. Carballido-López: Processive movement of MreB-associated cell wall biosynthetic complexes in bacteria. *Science*, 333, 225-8 (2011)

33. E. C. Garner, R. Bernard, W. Wang, X. Zhuang, D. Z. Rudner & T. Mitchison: Coupled, circumferential motions of the cell wall synthesis machinery and MreB filaments in *B. subtilis*. *Science*, 333, 222-5 (2011)

34. M. T. Swulius, S. Chen, H. Jane Ding, Z. Li, A. Briegel, M. Pilhofer, E. I. Tocheva, S. R. Lybarger, T. L. Johnson, M. Sandkvist & G. J. Jensen: Long helical filaments are not seen encircling cells in electron cryotomograms of rod-shaped bacteria. *Biochem Biophys Res Commun*, 407, 650-5 (2011)

35. S. van Teeffelen, S. Wang, L. Furchtgott, K. C. Huang, N. S. Wingreen, J. W. Shaevitz & Z. Gitai: The bacterial actin MreB rotates, and rotation depends on cell-wall assembly. *Proc Natl Acad Sci U S A*, 108, 15822-7 (2011)

36. A. Formstone & J. Errington: A magnesium-dependent *mreB* null mutant: implications for the role of *mreB* in *Bacillus subtilis*. *Mol Microbiol*, 55, 1646-57 (2005)

37. H. J. Defeu Soufo & P. L. Graumann: Dynamic localization and interaction with other *Bacillus subtilis* actin-like proteins are important for the function of MreB. *Mol Microbiol*, 62, 1340-56 (2006)

38. R. Carballio-López: The actin-like cytoskeleton. In: *Bacillus* cellular and molecular biology. Eds: Peter Graumann. Caister academic press, Wymondham (2007)

39. K. Schirner & J. Errington: The cell wall regulator {sigma}I specifically suppresses the lethal phenotype of mbl mutants in *Bacillus subtilis*. *J Bacteriol*, 191, 1404-13 (2009)

40. Y. Kawai, K. Asai & J. Errington: Partial functional redundancy of MreB isoforms, MreB, Mbl and MreBH, in cell morphogenesis of *Bacillus subtilis*. *Mol Microbiol*, 73, 719-31 (2009)

41. M. Leaver & J. Errington: Roles for MreC and MreD proteins in helical growth of the cylindrical cell wall in *Bacillus subtilis*. *Mol Microbiol*, 57, 1196-209 (2005)

42. H. J. Defeu Soufo & P. L. Graumann: Actin-like proteins MreB and Mbl from *Bacillus subtilis* are required for bipolar positioning of replication origins. *Curr Biol*, 13, 1916-20 (2003)

43. H. J. Defeu Soufo & P. L. Graumann: *Bacillus subtilis* actin-like protein MreB influences the positioning of the replication machinery and requires membrane proteins MreC/D and other actin-like proteins for proper localization. *BMC Cell Biol*, 6, 10 (2005)

44. Y. Abhayawardhane & G. C. Stewart: *Bacillus subtilis* possesses a second determinant with extensive sequence similarity to the *Escherichia coli mreB* morphogene. *J Bacteriol*, 177, 765-73 (1995)

45. L. Slamti, M. A. de Pedro, E. Guichet & M. Picardeau: Deciphering morphological determinants of the helix-shaped *Leptospira*. *J Bacteriol* (2011)

46. Y. Kawai, R. A. Daniel & J. Errington: Regulation of cell wall morphogenesis in *Bacillus subtilis* by recruitment of PBP1 to the MreB helix. *Mol Microbiol*, 71, 1131-44 (2009)

47. R. Carballido-Lopez & A. Formstone: Shape determination in *Bacillus subtilis*. *Curr Opin Microbiol*, 10, 611-6 (2007)

48. K. Schirner, J. Marles-Wright, R. J. Lewis & J. Errington: Distinct and essential morphogenic functions for wall- and lipo-teichoic acids in *Bacillus subtilis*. *Embo J*, 28, 830-42 (2009)

49. F. C. Neuhaus & J. Baddiley: A continuum of anionic charge: structures and functions of D-alanyl-teichoic acids in gram-positive bacteria. *Microbiol Mol Biol Rev*, 67, 686-723 (2003)

50. A. Grundling & O. Schneewind: Synthesis of glycerol phosphate lipoteichoic acid in *Staphylococcus aureus*. *Proc Natl Acad Sci U S A*, 104, 8478-83 (2007)

51. B. Gorke, E. Foulquier & A. Galinier: YvcK of *Bacillus subtilis* is required for a normal cell shape and for growth on Krebs cycle intermediates and substrates of the pentose phosphate pathway. *Microbiology*, 151, 3777-91 (2005)

52. U. Zuber, K. Drzewiecki & M. Hecker: Putative sigma factor SigI (YkoZ) of *Bacillus subtilis* is induced by heat shock. *J Bacteriol*, 183, 1472-5 (2001)

53. C. L. Tseng & G. C. Shaw: Genetic evidence for the actin homolog gene *mreBH* and the bacitracin resistance gene *bcrC* as targets of the alternative sigma factor SigI of *Bacillus subtilis*. *J Bacteriol*, 190, 1561-7 (2008)

54. C. L. Tseng, J. T. Chen, J. H. Lin, W. Z. Huang & G. C. Shaw: Genetic evidence for involvement of the alternative sigma factor SigI in controlling expression of the cell wall hydrolase gene *lytE* and contribution of LytE to heat survival of *Bacillus subtilis*. *Arch Microbiol*, 193, 677-85 (2011)

55. R. A. Daniel & J. Errington: Control of cell morphogenesis in bacteria: two distinct ways to make a rod-shaped cell. *Cell*, 113, 767-76 (2003)

56. K. Tiyanont, T. Doan, M. B. Lazarus, X. Fang, D. Z. Rudner & S. Walker: Imaging peptidoglycan biosynthesis in *Bacillus subtilis* with fluorescent antibiotics. *Proc Natl Acad Sci U S A*, 103, 11033-8 (2006)

57. A. P. Bhavsar & E. D. Brown: Cell wall assembly in *Bacillus subtilis*: how spirals and spaces challenge paradigms. *Mol Microbiol*, 60, 1077-90 (2006)

58. A. Formstone, R. Carballido-López, P. Noirot, J. Errington & D. J. Scheffers: Localization and interactions of teichoic acid synthetic enzymes in *Bacillus subtilis*. *J Bacteriol*, 190, 1812-21 (2008)

59. E. Sauvage, F. Kerff, M. Terrak, J. A. Ayala & P. Charlier: The penicillin-binding proteins: structure and role in peptidoglycan biosynthesis. *FEMS Microbiol Rev*, 32, 234-58 (2008)

60. D. C. McPherson & D. L. Popham: Peptidoglycan synthesis in the absence of class A penicillin-binding proteins in *Bacillus subtilis*. *J Bacteriol*, 185, 1423-31 (2003)

61. W. Vollmer, B. Joris, P. Charlier & S. Foster: Bacterial peptidoglycan (murein) hydrolases. *FEMS Microbiol Rev*, 32, 259-86 (2008)

62. J. V. Holtje: Growth of the stress-bearing and shape-maintaining murein sacculus of *Escherichia coli*. *Microbiol Mol Biol Rev*, 62, 181-203 (1998)

63. A. L. Koch: The origin of the rotation of one end of a cell relative to the other end during growth of gram-positive rods. *J Theor Biol*, 141, 391-402 (1989)

64. E. Scheurwater, C. W. Reid & A. J. Clarke: Lytic transglycosylases: bacterial space-making autolysins. *Int J Biochem Cell Biol*, 40, 586-91 (2008)

65. J. V. Holtje: Lytic transglycosylases. *Exs*, 75, 425-9 (1996)

66. R. Carballido-López: The bacterial actin-like cytoskeleton. *Microbiol Mol Biol Rev*, 70, 888-909 (2006)

67. G. M. Sheldrick, P. G. Jones, O. Kennard, D. H. Williams & G. A. Smith: Structure of vancomycin and its complex with acetyl-D-alanyl-D-alanine. *Nature*, 271, 223-5 (1978)

68. T. Murray, D. L. Popham & P. Setlow: Identification and characterization of *pbpA* encoding *Bacillus subtilis* penicillin-binding protein 2A. *J Bacteriol*, 179, 3021-9 (1997)

69. Y. Wei, T. Havasy, D. C. McPherson & D. L. Popham: Rod shape determination by the *Bacillus subtilis* class B penicillin-binding proteins encoded by *pbpA* and *pbpH. J Bacteriol*, 185, 4717-26 (2003)

70. J. C. Lee & G. C. Stewart: Essential nature of the *mreC* determinant of *Bacillus subtilis*. *J Bacteriol*, 185, 4490-8 (2003)

71. F. van den Ent, M. Leaver, F. Bendezu, J. Errington, P. de Boer & J. Lowe: Dimeric structure of the cell shape protein MreC and its functional implications. *Mol Microbiol*, 62, 1631-42 (2006)

72. T. Kruse, J. Bork-Jensen & K. Gerdes: The morphogenetic MreBCD proteins of *Escherichia coli* form an essential membrane-bound complex. *Mol Microbiol*, 55, 78-89 (2005)

73. D. Claessen, R. Emmins, L. W. Hamoen, R. A. Daniel, J. Errington & D. H. Edwards: Control of the cell elongation-division cycle by shuttling of PBP1 protein in *Bacillus subtilis*. *Mol Microbiol*, 68, 1029-46 (2008)

74. G. C. Stewart: Taking shape: control of bacterial cell wall biosynthesis. *Mol Microbiol*, 57, 1177-81 (2005)

75. A. O. Henriques, P. Glaser, P. J. Piggot & C. P. Moran, Jr.: Control of cell shape and elongation by the *rodA* gene in *Bacillus subtilis*. *Mol Microbiol*, 28, 235-47 (1998)

76. T. Mohammadi, V. van Dam, R. Sijbrandi, T. Vernet, A. Zapun, A. Bouhss, M. Diepeveen-de Bruin, M. Nguyen-Disteche, B. de Kruijff & E. Breukink: Identification of FtsW as a transporter of lipid-linked cell wall precursors across the membrane. *Embo J*, 30, 1425-32 (2011)

77. P. Margot, M. Wahlen, A. Gholamhoseinian, P. Piggot & D. Karamata: The *lytE* gene of *Bacillus subtilis* 168 encodes a cell wall hydrolase. *J Bacteriol*, 180, 749-52 (1998)

78. W. M. Huang, J. L. Libbey, P. van der Hoeven & S. X. Yu: Bipolar localization of *Bacillus subtilis* topoisomerase IV, an enzyme required for chromosome segregation. *Proc Natl Acad Sci U S A*, 95, 4652-7 (1998)

79. D. R. Zeigler, Z. Pragai, S. Rodriguez, B. Chevreux, A. Muffler, T. Albert, R. Bai, M. Wyss & J. B. Perkins: The origins of 168, W23, and other *Bacillus subtilis l*egacy strains. *J Bacteriol*, 190, 6983-95 (2008)

80. K. Boeneman & E. Crooke: Chromosomal replication and the cell membrane. *Curr Opin Microbiol*, 8, 143-8 (2005)

81. A. Bravo, G. Serrano-Heras & M. Salas: Compartmentalization of prokaryotic DNA replication. *FEMS Microbiol Rev*, 29, 25-47 (2005)

82. D. Muñoz-Espín , I. Holguera, D. Ballesteros-Plaza, R. Carballido-López & M. Salas: Viral terminal protein directs early organization of phage DNA replication at the bacterial nucleoid. *Proc Natl Acad Sci U S A*, 107, 16548-53 (2010)

83. J. A. Mayer & K. J. Amann: Assembly properties of the *Bacillus subtilis* actin, MreB. *Cell Motil Cytoskeleton*, 66, 109-18 (2009)

84. D. Popp, A. Narita, K. Maeda, T. Fujisawa, U. Ghoshdastider, M. Iwasa, Y. Maeda & R. C. Robinson: Filament structure, organization, and dynamics in MreB sheets. *J Biol Chem*, 285, 15858-65 (2010)

85. O. Esue, M. Cordero, D. Wirtz & Y. Tseng: The assembly of MreB, a prokaryotic homolog of Actin. *J Biol Chem*, 280, 2628-35 (2005)

86. O. Esue, D. Wirtz & Y. Tseng: GTPase activity, structure, and mechanical properties of filaments assembled from bacterial cytoskeleton protein MreB. *J Bacteriol*, 188, 968-76 (2006)

87. G. J. Bean & K. J. Amann: Polymerization properties of the *Thermotoga maritima* actin MreB: roles of temperature, nucleotides, and ions. *Biochemistry*, 47, 826-35 (2008)

88. K. K. Wen, X. Yao & P. A. Rubenstein: GTP-yeast actin. *J Biol Chem*, 277, 41101-9 (2002)

89. S. Y. Kim, Z. Gitai, A. Kinkhabwala, L. Shapiro & W. E. Moerner: Single molecules of the bacterial actin MreB undergo directed treadmilling motion in *Caulobacter crescentus*. *Proc Natl Acad Sci U S A*, 103, 10929-34 (2006)

90. Y. L. Shih, T. Le & L. Rothfield: Division site selection in *Escherichia coli* involves dynamic redistribution of Min proteins within coiled structures that extend between the two cell poles. *Proc Natl Acad Sci U S A*, 100, 7865-70 (2003)

91. H. Strahl & L. W. Hamoen: Membrane potential is important for bacterial cell division. *Proc Natl Acad Sci U S A*, 107, 12281-6 (2010)

92. P. Vats & L. Rothfield: Duplication and segregation of the actin (MreB) cytoskeleton during the prokaryotic cell cycle. *Proc Natl Acad Sci U S A*, 104, 17795-800 (2007)

93. P. Vats, Y. L. Shih & L. Rothfield: Assembly of the MreB-associated cytoskeletal ring of *Escherichia coli*. *Mol Microbiol*, 72, 170-82 (2009)

94. A. R. Paredez, C. R. Somerville & D. W. Ehrhardt: Visualization of cellulose synthase demonstrates functional association with microtubules. *Science*, 312, 1491-5 (2006)

95. A. V. Divakaruni, C. Baida, C. L. White & J. W. Gober: The cell shape proteins MreB and MreC control cell morphogenesis by positioning cell wall synthetic complexes. *Mol Microbiol*, 66, 174-88 (2007)

96. C. L. White, A. Kitich & J. W. Gober: Positioning cell wall synthetic complexes by the bacterial morphogenetic proteins MreB and MreD. *Mol Microbiol*, 76, 616-33 (2010)

97. N. A. Dye, Z. Pincus, I. C. Fisher, L. Shapiro & J. A. Theriot: Mutations in the nucleotide binding pocket of MreB can alter cell curvature and polar morphology in *Caulobacter*. *Mol Microbiol*, 81, 368-94 (2011)

98. B. Dmitriev, F. Toukach & S. Ehlers: Towards a comprehensive view of the bacterial cell wall. *Trends Microbiol*, 13, 569-74 (2005)

99. C. N. Takacs, S. Poggio, G. Charbon, M. Pucheault, W. Vollmer & C. Jacobs-Wagner: MreB drives de novo rod morphogenesis in *Caulobacter crescentus* via remodeling of the cell wall. *J Bacteriol*, 192, 1671-84 (2010)

100. F. A. Barr & U. Gruneberg: Cytokinesis: placing and making the final cut. *Cell*, 131, 847-60 (2007)

101. M. F. Carlier & D. Pantaloni: Control of actin assembly dynamics in cell motility. *J Biol Chem*, 282, 23005-9 (2007)

102. L. Lanzetti: Actin in membrane trafficking. *Curr Opin Cell Biol*, 19, 453-8 (2007)

103. E. Groves, A. E. Dart, V. Covarelli & E. Caron: Molecular mechanisms of phagocytic uptake in mammalian cells. *Cell Mol Life Sci*, 65, 1957-76 (2008)

104. C. Le Clainche & M. F. Carlier: Regulation of actin assembly associated with protrusion and adhesion in cell migration. *Physiol Rev*, 88, 489-513 (2008)

105. S. L. Bullock: Translocation of mRNAs by molecular motors: think complex? *Semin Cell Dev Biol*, 18, 194-201 (2007)

106. R. L. Frederick & J. M. Shaw: Moving mitochondria: establishing distribution of an essential organelle. *Traffic*, 8, 1668-75 (2007)

107. J. L. Ross, M. Y. Ali & D. M. Warshaw: Cargo transport: molecular motors navigate a complex cytoskeleton. *Curr Opin Cell Biol*, 20, 41-7 (2008)

108. S. H. Lee & R. Dominguez: Regulation of actin cytoskeleton dynamics in cells. *Mol Cells*, 29, 311-25 (2010)

109. P. J. Mattei, D. Neves & A. Dessen: Bridging cell wall biosynthesis and bacterial morphogenesis. *Curr Opin Struct Biol*, 20, 749-55 (2010)

110. J. W. Shaevitz & Z. Gitai: The structure and function of bacterial actin homologs. *Cold Spring Harb Perspect Biol*, 2, a000364 (2010)

111. F. O. Bendezu, C. A. Hale, T. G. Bernhardt & P. A. de Boer: RodZ (YfgA) is required for proper assembly of the MreB actin cytoskeleton and cell shape in *E. coli*. *Embo J*, 28, 193-204 (2009)

112. Y. L. Shih, I. Kawagishi & L. Rothfield: The MreB and Min cytoskeletal-like systems play independent roles in prokaryotic polar differentiation. *Mol Microbiol*, 58, 917-28 (2005)

113. D. Shiomi, M. Sakai & H. Niki: Determination of bacterial rod shape by a novel cytoskeletal membrane protein. *Embo J*, 27, 3081-91 (2008)

114. F. van den Ent, C. M. Johnson, L. Persons, P. de Boer & J. Lowe: Bacterial actin MreB assembles in complex with cell shape protein RodZ. *Embo J*, 29, 1081-90 (2010)

115. F. Ishino, W. Park, S. Tomioka, S. Tamaki, I. Takase, K. Kunugita, H. Matsuzawa, S. Asoh, T. Ohta, B. G. Spratt & *et al.*: Peptidoglycan synthetic activities in membranes of *Escherichia coli* caused by overproduction of penicillin-binding protein 2 and RodA protein. *J Biol Chem*, 261, 7024-31 (1986)

116. T. Mohammadi, A. Karczmarek, M. Crouvoisier, A. Bouhss, D. Mengin-Lecreulx & T. den Blaauwen: The essential peptidoglycan glycosyltransferase MurG forms a complex with proteins involved in lateral envelope growth as well as with proteins involved in cell division in *Escherichia coli*. *Mol Microbiol*, 65, 1106-21 (2007)

117. A. Karczmarek, R. Martinez-Arteaga, S. Alexeeva, F. G. Hansen, M. Vicente, N. Nanninga & T. den Blaauwen: DNA and origin region segregation are not affected by the transition from rod to sphere after inhibition of *Escherichia coli* MreB by A22. *Mol Microbiol*, 65, 51-63 (2007)

118. T. Kruse, B. Blagoev, A. Lobner-Olesen, M. Wachi, K. Sasaki, N. Iwai, M. Mann & K. Gerdes: Actin homolog MreB and RNA polymerase interact and are both required for chromosome segregation in *Escherichia coli*. *Genes Dev*, 20, 113-24 (2006)

119. R. Madabhushi & K. J. Marians: Actin homolog MreB affects chromosome segregation by regulating topoisomerase IV in *Escherichia coli*. *Mol Cell*, 33, 171-80 (2009)

120. X. Wang & D. J. Sherratt: Independent segregation of the two arms of the *Escherichia coli ori* region requires

neither RNA synthesis nor MreB dynamics. *J Bacteriol*, 192, 6143-53 (2010)

121. C. Engl, G. Jovanovic, L. J. Lloyd, H. Murray, M. Spitaler, L. Ying, J. Errington & M. Buck: *In vivo* localizations of membrane stress controllers PspA and PspG in *Escherichia coli*. *Mol Microbiol*, 73, 382-96 (2009)

122. N. A. Dye, Z. Pincus, J. A. Theriot, L. Shapiro & Z. Gitai: Two independent spiral structures control cell shape in *Caulobacter*. *Proc Natl Acad Sci U S A*, 102, 18608-13 (2005)

123. Z. Gitai, N. Dye & L. Shapiro: An actin-like gene can determine cell polarity in bacteria. *Proc Natl Acad Sci U S A*, 101, 8643-8 (2004)

124. A. V. Divakaruni, R. R. Loo, Y. Xie, J. A. Loo & J. W. Gober: The cell-shape protein MreC interacts with extracytoplasmic proteins including cell wall assembly complexes in *Caulobacter crescentus*. *Proc Natl Acad Sci U S A*, 102, 18602-7 (2005)

125. C. W. Shebelut, R. B. Jensen & Z. Gitai: Growth conditions regulate the requirements for *Caulobacter* chromosome segregation. *J Bacteriol*, 191, 1097-100 (2009)

126. E. Toro, S. H. Hong, H. H. McAdams & L. Shapiro: *Caulobacter* requires a dedicated mechanism to initiate chromosome segregation. *Proc Natl Acad Sci U S A*, 105, 15435-40 (2008)

127. J. K. Wagner, C. D. Galvani & Y. V. Brun: *Caulobacter crescentus* requires RodA and MreB for stalk synthesis and prevention of ectopic pole formation. *J Bacteriol*, 187, 544-53 (2005)

128. C. S. Costa & D. N. Anton: Round-cell mutants of *Salmonella typhimurium* produced by transposition mutagenesis: lethality of *rodA* and *mre* mutations. *Mol Gen Genet*, 236, 387-94 (1993)

129. E. G. Biondi, F. Marini, F. Altieri, L. Bonzi, M. Bazzicalupo & M. del Gallo: Extended phenotype of an *mreB*-like mutant in *Azospirillum brasilense*. *Microbiology*, 150, 2465-74 (2004)

130. B. Hu, G. Yang, W. Zhao, Y. Zhang & J. Zhao: MreB is important for cell shape but not for chromosome segregation of the filamentous cyanobacterium *Anabaena* sp. PCC 7120. *Mol Microbiol*, 63, 1640-52 (2007)

131. S. W. Chiu, S. Y. Chen & H. C. Wong: Dynamic localization of MreB in *Vibrio parahaemolyticus* and in the ectopic host bacterium *Escherichia coli*. *Appl Environ Microbiol*, 74, 6739-45 (2008)

132. P. Mazza, E. E. Noens, K. Schirner, N. Grantcharova, A. M. Mommaas, H. K. Koerten, G. Muth, K. Flardh, G. P. van Wezel & W. Wohlleben: MreB of *Streptomyces coelicolor* is not essential for vegetative growth but is required for the integrity of aerial hyphae and spores. *Mol Microbiol*, 60, 838-52 (2006)

133. A. K. Fenton, C. Lambert, P. C. Wagstaff & R. E. Sockett: Manipulating each MreB of *Bdellovibrio bacteriovorus* gives diverse morphological and predatory phenotypes. *J Bacteriol*, 192, 1299-311 (2010)

134. P. M. Slovak, G. H. Wadhams & J. P. Armitage: Localization of MreB in *Rhodobacter sphaeroides* under conditions causing changes in cell shape and membrane structure. *J Bacteriol*, 187, 54-64 (2005)

135. B. Waidner, M. Specht, F. Dempwolff, K. Haeberer, S. Schaetzle, V. Speth, M. Kist & P. L. Graumann: A novel system of cytoskeletal elements in the human pathogen *Helicobacter pylori*. *PLoS Pathog*, 5, e1000669 (2009)

136. K. N. Cowles & Z. Gitai: Surface association and the MreB cytoskeleton regulate pilus production, localization and function in *Pseudomonas aeruginosa*. *Mol Microbiol*, 76, 1411-26 (2010)

137. E. M. Mauriello, F. Mouhamar, B. Nan, A. Ducret, D. Dai, D. R. Zusman & T. Mignot: Bacterial motility complexes require the actin-like protein, MreB and the Ras homologue, MglA. *Embo J*, 29, 315-26 (2010)

138. P. Srivastava, G. Demarre, T. S. Karpova, J. McNally & D. K. Chattoraj: Changes in nucleoid morphology and origin localization upon inhibition or alteration of the actin homolog, MreB, of *Vibrio cholerae*. *J Bacteriol*, 189, 7450-63 (2007)

139. G. J. Bean, S. T. Flickinger, W. M. Westler, M. E. McCully, D. Sept, D. B. Weibel & K. J. Amann: A22 disrupts the bacterial actin cytoskeleton by directly binding and inducing a low-affinity state in MreB. *Biochemistry*, 48, 4852-7 (2009)

140. Z. Gitai, N. A. Dye, A. Reisenauer, M. Wachi & L. Shapiro: MreB actin-mediated segregation of a specific region of a bacterial chromosome. *Cell*, 120, 329-41 (2005)

141. N. Iwai, K. Nagai & M. Wachi: Novel S-benzylisothiourea compound that induces spherical cells in *Escherichia coli* probably by acting on a rod-shape-determining protein (s) other than penicillin-binding protein 2. *Biosci Biotechnol Biochem*, 66, 2658-62 (2002)

Key Words: Actin cytoskeleton, MreB, Cell Wall Elongation, Helix Localization, Bacterial Cell Shape, Review

Send correspondence to: Rut Carballido-Lopez, INRA, UMR1319 Micalis, F-78352 Jouy-en-Josas, France, Tel: 33-0-1 34-65-25-34, Fax: 33-0-1-34-65-25-21, E-mail: rut.carballido-lopez@jouy.inra.fr