

# RodZ, a key player of the bacterial morphogenic apparatus

José Sebastián Robalino-Espinosa <sup>(1,2)</sup>

## Abstract

Bacteria display a variety of cell shapes, including straight cylinders, banana-shaped cylinders, spirals and American footballs. Maintenance of cell shape is vital for cell growth and division. The distinct shape of most bacteria is maintained by their peptidoglycan layer (PG). Nowadays, we know how sub-unites of the PG are synthesized within the cell, but how the synthesis of PG is coordinated with cell division and growth remains elusive. The PG is inserted into the periplasm and covalently linked into the cell wall. This process is regulated by the morphogenic apparatus composed of MreBCD, PBP2 and RodA. Recently, RodZ an inner membrane protein has been shown to be part of the morphogenic apparatus. Studies using *Escherichia coli* and *Caulobacter crescentus* showed that RodZ has a key role in the morphogenesis of Gram-negative bacteria; however, its functions are still unclear in Gram-positive bacteria.

Rev Fac Cien Med (Quito) 2012; 37: 79-82

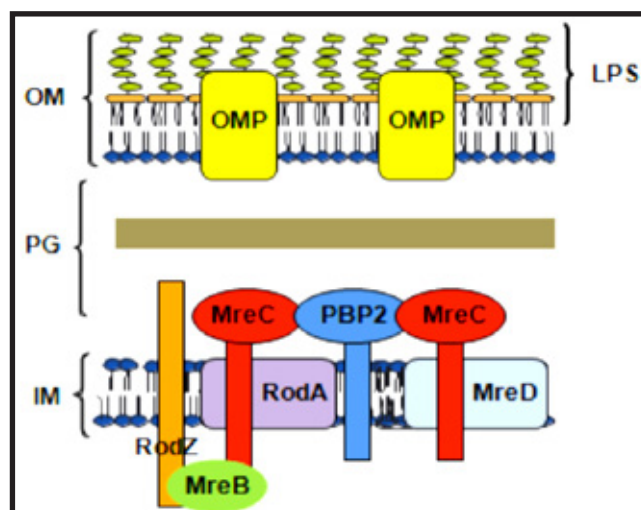
<sup>1</sup> Escuela de Biología, Universidad Internacional del Ecuador (UIDE); Quito-Ecuador  
<sup>2</sup> Corporación para la Biotecnología y Ciencias Biomoleculares (CBCB); Valle de los Chillos-Ecuador

**Correspondence:**  
Sebastián Robalino.  
Corporación para la Biotecnología y Ciencias Biomoleculares. Calle Ana de Ayala 236 y Mercedes Gonzales, Valle de los Chillos-Ecuador.  
sebastianrobalino@hotmail.com

**Keywords**  
Bacterial-proteins, Morphogenesis, Peptidoglycan, MreBCD, PBP2, RodA, RodZ  
**Received:**  
15 – Octubre – 2012  
**Accepted:**  
20 – Noviembre – 2012

## Background

The shape of bacteria is maintained by the shape of their peptidoglycan (PG) layer. Precursors of the PG are inserted into the periplasm. In this process the bacterial actin-homolog, MreB, may function as a cytoplasmatic track for murein synthases<sup>[1]</sup>. Depletion of mreB leads to loss of normal shape with the formation of spherical shapes. MreB forms helical structures beneath the cell surface and interacts with the inner membrane protein MreC. The latter protein appears to act as a bridge between MreB and the murein biosynthetic machinery, specifically PBP2<sup>[2,3]</sup>. In *Escherichia coli* PBP2 is the only murein-synthase that is specifically required for cell extension<sup>[4]</sup>. The sub-complex MreBC may interact with RodA which is likely to be important for the proper function of PBP2<sup>[5]</sup>. In addition, MreC interacts with another inner membrane protein, MreD<sup>[6]</sup>. MreC, MreD and PBP2 accumulate in a spoty or helical manner along the cell envelope in *E. coli*, *Bacillus subtilis* and *C. crescentus*<sup>[7-11]</sup>. These multiple interactions suggest that MreC intercalates between MreB and MreD to form the morphogenic apparatus; (Figure 1).



**Figure 1. Schematic diagram of the suggested morphogenic apparatus model for *E. coli*.**

RodZ, MreB, MreC, RodA, PBP2, MreD, inner-membrane, peptidoglycan, outer-membrane, lipopolysaccharide, outer-membrane protein are indicated in orange, green, red, purple, blue and sky blue, IM, PG, OM, LPS, OMP respectively.

### Role of RodZ in the morphogenic apparatus

Recently, RodZ has been shown to be critical for cell shape maintenance<sup>[12-14]</sup>. RodZ mutants are affected mainly in their length<sup>[12]</sup>. For instance, *E. coli* and *C. crescentus* cells that lacked RodZ are round or otherwise misshapen<sup>[12-14]</sup>. Remarkably, these defects were also imprinted in the isolated sacculi of *C. crescentus*<sup>[14]</sup>.

RodZ localizes in a spiral-type manner and this pattern is apparently dependent on MreB. The latter was probed using fluorescent tagged versions of MreB and RodZ. When these fluorescent fusions are co-expressed in *E. coli* they appear to co-localize in a spiral-type manner, but when RodZ is expressed in MreB-null cells it fails to adopt such localization<sup>[13]</sup>. However, the evidence of interdependent localization between RodZ and MreB is still controversial<sup>[12]</sup>. Likewise MreB and RodZ co-localize in time and space in *C. crescentus*, but the proper RodZ localization is also dependent on FtsZ. The latter is consistent with the unusual behavior of RodZ and MreB during the cell cycle of *C. crescentus*, in which RodZ and MreB have a patchy localization during the initial states of the cell cycle, while they are latter redistributed as a band at mid cell<sup>[14]</sup>. Remarkably, co-localization evidence of RodZ with FtsZ in *E. coli* was also reported<sup>[12]</sup>.

The growth properties of *rodZ*-null cells are complex and their cell shape depends to some extent on growth conditions. Just as the other

proteins, that form the morphogenic apparatus, RodZ is conditionally essential since an extra supply in *E. coli* of the tubulin homolog, FtsZ, allows *rodZ* mutants to propagate. In addition, *rodZ*-null cells exhibited a cold sensitive phenotype. At low temperatures, cells cannot divide anymore and form misshapen spheres. One notable difference between MreBCD, PBP2 and RodA mutants with *rodZ*-null cells is the mass doubling time since cells that lacked RodZ showed medium dependence for their growth<sup>[13]</sup>.

Over-expression in *E. coli* of either RodZ or MreB causes elongation of the long axis or loss of the rod shape depending on growth medium<sup>[12,13]</sup>. Similarly, overproduction of RodZ in *C. crescentus* resulted in straighter cells, but there has not been reported to our knowledge, any medium dependence<sup>[14]</sup>. Remarkably, co-overexpression of both RodZ and MreB in *E. coli* prevents loss of the rod shape. The latter indicates that proper MreB to RodZ ratio is essential for the normal cell shape maintenance<sup>[13]</sup>. In addition, evidences suggest that the over-production of RodZ may cause cell division defects. Cells that over-expressed RodZ showed bulges along the long axis and thin connections<sup>[13,14]</sup>.

RodZ is broadly conserved across bacterial phyla<sup>[14]</sup>. Studies of fractionated membrane confirmed that RodZ is an inner membrane protein and *phoA* fusion assays showed that its C-terminal part is exposed in the periplasm<sup>[12]</sup>. Predictions show that RodZ is a type II protein (N-in) with 337 and 354 residues in *E. coli* and *C. crescentus* respectively. RodZ possesses multiple putative domains<sup>[12-14]</sup>; (Figure 2). Regions of interest include: H-T-H motif, HTH Cro/CI-type (HTH), basic juxta membrane (JM), trans-membrane (TM), and periplasmatic (P) domains. Functionality and structure analysis of each domain in *E. coli* have shown the importance of P domain in maintaining the rod shape<sup>[12,13]</sup>. However, the JM domain is the only strictly required domain for cell shape maintenance and to accomplish its function this region must be membrane tethered and be accompanied by either the HTH or the P domains<sup>[13]</sup>.

In *E. coli* and *C. crescentus* the proper localization of RodZ is depended on the HTH domain membrane tethered<sup>[12-14]</sup>. The HTH domain in its N-terminus part has a classical helix-turn-helix motif (HTH motif). This fold comprises the first three  $\alpha$ -helices (H1, H2, and H3) arranged in a triangular manner. The HTH motif is followed by two  $\alpha$ -helices (H4 and H5); (Figure 3A)<sup>[15]</sup>.

Structural evidence for the interaction between MreB and RodZ was reported in *Thermatoga maritima*. The co-crystal structure of MreB and the HTH domain revealed an interesting packing, in which RodZ is in the middle of two MreB sub-

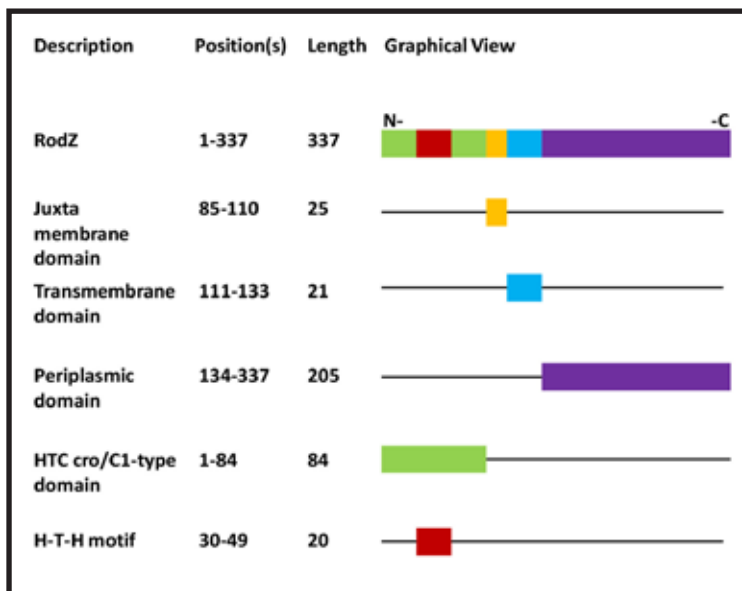


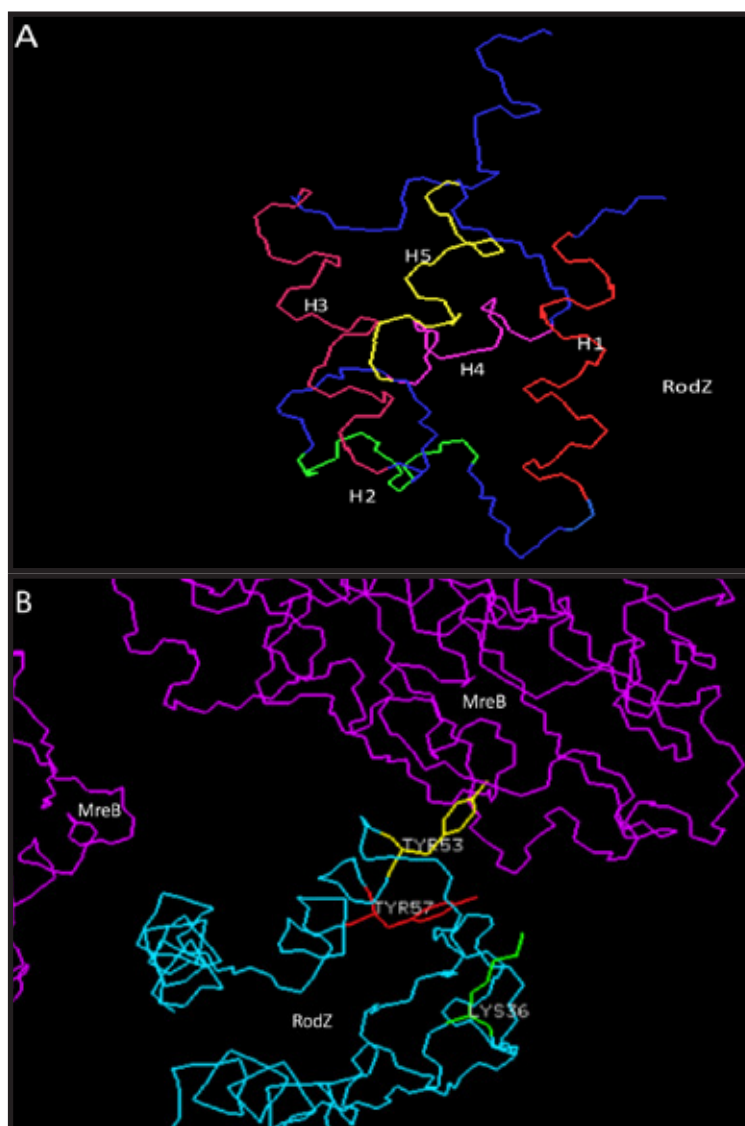
Figure 2. Predicted domain and motif organization of *E. coli*-RodZ.

The H-T-H motif, HTH Cro/CI-type (HTH), basic juxta membrane (JM), trans-membrane (TM), and peri-plasmatic (P) domains, N-terminal part, C-terminal part are illustrated in red, green, orange, purple, (N-) & (-C) respectively<sup>[13]</sup>.

domains. Hence, RodZ shared two distinct interfaces when interacting with MreB. Single amino-acids substitutions in RodZ showed that Lys36, Tyr57, and Tyr53 are essential for the interaction of this protein with MreB; (**Figure 3B**)<sup>[15]</sup>.

### Concluding Remarks

It is clear that RodZ plays a key role in maintaining the cell shape of Gram-negative bacteria. However, does RodZ have a similar role in Gram-positive bacteria? A screen for weak-organic acids susceptible genes with transposon mutagenesis identified *rodZ* (formally named *yfmM*) in *Bacillus subtilis*. Null-*yfmM* cells displayed modifications in membrane composition. *yfmM* transposon mutants show similar phenotype as *pgsA* conditional mutants on solid plates. Remarkably, the *pgsA* gene was also found in the same type of screening that leads with the discovery of *rodZ* in *B subtilis*. The *pgsA* gene is in the same operon of *yfmM*, and codes for the essential phosphatidyl-glycerol synthase, which is involved in lipid biosynthesis<sup>[14]</sup>. Moreover, it has been observed that the overproduction of RodZ inhibited growth in minimal medium unless magnesium is present. Removal of magnesium resulted in progressive cell widening and mid-cell bulging<sup>[14]</sup>. In summary, RodZ is a key player in the morphogenic apparatus and the discovery of its function in Gram-negative bacteria open new possibilities to uncover the mysteries of the biogenesis of the cell-wall in Gram-positive bacteria.



**Figure 3. MreB and RodZ ribbon diagrams.**

(Panel 3A) Ribbon representation of the crystal structure of the HTH domain of RodZ helices 1, 2, 3, 4, 5 are indicated in red, green, pink purple and yellow.

(Panel 3B) Binding mode of RodZ, residues Lys36, Tyr57 and Tyr53 are indicated in green, red and yellow. RodZ and sub-domains of MreB are indicated in sky blue and purple. Images were generated using Swiss-PDB Viewer 4.0.1. (PDB 2WUS)<sup>[15]</sup>

### References

- Daniel RA, Errington J. Control of cell morphogenesis in bacteria: two distinct ways to make a rod-shaped cell. *Cell* 2003; 113: 767–76.
- Jones LJ, Carballido-López R, Errington J. Control of cell shape in bacteria: helical, actin-like filaments in *Bacillus subtilis*. *Cell* 2001; 104: 913–922.
- Leaver M, Errington J. Roles for MreC and MreD proteins in helical growth of the cylindrical cell wall in *Bacillus subtilis*. *Mol Microbiol* 2005; 57: 1196 – 1209.
- Sprat BG. 1975. Distinct penicillin binding proteins involved in the division, elongation and shape of *Escherichia coli* K12. *Proc Natl Acad Sci USA* 1975; 72: 2999 – 3003.
- Ishino F, Park W, Tomioka S, Tamaki S, Takase I, Kunugita K; et al. Peptidoglycan synthetic activities in membranes of *Escherichia coli* caused by overproduction of penicillin binding protein 2 and RodA protein. *J Biol Chem* 1986; 261: 7024 – 31.
- Kruse T, Bork-Jensen J, Gerdes K. The morphogenetic MreB-CD proteins of *Escherichia coli* form an essential membrane-bound complex. *Mol Microbiol* 2005; 55: 78 – 89.

7. den Blaauwen T, Aarsman ME, Vischer NO, Nanninga N. Penicillin binding protein PBP2 of *Escherichia coli* localizes preferentially to the lateral wall and a mid cell in comparison with the old cell pole. *Mol Microbiol* 2003; 47: 539 – 47.
8. Figge RM, Divakaruni AV, Gober JW. MreB, the cell shape-determining bacterial actin homologue, co-ordinates cell wall morphogenesis in *Caulobacter crescentus*. *Mol Microbiol* 2004; 51: 1321–32.
9. Divakaruni AV, Loo RR, Xie Y, Loo JA, Gober JW. The cell-shape protein MreC interacts with extracytoplasmic proteins including cell wall assembly complexes in *Caulobacter crescentus*. *Proc Natl Acad Sci USA* 2005; 102: 18602 – 607.
10. Dye NA, Pincus Z, Theriot JA, Shapiro L, Gitai Z. Two independent spiral structures control cell shape in *Caulobacter*. *Proc Natl Acad Sci USA* 2005 102: 18608 –613.
11. Leaver M, Errington J. Roles for MreC and MreD proteins in helical growth of the cylindrical cell wall in *Bacillus subtilis*. *Mol. Microbiol* 2005; 57: 1196–09.
12. Shiomi D, Sakai M, Niki H. Determination of bacterial rod shape by a novel cytoskeletal membrane protein. *EMBO J* 2008; 27: 3081 - 91.
13. Bendezu F, Hale CA, Bernhardt TG, De Boer P. RodZ (YfgA) is required for proper assembly of the MreB actin cyoskeleton and cell shape in *Escherichia coli*. *EMBO J* 2009; 28: 193 – 204.
14. Alyahya SA, Alexander R, Costa T, Henriques AO, Emonet T, Jacobs-Wagner C. RodZ, a component of the bacterial core morphogenic apparatus. *Proc Natl Acad Sci USA* 2009 106:1239 – 44.
15. van den Ent F, Johnson MC, Persons L, de Boer P, Löwe J. Bacterial actin MreB assembles in complex with cell shape protein RodZ. *EMBO J* 2010; 29: 1081 – 90.
16. Ter Beek AS. Weak organic stress in *Bacillus subtilis* [Thesis]. Amsterdam: University Van Amsterdam; 2009.

## RodZ, un actor importante en el aparato morfogénico bacteriano

Robalino-Espinosa JS.  
*Rev Fac Cien Med (Quito)* 2012; 37: 79-82

### Resumen

**Palabras clave**  
Proteínas bacterianas,  
Morfogénesis,  
Peptidoglucano, MreBCD,  
PBP2, RodA, RodZ

Las bacterias presentan una gran variedad de formas entre ellas se incluyen cilindros no curvos, cilindros tipo bananas, espirales, y círculos tipo balones de fútbol. El mantenimiento de las diferentes formas celulares está mediado por su peptidoglucano (PG). En la actualidad es un misterio como la síntesis de PG es coordinada con la división y el crecimiento celular. El PG es insertado en el periplasma y unido de manera covalente a la pared celular. Este proceso es regulado por el aparato morfogénico compuesto por las proteínas: MreBCD, PBP2, y RodA. Recientemente RodZ fue descubierta como una proteína de membrana interna que además forma parte del aparato morfogénico. Estudios usando células de *Escherichia coli* y *Caulobacter crescentus* han mostrado que RodZ tiene un rol importante en la morfogénesis de bacterias Gram-negativas; no obstante, las funciones de esta proteína en organismos Gram-positivos no han sido esclarecidas hasta el momento.