A bacterial actin unites to divide bacterial cells

Jennifer R Juarez and William Margolin*

Department of Microbiology and Molecular Genetics, University of Texas Medical School at Houston, Houston TX, USA

*Correspondence to: William.Margolin@uth.tmc.edu

The EMBO Journal (2012) 31, 2235–2236. doi:10.1038/emboj.2012.113; Published online 17 April 2012

Once thought to exist only in eukaryotic cells, the highly conserved bacterial cytoskeleton is now known to function analogously to its eukaryotic counterparts, particularly in cell shape and division. For instance, the actin-like MreB protein and its homologs are important to maintain cell shape in many rod-shaped bacteria, probably by organizing how peptidoglycan is synthesized. FtsZ, a tubulin homolog, forms a scaffold for the cytokinetic ring, or divisome, by GTP-dependent polymerization into protofilaments. In this issue of *The EMBO Journal*, Szwedziak *et al* (2012) reveal the first crystal structures of cell division protein FtsA polymerizing into actin-like filaments, along with *in vivo* evidence that this self-interaction is crucial for proper cell division.

FtsA is an actin homolog required for cytokinesis in many bacterial species and has several key roles in cell division, including helping to tether FtsZ to the cytoplasmic membrane via a membrane-targeting sequence (MTS), recruiting other essential proteins to the divisome, and perhaps promoting divisome constriction (de Boer, 2010). Szwedziak *et al* (2012) recapitulate the FtsZ-FtsA-membrane association *in vitro* using liposomes with FtsZ and FtsA proteins from *Thermotoga maritima*. To get a closer look at the FtsA-FtsZ interface, the authors co-crystallize FtsA with the carboxy-terminal tail of FtsZ, which is known to interact with FtsA. Intriguingly, the crystal reveals an FtsA homodimer. Contrary to the previous bioinformatics model of FtsA self-interaction that proposed a 180° rotation between the two subunits (Carettoni *et al*, 2003), the FtsA-FtsA interface in the crystal structure shows no rotation, similar to F-actin. Szwedziak *et al* (2012) also show that FtsA can form longer, actin-like polymers in the presence of non-hydrolysable ATP or on lipid monolayers. These results are surprising because FtsA has a divergent subdomain architecture compared to other actin-family proteins (van den Ent and Löwe, 2000).

A critical question now is whether FtsA needs to form polymers *in vivo* to function properly. Purified *Streptococcus pneumoniae* FtsA assembles into large polymers that are not like F-actin, and it remains unclear if these structures are relevant *in vivo* (Krupka *et al*, 2012). Wild-type FtsA proteins do not form detectable filaments in cells, but C-terminal truncations of FtsA that remove the MTS form polymers quite readily in cells when overproduced, although they are not functional (Pichoff and Lutkenhaus, 2007). Even so, starting with an MTS truncation derivative of FtsA to

visualize *in vivo* polymers, Szwedziak *et al* (2012) design site-directed mutants of *Bacillus subtilis* FtsA based on the FtsA-FtsA interface of their crystals; these fail to assemble into polymers *in vivo*. Using a similar MTS truncation derivative, Pichoff *et al* (2012) created random mutations in *Escherichia coli* FtsA, and found that those mapping to the same interface found by Szwedziak *et al* (2012) also disrupted polymer formation. Together, these data suggest that these residues are needed for FtsA self-interaction. Perplexingly, when these mutants were subsequently tested for functionality in the context of full-length FtsA, the results were mixed. Pichoff *et al* (2012) showed that FtsA mutants deficient for self-interaction in *E. coli* have a gain-of-function

Figure 1 Bacterial actin and tubulin filaments involved in cell growth and division. (A) MreB (purple) has long been thought of as a spiral filament twisting along the cell length to control cell shape. Likewise, FtsZ protofilaments (blue) were once thought to wrap around the cell midpoint to organize the divisome. (B) Recent work using high-resolution microscopy has revealed that long cytoskeletal filaments are more likely to be short patches of polymers. The present work by Szwedziak *et al* (2012) has added FtsA actin-like filaments (green) to the model of possible divisome architecture.

phenotype, whereas Szwedziak *et al* (2012) report that analogous mutants in *B. subtilis* FtsA suffer a loss of function. These results support the idea that FtsA self-association is related to its activity (Shiomi and Margolin, 2007), yet understanding how self-interaction regulates FtsA function clearly requires further study.

The ability of eukaryotic cytoskeletal proteins to form long polymers is essential to their function, but the physiological relevance of long polymer formation by bacterial cytoskeletal proteins is now a topic of debate (Figure 1). For example, it has been hypothesized that FtsZ protofilaments wrap around the entire circumference of the cell to form the cytokinetic ring. However, recent studies using photoactivated localization microscopy (PALM) and electron cryotomography reveal a different model in which FtsZ forms a series of very short polymers that overlap to encompass the diameter of the cell (Li *et al*, 2007; Fu *et al*, 2010). MreB was also originally thought to form long-range helical polymers extending the length of the cell, but recent data obtained with more

sophisticated microscopic techniques suggest that MreB is distributed in patches that move circumferentially and independently (White and Gober, 2012). It is not yet clear which of these models represents the true cellular architecture of MreB, although it is likely that some degree of MreB polymerization is still needed for function. It is notable that other bacterial homologs of actin and tubulin used for generating scaffolds or partitioning plasmid DNA, but not for essential cellular processes such as cell division and growth, tend to form long polymers that extend throughout the cell (Pogliano, 2008). The continued combined use of microscopic, biochemical, and genetic methods, as demonstrated by Szwedziak *et al* (2012) will enhance future understanding of ancestral tubulin and actin proteins in prokaryotes.

Conflict of interest

The authors declare that they have no conflict of interest.

References

- Carettoni D, Gomez-Puertas P, Yim L, Mingorance J, Massidda O, Vicente M, Valencia A, Domenici E, Anderluzzi D (2003) Phage-display and correlated mutations identify an essential region of subdomain 1C involved in homodimerization of *Escherichia coli* FtsA. *Proteins* **50**: 192–206
- de Boer PA (2010) Advances in understanding *E. coli* cell fission. *Curr Opin Microbiol* **13**: 730–737
- Fu G, Huang T, Buss J, Coltharp C, Hensel Z, Xiao J (2010) *In vivo* structure of the *E. coli* FtsZ-ring revealed by photoactivated localization microscopy (PALM). *PLoS One* **5**: e12682
- Krupka M, Rivas G, Rico AI, Vicente M (2012) Key Role of Two Terminal Domains in the Bidirectional Polymerization of FtsA Protein. *J Biol Chem* **287**: 7756–7765
- Li Z, Trimble MJ, Brun YV, Jensen GJ (2007) The structure of FtsZ filaments *in vivo* suggests a force-generating role in cell division. *EMBO J* **26**: 4694–4708
- Pichoff S, Lutkenhaus J (2007) Identification of a region of FtsA required for interaction with FtsZ. *Mol Microbiol* **64**: 1129–1138
- Pichoff S, Shen B, Sullivan B, Lutkenhaus J (2012) FtsA mutants impaired for self-interaction bypass ZipA suggesting a model in which FtsA's self-interaction competes with its ability to recruit downstream division proteins. *Mol Microbiol* **83**: 151–167
- Pogliano J (2008) The bacterial cytoskeleton. *Curr Opin Cell Biol* **20**: 19–27
- Shiomi D, Margolin W (2007) Dimerization or oligomerization of the actin-like FtsA protein enhances the integrity of the cytokinetic Z ring. *Mol Microbiol* **66**: 1396–1415
- Szwedziak P, Wang Q, Freund SMV, Löwe J (2012) FtsA forms actin-like protofilaments. *EMBO J* **31**: 2249–2260
- van den Ent F, Löwe J (2000) Crystal structure of the cell division protein FtsA from *Thermotoga maritima*. *EMBO J* **19**: 5300–5307
- White CL, Gober JW (2012) MreB: pilot or passenger of cell wall synthesis? *Trends Microbiol* **20**: 74–79