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MicroCommentary

In long bacterial cells, the Min system can act off-center

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Summary

In many rod-shaped bacteria, the Min system is well-known for generating a cell-pole to cell-pole standing wave oscillation with a single node at mid-cell to align cell division. In filamentous *E. coli* cells, the single-node standing wave transitions into a multi-nodal oscillation. These multi-nodal dynamics have largely been treated simply as an interesting byproduct of artificially elongated cells. However, a recent *in vivo* study by Muraleedharan *et al.* shows how multi-nodal Min dynamics are used to align non-mid-cell divisions in the elongated swarmer cells of *Vibrio parahaemolyticus* . The authors propose a model where the combined actions of cell-length dependent Min dynamics, in concert with nucleoid occlusion along the cell length and regulation of FtsZ levels ensures Z ring formation and complete chromosome segregation at a single off-center position. By limiting the number of cell division events to one per cell at an off-center position, long swarmer cells are preserved within a multiplying population. The findings unveil an elegant mechanism of cell-division regulation by the Min system that allows long swarmer cells to divide without the need to 'dedifferentiate'.

Multi-nodal Min oscillations and asymmetric cell division

Bacterial cells have intricate subcellular organization with various mechanisms for growth and division that result in a rich diversity of cell shapes and sizes (Kysela *et al.*, 2016). Rod-shaped bacteria, such as *E. coli*, have mechanisms to position their future division site precisely at mid-cell. Two well-studied spatial regulators of cell division positioning in *E. coli* and *B. subtilis* are the Min system and nucleoid occlusion (Schumacher, 2017). Whereas nucleoid occlusion prevents cell division over nucleoids, the Min system forms a bipolar gradient that inhibits cell division at the cell poles by acting on FtsZ.

The spatial regulation of bacterial cell division provided by the Min/FtsZ system is a remarkable example of dynamic subcellular organization found throughout the microbial world as well as in the vast majority of eukaryotic plastids, such as chloroplasts (Leger *et al.*, 2015) and mitochondria (Chen *et al.*, 2018). FtsZ is a conserved tubulin-like GTPase that can organize into a polymeric ring-like structure called the Z ring (Bi and Lutkenhaus, 1991). The Z ring acts as a scaffold for the recruitment of several other proteins required for cell division (Busiek and Margolin, 2015). The MinCDE system is composed of three proteins that self-organize into a standing-wave oscillator on the inner membrane and spatially regulates where FtsZ polymerizes into a Z ring (Szwedziak and Ghosal, 2017). MinD is an ATPase that associates with the inner membrane when bound to ATP (Hu and Lutkenhaus, 2003; Zhou and Lutkenhaus, 2003). MinE regulates MinD association with membrane in many ways (Vecchiarelli *et al.*, 2016; Kiyoshi and Vecchiarelli, 2018), including stimulating MinD release from the membrane (Hu *et al.*, 2002). The dynamic interplay between MinD and MinE on the inner-membrane results in a cell-pole to cell-pole standing wave oscillation (Raskin and de Boer, 1999). The final protein MinC is not required for oscillation, but directly associates with MinD on the membrane as well as FtsZ to inhibit its polymerization into a Z ring (Hu *et al.*, 1999; Dajkovic *et al.*, 2008; Shen and Lutkenhaus, 2009; Shen and Lutkenhaus, 2010). The pole-to-pole oscillation creates a time-averaged concentration gradient of MinD (and MinC) that is highest at the poles and lowest at mid-cell (Meinhardt and de Boer, 2001). The Z ring is therefore allowed to polymerize within this single node where MinD and MinC concentrations are at a minimum, thus promoting symmetric binary fission that generates two daughter cells roughly equal in size (Lutkenhaus, 2007).

It is well established that the 'single-node' dynamics of the Min system act to align mid-cell division in small rod-shaped bacteria like *E. coli* (Raskin and de Boer, 1999), and more

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recently in the cyanobacterium *S. elongatus* (MacCready *et al*., 2017). It is also known that Min oscillation is strongly influenced by cell shape (Raskin and de Boer, 1999; Hu and Lutkenhaus, 2001; Bonny *et al*., 2013). In elongated *E. coli* cells, such as in division-defective mutants, oscillation no longer extends from pole-to-pole with a single node at mid-cell. Once the cell length exceeds that of the oscillation wavelength, the pattern changes to a multi-nodal standing wave. The number and position of these nodes depends on cell length. Recent work by Muraleedharan *et al*. shows how multi-nodal dynamics of the Min system that emerge in elongated cells are used to align non-mid-cell divisions (Muraleedharan *et al*., 2018).

The Gram-negative bacterium *Vibrio parahaemolyticus* can exist as a swimmer cell that grows in liquid culture or as a swarmer cell that is specialized for growth on surfaces (McCarter and Silverman, 1990). Swarmer cells suppress cell division and are therefore highly elongated. However, division is not completely halted in these cells because cell proliferation is still needed to expand the colony. The mechanism used by long swarmer cells to divide without diminishing the population of long cells required for swarming behaviour is unknown. Is there a switch in gene expression that differentiates a subset of swarmer cells in order to promote cell division? Or is there regulation at the post-translational level, where cell division is asymmetrically positioned to make both long and short cells without the need to 'dedifferentiate'? Muraleedharan *et al*. shows that swarmer cells indeed divide, but positioning of the cell division machinery is dependent upon cell length – small

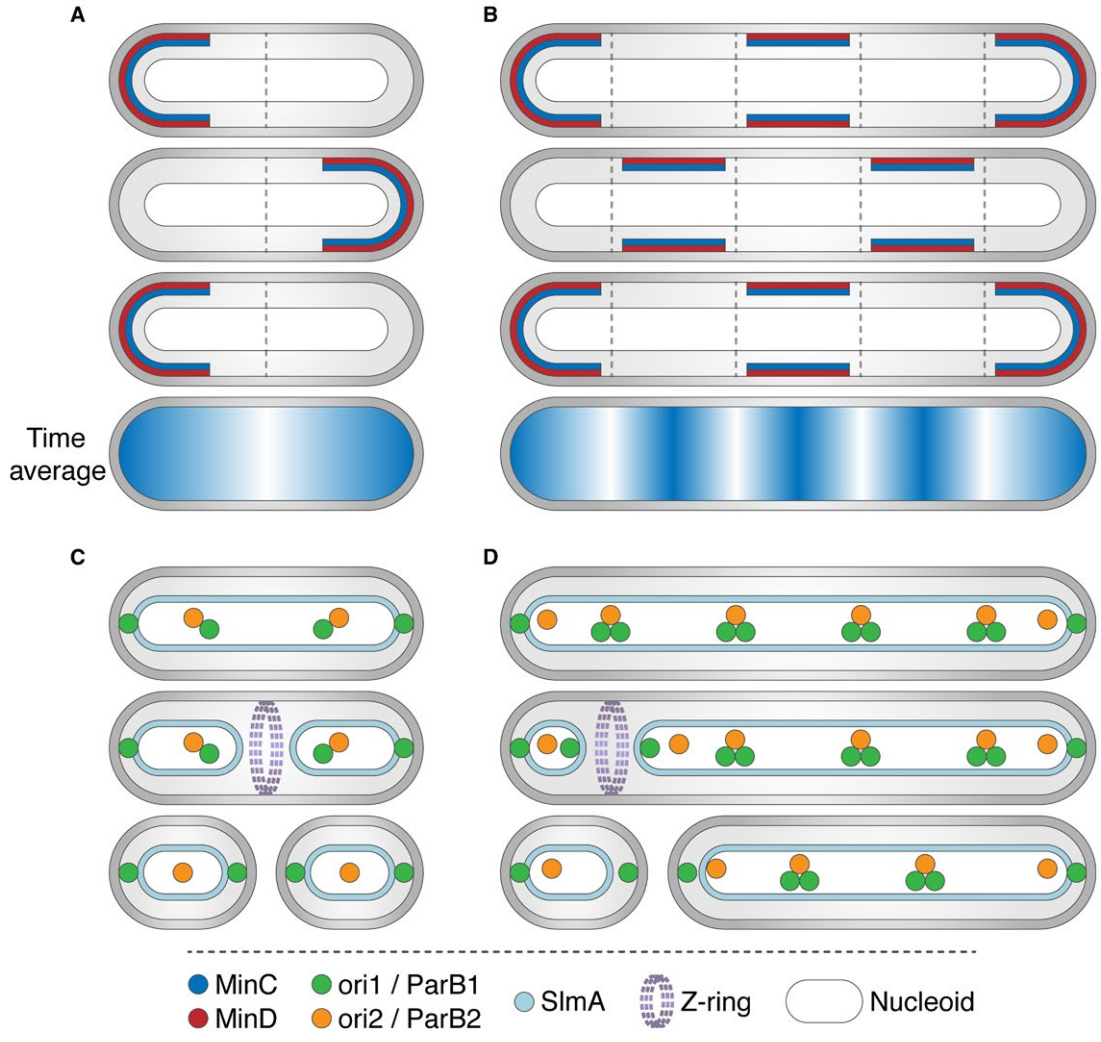

Fig. 1. Cell-length dependent localization of the division plane in *V. parahaemolyticus*.
A. MinC (blue) and MinD (red) oscillate from pole-to-pole to form a time averaged gradient of anti-FtsZ activity that is highest towards the polar regions of the cell, inhibiting FtsZ assembly, and lowest at midcell, allowing Z-ring (purple) assembly.
B. In elongated swarmer cells, MinCD form a dynamic standing wave that forms multiple possible sites for Z-ring assembly.
C. SlmA (light blue) prevents Z-ring formation atop the nucleoid. Z-ring assembly occurs where chromosomes completely segregate.
D. In elongated swarmer cells, FtsZ assembles towards the polar region of the cell (mimicking an averaged size cell) following chromosome segregation.

cells (less than 10 microns in length) divide at mid-cell, while long cells (greater than 10 microns) transition to an off-center division site (Muraleedharan *et al*., 2018).

The authors find that a switch to non-mid-cell division is mediated by a cell-length-dependent transition in the number and position of nodes generated by the Min system (Muraleedharan *et al*., 2018). Consistent with the previously studied Min systems of *E. coli, B. subtilus* and *S. elongatus*, both swimmer- and swarmer-cells less than 10 microns in length support a single node where MinD/MinC concentrations are minimum at mid-cell (Fig. 1A), thus promoting symmetric division. However, in swarmer cells longer than 10 microns, Min oscillation transitions from a single node at mid-cell to two nodes at the quarter positions (Fig. 1B). Therefore, in these longer cells, the Min system promotes asymmetric positioning of the Z ring at one of these off-center nodes. In artificially elongated *E.coli* cells, Min nodes occur with a spacing of ~ 7 μm (Raskin and de Boer, 1999; Meinhardt and de Boer, 2001). Therefore, the maximum Min wavelength and cell-length at which a transition to multi-node dynamics occur are very similar between *E. coli* and *V. parahaemolyticus*. Similar length-dependent transitions in Min oscillation have also been reconstituted in rod-shaped compartments covered with membranes (Zieske and Schwille, 2013; Caspi and Dekker, 2016). Together the findings show that it is a cell length-dependent intrinsic property of the Min system that triggers the transition in localization dynamics.

Regulation of FtsZ levels and Nucleoid Occlusion limits division to a single site

Two nodes provide the cell with two potential sites for a functional Z ring, however, the authors find that only one of these quarter positions are selected for Z ring formation and cell division (Muraleedharan *et al*., 2018). In very long swarmer cells, Min dynamics transition from 2 to 4 nodes for cells between 20 and 30 microns in length, and then from 4 to 6 nodes for cells 30–40 microns in length. Even in these multi-nodal cells, only one functional Z ring is formed, allowing for a single septation event. How do swarmer cells with multi-nodal Min dynamics restrict Z ring formation and cell division to a single node? The authors find that FtsZ levels in long swarmer cells are regulated to match that of swimmer cells. Consistently, short swimmer cells that were chemically elongated had significantly higher levels of FtsZ and, combined with multi-nodal Min dynamics, displayed equidistant positioning of multiple Z rings. Therefore, in long swarmer cells, multi-nodal Min dynamics provide several choices for Z ring positioning, but there is only enough FtsZ to form a single Z ring.

The authors also determined the relationship between cell division and chromosome segregation in swarmer cells. *V. parahaemolyticus* has two chromosomes that are actively partitioned by two independent Par systems (Yamaichi *et al*., 1999). Swarmer cells are polyploid, and the copy number of both chromosomes correlate with cell length. By tagging the *ori* regions, the authors find both chromosomes are equidistantly segregated from their respective copies along the cell length. However, upon DAPI staining, it was found that these nucleoid copies are not spatially resolved from one another; that is, there were no cytoplasmic gaps in between chromosome copies spanning the cell length. Intriguingly, a single cytoplasmic gap would only emerge between nucleoids at a site that correlated with the position of a functional Z ring. This 'complete chromosome segregation' event followed a similar cell length-dependent transition from a mid-cell position in short swarmer cells (Fig. 1C) to an off-center position in long cells (Fig. 1D). In contrast, all DAPI-stained nucleoids of chemically-elongated swimmer cells were completely segregated from one another with cytoplasmic gaps that correlated with the positioning of multiple Z rings. Together, the data suggests that positioning of the Z ring by the Min system is a prerequisite to promoting complete chromosome segregation. Together, the paper highlights the importance of node positioning by the Min system as a key first step in a cascade of spatial regulation for Z ring positioning, and the completion of chromosome segregation at the future division site.

The indication that small swimmer and long swarmer cells possess the same concentration of FtsZ presents a problem in concentrating all FtsZ molecules into a single functional Z ring. How do all FtsZ molecules concentrate to form a single Z ring at one Min node, and avoid getting sequestered as non-functional subpopulations at other nodes? To ensure that all FtsZ molecules are available for a single Z ring in long cells, the data implicate nucleoid occlusion. In *E. coli*, the nucleoid occlusion effector protein is SlmA (Bernhardt and de Boer, 2005), which binds to specific DNA sequences and directly interacts with FtsZ to block Z ring formation over regions of the cell occupied by the nucleoid (Tonthat *et al*., 2013; Cho *et al*., 2011). This has also been shown for SlmA of *V. Cholerae* (Galli *et al*., 2016). In the absence of SlmA in *V. parahaemolyticus*, the authors found that FtsZ forms division-deficient clusters along the cell length rather than a single, division-competent Z ring (Muraleedharan *et al*., 2018). Therefore, FtsZ is inhibited from accumulating along the cell length by SlmA, which is bound to the multiple copies of nucleoids occupying the entire length of the cell. But once a 'complete chromosome segregation' event occurs at one of the Min-nodes, all FtsZ molecules can congregate in this cytoplasmic gap to form a Z ring, free from SlmA inhibition (Fig. 1CD). Together, the authors propose a model where the combined actions of cell-length dependent Min dynamics, a uniform distribution of nucleoids and SlmA function along the cell length, and regulation of FtsZ levels ensures Z ring formation and complete chromosome segregation at a single off-center position.

Concluding remarks and future directions

Very long swarmer cells with more than two Min nodes showed a preference for pole-proximal nodes over pole-distal nodes for Z ring formation and cell division, suggesting there is an additional level of regulation for cell division positioning not treated in this work. No preference was found for one cell pole over the other in the placement of the division site with respect to the previous division. Recently, unique positive regulators of Z ring formation have been identified in bacteria that can work in concert with nucleoid occlusion and the Min system (Huang *et al*., 2013); highlighting the diversity and redundancy of division site selection mechanisms that may play a role in positioning the Z ring at pole-proximal Min nodes. When *E. coli* cells are grown in rich media, deletions of the Min system and SlmA results in too many potential locations for Z rings, preventing assembly of a single functional Z ring at mid-cell (Bernhardt and De Boer, 2004; Yu and Margolin, 1999). However, in minimal medium, these cells can divide with surprisingly robust Z ring placement at mid-cell (Bailey *et al*., 2014). There may be other regulators outside of Min and SlmA contributing to Z ring positioning at pole-proximal Min nodes in swarmer cells. One possibility not pursued in this paper is the positioning of the Ter macrodomain of chromosomes at the cell poles relative to those found along the cell length. In *E. coli*, the Ter macrodomain has been implicated as part of a positive regulatory system for Z ring positioning (Bailey *et al*., 2014). In this system, MatP serves to connect the Ter macrodomain to the divisome through interaction with ZapB (Espéli *et al*., 2012). It is attractive to speculate that the Ter macrodomains of chromosomes at the poles are oriented differently to those down the length of the cell, such that pole-proximal Min nodes are preferentially chosen for Z ring positioning.

Although it is fairly well understood how the Min system positions the Z ring during vegetative growth, relatively little is known about the requirements for Z ring placement near the cell poles in preparation for asymmetric septation in endospore-forming bacilli. When fluorescently-tagged MinD and MinE from *E. coli* was reconstituted in *B. subtilis*, the reconstituted pole-to-pole oscillation inhibited sporulation (Jamroškovič *et al*., 2012). It was proposed that Min oscillations likely prevented the assembly of Z rings near cell poles required for endospore formation. Min oscillation was therefore suggested to be a system optimized for mid-cell divisions in cells like *E. coli* that only grow vegetatively, and problematic for cells that switch to asymmetric division (Barak, 2013). Although this is still likely true for cells smaller than the wavelength of the Min system, the work by Muraleedharan *et al*. show how multi-nodal Min dynamics that emerge in elongated cells can be used to align non-mid-cell divisions (Muraleedharan *et al*., 2018).

Wehrens and Ershove et al. recently published highly complementary findings in *E. coli* cells that were elongated due to stress (Wehrens *et al*., 2018). In these growing filamentous cells, multiple Fts rings were evenly distributed in response to the multi-nodal dynamics of the Min system. Upon removal of filamentation stress, single division events would sequentially occur at one of the Fts rings. But unlike the pole proximal preference of cell division in the elongated swarmer cells of *V. parahaemolyticus*, cell division position was chosen at random from one of the pre-positioned Fts ring sites in these elongated *E. coli* cells. How these long *E. coli* cells divide just once, despite having several Fts rings, remains an open question. The findings by Muraleedharan *et al.* suggest several other mechanisms could be at play, outside of FtsZ depletion (Muraleedharan *et al*., 2018). Taken together, these studies reveal an underappreciated function of the Min/Fts system in the off-center positioning of cell division. How many of other bacterial species that undergo a filamentous lifestyle employ this Min dependent mode of asymmetric cell-division?

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