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Probing the mechanistic principles of bacterial cell division with super-resolution microscopy

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Bacterial cell division takes place almost entirely below the diffraction limit of light microscopy, making super-resolution microscopy ideally suited to interrogating this process. I review how super-resolution microscopy has advanced our understanding of bacterial cell division. I discuss the mechanistic implications of these findings, propose physical models for cell division compatible with recent data, and discuss key outstanding questions and future research directions.

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Introduction

How do you cut a balloon in half without bursting it? This is the remarkable challenge that bacteria must overcome in order to divide and multiply. How bacterial cell division proteins work together to cut the cell in two has remained mysterious for decades, because it is difficult to measure protein organization and dynamics in cells not much larger than the diffraction limit of visible light. Now, single molecule imaging and super-resolution microscopy, together with *in vitro* reconstitution and cryo-electron microscopy, are allowing us to make major steps forward in our understanding of bacterial cell division.

In this commentary, I will focus on how single molecule and super-resolution microscopies have helped us understand how the bacterial division machinery constricts the cell. Owing to space constraints, I do not discuss excellent work using super-resolution microscopy to study other aspects of division, including positioning of the division machine [1], coordinated disassembly of the division machine [2], and the role of divisome regulatory proteins [3,4].

The divisome, a multi-protein nanomachine

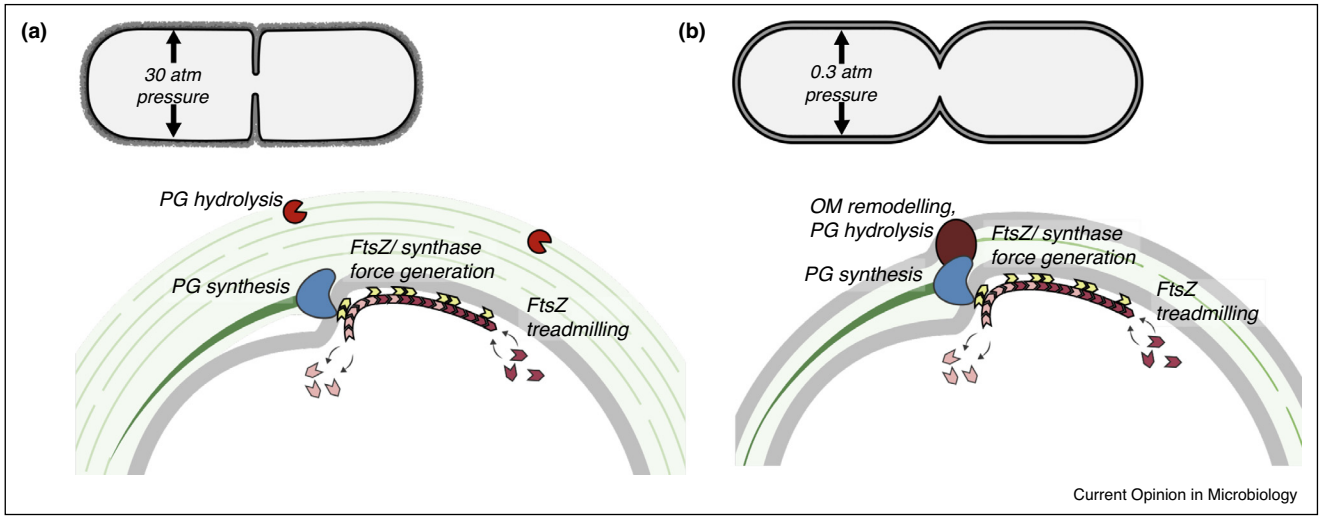
Bacteria are surrounded by a mesh-like peptidoglycan cell wall which opposes the cell's high osmotic pressure. Gram-positive bacteria have a single plasma membrane surrounded by a thick multi-layered cell wall and maintain an osmotic pressure of approximately 20 atmospheres [5] (Figure 1a). To divide, Gram-positives build a cross-wall or septum across the mid-cell [6]. Gram-negative bacteria have both an inner and outer membrane surrounding a thin, mainly single layered cell wall [7] (Figure 1b), and maintain a lower osmotic pressure of about 0.3 atmospheres [8]. Instead of building a division septum, Gram-negative cells form a constricting invagination at mid-cell, probably by simultaneously building new inner layers of cell wall at mid-cell, hydrolysing the old outer layers and remodelling the outer membrane [9,10•].

Division is carried out by a network of proteins called the divisome [11]. The core of the divisome is the almost universally essential tubulin homologue FtsZ, which polymerizes to form a mid-cell band, and recruits the other divisome proteins to mid-cell. FtsZ is tethered to the cell membrane by redundant membrane anchors, including the widely conserved actin homologue FtsA. FtsZ recruits the cell-wall synthesis machinery to mid-cell, including a glycosyltransferase, FtsW or PBP1B (*Escherichai coli*)/PBP1 (*Bacillus subtilis*), which elongates glycan strands, and a transpeptidase, PBP3 (*E. coli*)/PBP2B (*B. subtilis*), which attaches new glycan strands to existing peptidoglycan. These core proteins are supplemented by multiple additional factors, mostly with regulatory or scaffolding roles [6,9].

How the divisome works mechanistically has remained a great unsolved mystery of cell division since the discovery that FtsZ localizes to mid-cell 25 years ago [12]. Key mechanistic questions are: how is constrictive force generated to oppose turgor pressure, how is septal cell wall synthesis organized, and how are synthesis and force generation coupled? Recently, super-resolution and single molecule microscopies have made important contributions to these questions thanks to their high resolution and sensitivity (Box 1).

How does the divisome generate constrictive force? A longstanding model for cytokinesis is the contractile ring

Figure 1



Mechanistic principles of Gram-positive *B. subtilis* and Gram-negative *E. coli* cell division. **(a)** Key physical tasks required for division of Gram-positive *B. subtilis* by septal cross-wall building. **(b)** Key physical tasks required for invagination of the *E. coli* mid-cell. *PG*, peptidoglycan; *OM*, outer membrane.

model [13] where FtsZ forms a continuous 'Z-ring' around the mid cell, made up of either a single continuous FtsZ filament, or multiple overlapping short filaments. The Z-ring would generate sustained constrictive force around the entire circumference of the plasma membrane, similar to the actin–myosin contractile ring in eukaryotes. This could be achieved either through GTP-hydrolysis induced bending of the Z-ring, or by sliding of multiple overlapping short FtsZ filaments within the Z-ring [14]. This was supported by the fact that FtsZ forms an apparently continuous ring when observed via widefield fluorescence microscopy, and that reconstituted FtsZ forms helices or rings capable of constricting artificial lipid vesicles [15,16].

The contractile ring model makes the strong prediction that FtsZ should form continuous ring-like structures *in vivo*. However, widefield fluorescence microscopy of FtsZ has insufficient resolution to test this hypothesis, and transmission electron microscopy failed to detect FtsZ filaments at mid-cell.

Divisome ultrastructure

The first major step forward in determining the *in vivo* ultrastructure of FtsZ came from cryo-electron tomography (cryo-ET). In 2007 Li and co-workers used cryo-electron tomography to detect multiple short scattered FtsZ filaments around the mid-cell of *Caulobacter crescentus* [17•]. What they did not see was the predicted continuous FtsZ ring, intriguingly suggesting that cell division might not in fact be driven by a contractile Z-ring. However at the time it was unclear whether this was because a continuous Z-ring was genuinely absent, or whether cryo-ET's relatively low contrast and missing wedge (incomplete imaging around the cell circumference) meant that only a small fraction of polymerized FtsZ was detected.

The ultrastructure of the FtsZ ring was therefore an ideal target for the nascent field of super-resolution microscopy, as it offered high *in vivo* resolution, protein specific high contrast, single molecule sensitivity, and the ability to image around the entire cell.

In 2010, Fu and co-workers presented a pioneering first study of FtsZ ultrastructure by super-resolution microscopy [18••] (Figure 2a). They used 2D localization microscopy at 30 nm resolution to discover that *E. coli* FtsZ rings are surprisingly thick structures, about 100 nm thick axially. As it would be hard to build such a thick structure from a single continuous FtsZ filament, due to low cellular FtsZ concentration, this instead suggested a loosely packed Z-ring composed of multiple overlapping filaments. However, this early study lacked the crucial third dimension required to resolve the overall architecture of the Z-ring.

Shortly after, Strauss and co-workers used structured illumination microscopy to perform the first 3D super-resolution study of FtsZ ultrastructure and dynamics, allowing the overall architecture of the entire Z-ring to be resolved [19••] (Figure 2b). Structured illumination microscopy uses patterned illumination to double the resolution of conventional widefield microscopy, to 100 nm laterally and 300 nm axially. Strauss and co-workers examined FtsZ localization in *B. subtilis* and

Box 1 Super-resolution/advanced microscopy approaches for cell division imaging

Method	*XY* resolution	*Z* resolution	Principle/technical notes	Advantages/disadvantages for cell division imaging
Localization microscopy (PALM/STORM)	10–40 nm	20–100 nm	Circumvents diffraction limit via stochastic localization of single blinking fluorophores [50,51].	Highest fluorescence-based resolution. Mainly limited to fixed cells or single snapshots in live cells.
Structured illumination microscopy (SIM)	100 nm	300 nm	Doubles resolution through spatially patterned illumination [52]. Related image scanning microscopy techniques [53] achieve similar resolution inc. Airyscanning/ HyVolution/iSIM.	Only doubles resolution compared to epifluorescence, but relatively low light intensity makes SIM ideal for live cell imaging of division. Can be further combined with TIRF to reduce background or vertical immobilization to increase effective resolution.
Stimulated emission depletion microscopy (STED)	~50 nm	~100 nm	Narrows effective confocal microscope spot size with a stimulated emission doughnut beam surrounding the excitation spot [54].	High illumination intensity effectively limits this approach to fixed cells. Requires dye/ antibody labelling for optimal image quality.
Single molecule tracking	<30 nm	<100 nm	Tracking of small numbers of isolated individual fluorescently labelled molecules within a cell. Often illuminated in TIRF, or by combined widefield illumination and photoactivation (sptPALM) [55].	Allows measurement of overall motion, organization and subpopulation heterogeneity for individual proteins.
TIRF microscopy	250 nm	50–150 nm	Evanescent field illumination of a small slice within about 100 nm from the cover glass-sample interface. Can be combined with other SR methods.	Restricted illumination depth greatly increases signal to noise ratio, making TIRF ideal for live cell imaging of membrane bound proteins. Can only be used to interrogate early cell division as septal constriction eventually moves the division machinery out of the illumination area.
Vertical immobilization	250 nm	500 nm	Vertical immobilization of rod-shaped bacteria in patterned microholes [30••]. This rotates the cell division plane parallel to the 2D imaging plane, increasing effective resolution. Can be combined with other SR methods.	Allows simultaneous live cell imaging of the entire division site, throughout the division cycle at increased effective resolution and with less photobleaching than 3D microscopy. However, signal to noise ratio is lower than TIRF.

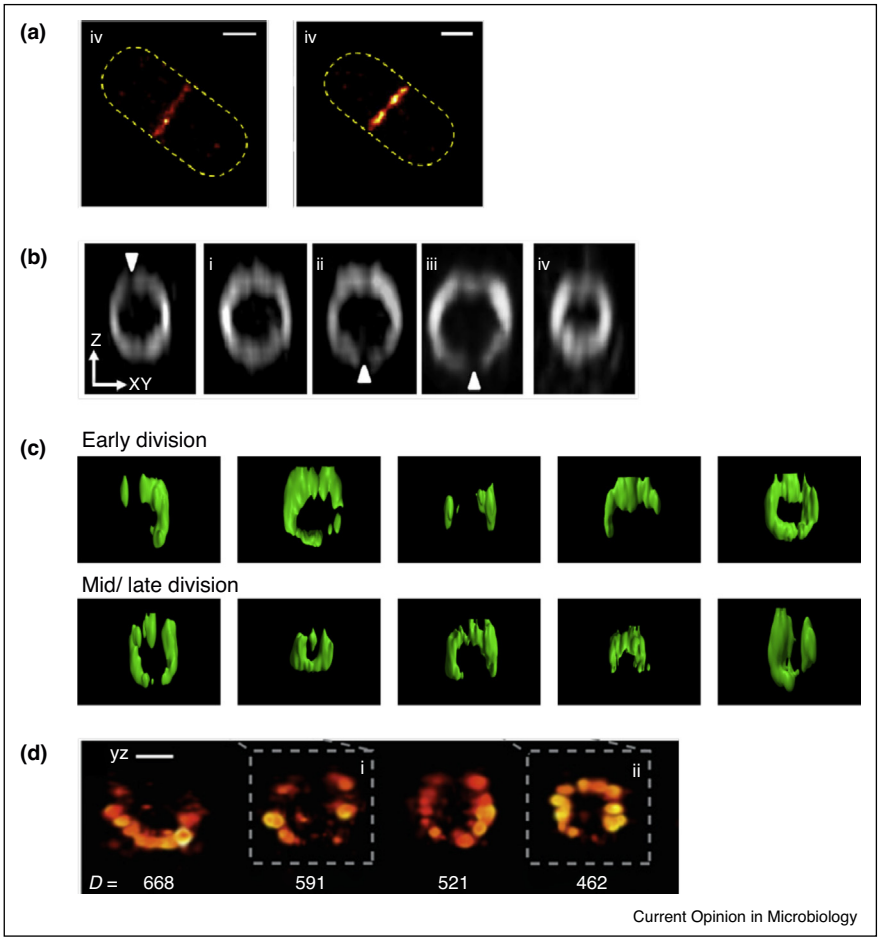
Staphylococcus aureus, notably using a *B. subtilis* strain with FtsZ-GFP as the sole source of cellular FtsZ. Remarkably, they found that FtsZ formed highly heterogeneous rings, containing gaps of >120 nm, calling into question the contractile Z-ring model. Subsequent 3D-SIM in *E. coli* also presented a similar picture of patchy FtsZ organization [20].

Biteen and co-workers presented the first proof of concept imaging of FtsZ by 3D localization microscopy, allowing imaging around the entire *C. crescentus* Z-ring at 30 nm lateral resolution and 100 nm axial resolution [21•]. In preliminary measurements of 3D Z-ring organization in two cells they observed Z-rings composed of a loosely packed band of FtsZ.

In 2014 we presented a systematic study of FtsZ ultrastructure by 3D localization microscopy, again at 30 nm lateral and 100 nm axial resolution, using an automated high-throughput 3D PALM microscope which we developed [22••] (Figure 2c). We analysed 3D Z-ring organization in hundreds of cells throughout the *C. crescentus*cell cycle and observed that the Z-ring consistently showed large gaps of several hundred nanometres. Similar organization of the *E. coli* Z-ring has subsequently been observed by 3D PALM [23••,24] (Figure 2d). Taken together with 3D-SIM studies [19••,20], these data from multiple organisms and imaging techniques form a consistent picture of the Z-ring as a patchy heterogeneous structure, inconsistent with the model of a continuous Z-ring which generates stable sustained force around the entire division site [13].

In the most recent of several cryo-ET studies of FtsZ ultrastructure [17•,25•,26•], FtsZ was imaged throughout the cell cycle in multiple Gram-negative species, and was found to form largely patchy Z-rings made up of short FtsZ filaments [26•], fairly consistent with super-resolution studies. However, the precise ultrastructure of the Z-ring still remains unclear. Super-resolution studies

Figure 2



Super-resolution imaging of FtsZ ultrastructure. **(a)** 2D localization microscopy of *E. coli* FtsZ showing axially thick Z-rings. Scale bar, 500 nm. (b–d) 3D super-resolution imaging of mid-cell FtsZ showing patchy Z-rings. **(b)** 3D-SIM of *B. subtilis* FtsZ; mid-cell Z cross-sections. **(c)** 3D-PALM of *C. crescentus* FtsZ; mid-cell volume renderings. **(d)** 3D-PALM of *E. coli* FtsZ; mid-cell Z cross-sections. Scale bar, 300 nm.
(a–d) adapted from [18••,19••,22••,23••] with permission.

suggest a loose, patchy, toroidal FtsZ band, about 80–100 nm thick radially [22••,24] — possibly exaggerated by the historic use of ectopically expressed FtsZ fluorescent protein fusions for PALM. Cryo-ET studies have only detected FtsZ in a thin layer about 15 nm from the inner membrane [25•] (which fits with FtsA membrane anchor spacing), but due to lower contrast, could miss short FtsZ filaments further away from the membrane. I speculate the truth may be somewhere in between; treadmilling FtsAZ filaments (see below) should be strongly membrane associated, but a sparse halo of short FtsZ filaments or dimers extending further into the cytoplasm could surround the division site, possibly acting as nucleators for polymerization of membrane-associated FtsAZ.

Pairing super-resolution and cryo-ET as complementary techniques could be an exciting future direction to study cytoskeletal ultrastructure. Each technique can in principle complement the advantages of the other: cryo-ET offers ultimate resolution and minimal perturbation, and super-resolution offers high (single molecule) protein specific contrast, imaging throughout the entire cell, and higher throughput. However, fluorescence super-resolution methods also have a further key application beyond imaging of ultrastructure — high resolution imaging in living cells.

Divisome dynamics

Division is a necessarily dynamic process involving rapid remodelling of the bacterial cell wall. It has been known for some time that FtsZ is highly dynamic, with the entire Z-ring turning over in <10 s [27], but the purpose of this dynamic turnover has until recently remained unclear. Super-resolution and single molecule fluorescence microscopy have proved ideal for investigating the organization and function of the dynamics of cell division at sufficient resolution *in vivo*.

In a pioneering first study of divisome dynamics at the single molecule level, in 2008 Niu and Yu carried out single molecule tracking of FtsZ, and found that individual FtsZ molecules within the *E. coli* Z-ring are immobile [28••]. This provided strong evidence against filament sliding models of FtsZ force generation [14].

In their 2012 3D-SIM paper discussed above, Strauss and co-workers [19••] also intriguingly found that *B. subtilis* and *S. aureus* Z-rings displayed dynamic structural rearrangements throughout constriction, suggesting that the high rate of FtsZ turnover involves not only Z-ring subunit exchange, but large scale structural rearrangement.

In 2016, Coltharp and co-workers used localization microscopy, combined with brightfield imaging of constriction site diameter, to measure constriction site dynamics in live *E. coli* [23••]. Surprisingly, perturbation of FtsZ GTP-hydrolysis activity did not affect constriction rate in *E. coli*, that is, FtsZ turnover does not appear to be rate limiting for *E. coli* cytokinesis. Furthermore, they found that cell wall synthesis is likely rate limiting for constriction, and that MatP, a regulator of chromosome segregation, may act as a brake on division, perhaps to allow sufficient time for chromosome segregation.

This question of divisome dynamics and rate-limiting steps in cytokinesis has recently proved crucial in elucidating key mechanistic principles of division. In 2014, Loose and Mitchison made the remarkable discovery that FtsZ can treadmill *in vitro* on supported lipid bilayers [29••], that is, that FtsZ filaments can move by GTP-hydrolysis-driven asymmetric filament polymerization and depolymerization. This inspired us and others to revisit the longstanding puzzle of why mid-cell FtsZ turns over rapidly *in vivo* [27].

Together with collaborators, we recently showed in *B. subtilis* [30••], and Yang and co-workers showed in *E. coli* [31••], that FtsZ forms motile filaments which treadmill *in vivo*, and that the treadmilling motion of FtsZ drives cell division and septal cell wall synthesis. This was demonstrated by TIRF and SIM microscopy for FtsZ localized to predivisional FtsZ-rings in a range of wildtype and GTP-hydrolysis impaired mutants [30••,31••]. In *B. subtilis*, we further showed that FtsZ treadmills even in highly constricted, actively dividing cells, using a novel method for imaging bacteria immobilized in vertical agarose microholes [30••]. In each case, high resolution live cell imaging of FtsZ dynamics, by TIRF, SIM, or vertical cell immobilization was crucial in providing sufficient spatiotemporal resolution and image contrast to successfully observe FtsZ treadmilling despite the near-diffraction-limited size of the division site.

Furthermore, SIM imaging of newly synthesized peptidoglycan showed that septal cell wall synthesis occurred at discrete mobile sites [30••], and single molecule tracking showed that single cell wall synthases PBP2B (*B. subtilis*)/PBP3 (*E. coli*) moved processively around the division site [30••,31••]. Surprisingly, the treadmilling motion of FtsZ was required for synthase motion, and the speed of individual synthases was set by the treadmilling speed of guiding FtsZ filaments [30••,31••]. In *B. subtilis*, FtsZ treadmilling further set the rate of division, which seems intuitive: treadmilling limits synthase speed, which thus limits constriction [30••].

Remarkably, in *E. coli*, treadmilling does not appear to set the rate of division. Although in *E. coli* the FtsZ GTP hydrolysis rate/treadmilling speed sets the speed of individual synthases, perturbations to FtsZ treadmilling do not affect the overall speed of division [31••]. Perhaps this is because *E. coli* has a more complex job than *B. subtilis*: it must maintain a thin cell wall at the division site, which presumably requires much more careful control than for the multi-layer *B. subtilis* cell wall in order to avoid lysis [9]. *E. coli* must also coordinate constriction with remodelling of the outer membrane [9]. These additional processes may act as brakes on division, such that *E. coli* FtsZ is the rate-limiting guide of individual synthases, but not of overall constriction rate. Together with previous work [23••], this then begs the intriguing question of what exactly is the key rate-limiting step for *E. coli* constriction?

These discoveries provide important new insight into the basic mechanism of cell division; that cell wall remodelling at mid-cell is carried out by multiple motile cytoskeleton-synthase complexes, driven by FtsZ treadmilling. However, multiple key questions about the mechanistic principles of cell division remain unsolved.

Implications for physical mechanisms of bacterial cell division

It is still unclear how the division machine generates constrictive force to oppose the cell's high turgor pressure. Although FtsZ treadmilling rate limits the motion of cell wall synthases, this does not mean that FtsZ generates force — it merely says FtsZ treadmilling is the slowest chemical/mechanistic step in the division process.

However, the treadmilling-driven cytokinesis model, where cell division is orchestrated by motile cytoskeleton-synthase complexes driven by FtsZ treadmilling and localized in a patchy 'Z-ring', does place significant constraints on how constrictive force could be generated. There are three primary possibilities consistent with treadmilling-driven cytokinesis (Figure 1): *First, FtsZ pulling:* GTP-hydrolysis-induced bending of short treadmilling FtsZ filaments could create a moving membrane deformation which the synthesis complex can surf along as it builds the cell wall. Importantly, this model requires only a localized and transient membrane deformation by

FtsZ filaments, subsequently rectified by ratchet-like cell wall synthesis. This significantly reduces force generation requirements compared to models requiring sustained force generation around the entire Z-ring [32•,33]. Assuming typical turgor pressure of 0.3 atm (Gram-negative) or 20 atm (Gram-positive) [5,8] and a 2 nm^2 membrane deformation as sufficient to accommodate one peptidoglycan subunit [34], the minimum force to locally oppose turgor pressure is 0.05 pN or 4 pN for Gram-negative or Gram-positive bacteria respectively. This is well within the plausible range of force generation by GTP-hydrolysis-induced FtsZ bending [35,36]. The surprisingly low force estimate for Gram-negatives might even explain why FtsZ treadmilling is rate limiting for division in *B. subtilis* but not for *E. coli*. *Second, synthase pushing:* Alternatively, the synthesis complex could be strong enough to displace the membrane inwards as it synthesizes new glycan strands. *Third, combined push-pulling:* Finally, both FtsZ and the synthesis complex could generate constrictive force, working together to drive division.

It was recently proposed that the divisome might not need to overcome turgor pressure, due to evidence from bulk studies of cytoplasmic and periplasmic volume that the periplasm/periplasmic space may be isosmotic with the cytoplasm [10•]. For Gram-negatives, the isotonic periplasm/cytoplasm hypothesis would move the key osmotic barrier to the periplasm-peptidoglycan interface [10•]. A problem with this hypothesis is that Gram-negative constriction site building necessarily reduces the volume of both periplasm and cytoplasm. Therefore, even in an isotonic periplasm/cytoplasm, the divisome would still need to overcome turgor pressure at the leading edge of the constriction site, leaving the physical constraints on Gram-negative cell division similar to the case of a non-isotonic cytoplasm. However, as discussed above, the force required to locally oppose Gram-negative turgor pressure could actually be rather small, and less of a critical factor than for higher turgor Gram-positives.

In Gram-positives, evidence that turgor pressure might not act on the plasma membrane comes from cryo-electron microscopy of frozen-hydrated cell sections by Beveridge and co-workers, which appear to support the existence of a periplasmic space in *B. subtilis* and *S. aureus* between the membrane and the peptidoglycan [37,38]. However, less perturbative cryo-electron tomography of flash frozen *B. subtilis* has subsequently not observed a periplasmic space [39,40] suggesting that the apparent periplasmic space seen in the Beveridge data may have been an artefact of high pressure freezing or cryoprotectant-induced osmotic upshock [39]. Current evidence therefore favours a model where the Gram-positive turgor pressure barrier is indeed the plasma membrane, and where the Gram-positive divisome must directly overcome turgor pressure under the physical constraints discussed above. However, further research into the role of turgor pressure in bacterial division and growth is required.

Beyond the question of force generation, treadmilling-driven cytokinesis raises many other exciting new questions. The molecular mechanism behind the coupled motion of the processive cell wall synthases and treadmilling FtsAZ filaments is unknown. Could one of the divisome components couple the synthesis machinery to moving FtsAZ filaments by acting as a filament end-tracker, like eukaryotic formins? Or perhaps the synthesis machinery uses FtsAZ filaments as a track for processive sliding, more like DNA polymerases?

Additionally, the tight coupling between FtsZ dynamics and cell wall synthesis identifies a new potential role for the various FtsZ-associated proteins. By modulating the organization and dynamics of FtsZ dynamics, Z-associated proteins could have a key functional role in regulating septal cell wall synthesis. It was recently shown that the *C. crescentus* Z-associated protein FzlA likely has exactly this role [41•]. In future, it will be exciting to examine the possible regulatory roles of the other Z-associated proteins.

Outlook

Over the last decade, single molecule and super-resolution microscopies have delivered exciting new insights into bacterial cytokinesis. Future cell division studies should benefit from recent developments in high resolution localization microscopy [42], new cell permeable dyes for live cell imaging [43,44], and new probes for super-resolution imaging of the bacterial cell wall [45,46].

Owing to their ability to interrogate the nanoscale motion and organization of division proteins directly in living cells, super-resolution microscopies should be crucial in the continuing quest to understand how bacteria cut themselves in two. They should be especially useful in testing physical models of cell division proposed here and elsewhere [10•,32•,47], in further resolving the organization, dynamics and function of FtsZ and the Z-associated proteins, and in revealing the mechanistic details of how FtsZ and its anchors guide and are coupled to the cell wall synthesis machinery. Investigation into the division mechanisms of non-canonical bacterial model organisms should be another exciting future application of super-resolution microscopy [48,49].

Conflict of interest

None declared.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Eswaramoorthy P, Erb ML, Gregory JA, Silverman J, Pogliano K, Pogliano J, Ramamurthi KS: **Cellular architecture mediates DivIVA ultrastructure and regulates min activity in *Bacillus subtilis***. *mBio* 2011, **2**.

2. Söderström B, Mirzadeh K, Toddo S, von Heijne G, Skoglund U, Daley DO: **Coordinated disassembly of the divisome complex in *Escherichia coli***. *Mol Microbiol* 2016, **101**:425-438.

3. Buss J, Coltharp C, Huang T, Pohlmeyer C, Wang S-C, Hatem C, Xiao J: **In vivo organization of the FtsZ-ring by ZapA and ZapB revealed by quantitative super-resolution microscopy**. *Mol Microbiol* 2013, **89**:1099-1120.

4. Buss J, Coltharp C, Shtengel G, Yang X, Hess H, Xiao J: **A multi-layered protein network stabilizes the *Escherichia coli* FtsZ-ring and Modulates constriction dynamics**. *PLoS Genet* 2015, **11**:e1005128.

5. Whatmore AM, Reed RH: **Determination of turgor pressure in *Bacillus subtilis*: a possible role for K+ in turgor regulation**. *J Gen Microbiol* 1990, **136**:2521-2526.

6. Gueiros-Filho F: **Cell division**. *Bacillus Cell Mol Biol*. Norfolk, UK: Caister Acad Press; 2007.

7. Labischinski H, Goodell EW, Goodell A, Hochberg ML: **Direct proof of a "more-than-single-layered" peptidoglycan architecture of Escherichia coli W7: a neutron small-angle scattering study**. *J Bacteriol* 1991, **173**:751-756.

8. Deng Y, Sun M, Shaevitz JW: **Direct measurement of cell wall stress stiffening and turgor pressure in live bacterial cells**. *Phys Rev Lett* 2011, **107**:158101.

9. Egan AJF, Vollmer W: **The physiology of bacterial cell division**. *Ann N Y Acad Sci* 2013, **1277**:8-28.

10. • Erickson HP: **How bacterial cell division might cheat turgor pressure — a unified mechanism of septal division in Gram-positive and Gram-negative bacteria**. *BioEssays* 2017, **39**.
Discusses several mechanistic models of bacterial cell division, and proposes that the Gram-positive and Gram-negative division machinery might not need to overcome turgor pressure to constrict the cell.

11. Harry E, Monahan L, Thompson L: **Bacterial cell division: the mechanism and its precision**. *Int Rev Cytol* 2006, **253**:27-94.

12. Bi E, Lutkenhaus J: **FtsZ ring structure associated with division in *Escherichia coli***. *Nature* 1991, **354**:161-164.

13. Erickson HP, Anderson DE, Osawa M: **FtsZ in bacterial cytokinesis: cytoskeleton and force generator all in one**. *Microbiol Mol Biol Rev* 2010, **74**:504-528.

14. Erickson HP: **Modeling the physics of FtsZ assembly and force generation**. *Proc Natl Acad Sci U S A* 2009, **106**:9238-9243.

15. Osawa M, Erickson HP: **Liposome division by a simple bacterial division machinery**. *Proc Natl Acad Sci U S A* 2013, **110**:11000-11004.

16. Osawa M, Anderson DE, Erickson HP: **Reconstitution of contractile FtsZ rings in liposomes**. *Science* 2008, **320**:792-794.

17. • Li Z, Trimble MJ, Brun YV, Jensen GJ: **The structure of FtsZ filaments in vivo suggests a force-generating role in cell division**. *EMBO J* 2007, **26**:4694-4708.
First in vivo cryo-electron tomography study of FtsZ reveals evidence for a patchy Z-ring in C. crescentus.

18. •• Fu G, Huang T, Buss J, Coltharp C, Hensel Z, Xiao J: **In vivo structure of the *E. coli* FtsZ-ring revealed by photoactivated localization microscopy (PALM)**. *PLoS ONE* 2010, **5**:e12680.
First super-resolution study of FtsZ ultrastructure revealed that Z-rings are axially thick.

19. •• Strauss MP, Liew ATF, Turnbull L, Whitchurch CB, Monahan LG, Harry EJ: **3D-SIM super resolution microscopy reveals a bead-like arrangement for FtsZ and the division machinery: implications for triggering cytokinesis**. *PLoS Biol* 2012, **10**: e1001389.
First 3D-SIM super-resolution study of FtsZ organization revealed that Z-rings are unexpectedly heterogeneous and dynamic in *B. subtilis* and *S. aureus*.

20. Rowlett VW, Margolin W: **3D-SIM super-resolution of FtsZ and its membrane tethers in *Escherichia coli* cells**. *Biophys J* 2014, **107**:L17-L20.

21. • Biteen JS, Goley ED, Shapiro L, Moerner WE: **Three-dimensional super-resolution imaging of the midplane protein FtsZ in live *Caulobacter crescentus* cells using astigmatism**. *ChemPhysChem* 2012, **13**:1007-1012.
First imaging of FtsZ by 3D PALM super-resolution microscopy.

22. •• Holden SJ, Pengo T, Meibom KL, Fernandez CF, Collier J, Manley S: **High throughput 3D super-resolution microscopy reveals *Caulobacter crescentus* in vivo Z-ring organization**. *Proc Natl Acad Sci U S A* 2014, **111**:4566-4571.
High-throughput 3D PALM microscopy of FtsZ organization throughout the C. crescentus cell cycle revealed that Z-rings consistently contain large gaps.

23. •• Coltharp C, Buss J, Plumer TM, Xiao J: **Defining the rate-limiting processes of bacterial cytokinesis**. *Proc Natl Acad Sci U S A* 2016, **113**:E1044-E1053.
Super-resolution microscopy combined with time-lapse imaging shows that FtsZ GTP hydrolysis is probably not rate limiting for *E. coli* constriction, but that cell wall synthesis is rate limiting.

24. Lyu Z, Coltharp C, Yang X, Xiao J: **Influence of FtsZ GTPase activity and concentration on nanoscale Z-ring structure in vivo revealed by three-dimensional superresolution imaging**. *Biopolymers* 2016, **105**:725-734.

25. • Szwedziak P, Wang Q, Bharat TAM, Tsim M, Löwe J: **Architecture of the ring formed by the tubulin homologue FtsZ in bacterial cell division**. *eLife* 2015, **3**:e04601.
Evidence via cryo-ET for thin, continuous Z-rings in C. crescentus and *E. coli*.

26. • Yao Q, Jewett AI, Chang Y-W, Oikonomou CM, Beeby M, Iancu CV, Briegel A, Ghosal D, Jensen GJ: **Short FtsZ filaments can drive asymmetric cell envelope constriction at the onset of bacterial cytokinesis**. *EMBO J* 2017 http://dx.doi.org/10.15252/embj.201696235.
Cryo-ET study showing that FtsZ forms mainly short filaments, rather than continuous rings, for majority of cell cycle in several Gram-negative organisms.

27. Stricker J, Maddox P, Salmon ED, Erickson HP: **Rapid assembly dynamics of the *Escherichia coli* FtsZ-ring demonstrated by fluorescence recovery after photobleaching**. *Proc Natl Acad Sci U S A* 2002, **99**:3171-3175.

28. •• Niu L, Yu J: **Investigating intracellular dynamics of FtsZ cytoskeleton with photoactivation single-molecule tracking**. *Biophys J* 2008, **95**:2009-2016.
First single molecule study of FtsZ presented evidence against sliding models of Z-ring constriction.

29. •• Loose M, Mitchison TJ: **The bacterial cell division proteins FtsA and FtsZ self-organize into dynamic cytoskeletal patterns**. *Nat Cell Biol* 2014, **16**:38-46.
Discovery that FtsZ can treadmill *in vitro*.

30. •• Bisson-Filho AW, Hsu Y-P, Squyres GR, Kuru E, Wu F, Jukes C, Sun Y, Dekker C, Holden S, VanNieuwenhze MS *et al.*: **Treadmilling by FtsZ filaments drives peptidoglycan synthesis and bacterial cell division**. *Science* 2017, **355**:739-743.
Together with [31••], shows that a motile cytoskeleton-synthase complex drives bacterial cell division.

31. •• Yang X, Lyu Z, Miguel A, McQuillen R, Huang KC, Xiao J: **GTPase activity-coupled treadmilling of the bacterial tubulin FtsZ organizes septal cell wall synthesis**. *Science* 2017, **355**:744-747.
See annotation to [30••].

32. • Xiao J, Goley ED: **Redefining the roles of the FtsZ-ring in bacterial cytokinesis**. *Curr Opin Microbiol* 2016, **34**:90-96.
Discusses physical constraints on bacterial cell division, and possible mechanisms for constrictive force generation.

33. Lan G, Wolgemuth CW, Sun SX: **Z-ring force and cell shape during division in rod-like bacteria**. *Proc Natl Acad Sci U S A* 2007, **104**:16110-16115.

34. Braun V: **Molecular organization of the rigid layer and the cell wall of *Escherichia coli***. *J Infect Dis* 1973, **128**:S9-S16.

35. Li Y, Hsin J, Zhao L, Cheng Y, Shang W, Huang KC, Wang H-W, Ye S: **FtsZ protofilaments use a hinge-opening mechanism for constrictive force generation**. *Science* 2013, **341**:392-395.

36. Hsin J, Gopinathan A, Huang KC: **Nucleotide-dependent conformations of FtsZ dimers and force generation observed through molecular dynamics simulations**. *Proc Natl Acad Sci U S A* 2012, **109**:9432-9437.

37. Matias VRF, Beveridge TJ: **Cryo-electron microscopy reveals native polymeric cell wall structure in *Bacillus subtilis* 168 and the existence of a periplasmic space**. *Mol Microbiol* 2005, **56**:240-251.

38. Matias VRF, Beveridge TJ: **Cryo-electron microscopy of cell division in *Staphylococcus aureus* reveals a mid-zone between nascent cross walls**. *Mol Microbiol* 2007, **64**:195-206.

39. Beeby M, Gumbart JC, Roux B, Jensen GJ: **Architecture and assembly of the Gram-positive cell wall**. *Mol Microbiol* 2013, **88**:664-672.

40. Tocheva EI, López-Garrido J, Hughes HV, Fredlund J, Kuru E, Vannieuwenhze MS, Brun YV, Pogliano K, Jensen GJ: **Peptidoglycan transformations during *Bacillus subtilis* sporulation**. *Mol Microbiol* 2013, **88**:673-686.

41. • Lariviere PJ, Szwedziak P, Mahone CR, Löwe J, Goley ED: **FzlA, an essential regulator of FtsZ filament curvature, controls constriction rate during Caulobacter division**. *Mol Microbiol* 2017 http://dx.doi.org/10.1111/mmi.13876.
Discovery that FtsZ-associated protein FzlA can regulate constriction rate by modulating FtsZ filament organization in *C. crescentus*.

42. Dai M, Jungmann R, Yin P: **Optical imaging of individual biomolecules in densely packed clusters**. *Nat Nanotechnol* 2016, **11**:798-807.

43. Grimm JB, English BP, Choi H, Muthusamy AK, Mehl BP, Dong P, Brown TA, Lippincott-Schwartz J, Liu Z, Lionnet T *et al.*: **Bright photoactivatable fluorophores for single-molecule imaging**. *Nat Methods* 2016, **13**:985-988.

44. Grimm JB, English BP, Chen J, Slaughter JP, Zhang Z, Revyakin A, Patel R, Macklin JJ, Normanno D, Singer RH *et al.*: **A general method to improve fluorophores for live-cell and single-molecule microscopy**. *Nat Methods* 2015, **12**:244-250.

45. Kuru E, Hughes HV, Brown PJ, Hall E, Tekkam S, Cava F, de Pedro MA, Brun YV, VanNieuwenhze MS: **In situ probing of newly synthesized peptidoglycan in live bacteria with fluorescent D-amino acids**. *Angew Chem Int Ed* 2012, **51**:12519-12523.

46. Liang H, DeMeester KE, Hou C-W, Parent MA, Caplan JL, Grimes CL: **Metabolic labelling of the carbohydrate core in bacterial peptidoglycan and its applications**. *Nat Commun* 2017, **8** ncomms15015.

47. den Blaauwen T, Hamoen LW, Levin PA: **The divisome at 25: the road ahead**. *Curr Opin Microbiol* 2017, **36**:85-94.

48. Jacq M, Adam V, Bourgeois D, Moriscot C, Guilmi A-MD, Vernet T, Morlot C: **Remodeling of the Z-ring nanostructure during the *Streptococcus pneumoniae* cell cycle revealed by photoactivated localization microscopy**. *mBio* 2015, **6**: e01108-e1115.

49. Eswara PJ, Ramamurthi KS: **Bacterial cell division: nonmodels poised to take the spotlight**. *Annu Rev Microbiol* 2017, **71** null.

50. Betzig E: **Imaging intracellular fluorescent proteins at nanometer resolution**. *Science* 2006, **313**:1642-1645.

51. Rust MJ, Bates M, Zhuang X: **Sub-diffraction-limit imaging by stochastic optical reconstruction microscopy (STORM)**. *Nat Methods* 2006, **3**:793-795.

52. Gustafsson MGL: **Surpassing the lateral resolution limit by a factor of two using structured illumination microscopy**. *J Microsc* 2000, **198**:82-87.

53. Sheppard CJR, Mehta SB, Heintzmann R: **Superresolution by image scanning microscopy using pixel reassignment**. *Opt Lett* 2013, **38**:2889-2892.

54. Hell SW, Wichmann J: **Breaking the diffraction resolution limit by stimulated emission: stimulated-emission-depletion fluorescence microscopy**. *Opt Lett* 1994, **19**:780-782.

55. Manley S, Gillette JM, Patterson GH, Shroff H, Hess HF, Betzig E, Lippincott-Schwartz J: **High-density mapping of single-molecule trajectories with photoactivated localization microscopy**. *Nat Methods* 2008, **5**:155-157.