Peer Review Information

Journal: Nature Microbiology
Manuscript Title: Single-molecule imaging reveals that Z ring condensation is essential for cell division in Bacillus subtilis
Corresponding author name(s): Ethan Garner

Reviewer Comments & Decisions:

Decision Letter, initial version:

Dear Ethan,

Thank you for your patience while your manuscript "Dynamics of bacterial cell division: Z ring condensation is essential for bacterial cell division" was under peer-review at Nature Microbiology. It has now been seen by 4 referees, whose expertise and comments you will find at the of this email. Although they find your work of some potential interest, they have raised a number of concerns that will need to be addressed before we can consider publication of the work in Nature Microbiology.

In particular, referee#3 feels that your findings are not convincing enough to prove the role of ZPB in Z-ring condensation and feels that additional data might reinforce your conclusions. Referees#1 and #2 noted the absence of FtsA in your study while referee#3 highlighted the missing Z-ring width in single ZPB mutant. Referee#2 raised some concerns about the conclusion drawn in the K86E mutant and suggests additional work to experimentally validate your model. Editorially, we feel that it will be important to address these concerns. Beyond those points, an in-depth description of ZPB in B. subtilis is missing in the introduction (referee#1), the Z-ring condensation part lacks clarity (referees#1 and #2) and some figures should be reworked (referee#4). Finally, all referees raised a number of issues and comments that we would ask you to address and clarify.

Should further experimental data allow you to address these criticisms, we would be happy to look at a revised manuscript.

We are committed to providing a fair and constructive peer-review process. Please do not hesitate to contact us if there are specific requests from the reviewers that you believe are technically impossible or unlikely to yield a meaningful outcome.

We strongly support public availability of data. Please place the data used in your paper into a public

data repository, if one exists, or alternatively, present the data as Source Data or Supplementary Information. If data can only be shared on request, please explain why in your Data Availability Statement, and also in the correspondence with your editor. For some data types, deposition in a public repository is mandatory - more information on our data deposition policies and available repositories can be found at https://www.nature.com/nature-research/editorial-policies/reporting-standards#availability-of-data.

Please include a data availability statement as a separate section after Methods but before references, under the heading "Data Availability". This section should inform readers about the availability of the data used to support the conclusions of your study. This information includes accession codes to public

repositories (data banks for protein, DNA or RNA sequences, microarray, proteomics data etc…), references to source data published alongside the paper, unique identifiers such as URLs to data repository entries, or data set DOIs, and any other statement about data availability. At a minimum, you should include the following statement: "The data that support the findings of this study are available from the corresponding author upon request", mentioning any restrictions on availability. If DOIs are provided, we also strongly encourage including these in the Reference list (authors, title, publisher (repository name), identifier, year). For more guidance on how to write this section please see: http://www.nature.com/authors/policies/data/data-availability-statements-data-citations.pdf

If revising your manuscript:

* Include a "Response to referees" document detailing, point-by-point, how you addressed each referee comment. If no action was taken to address a point, you must provide a compelling argument. This response will be sent back to the referees along with the revised manuscript.

* If you have not done so already we suggest that you begin to revise your manuscript so that it conforms to our Letter format instructions at http://www.nature.com/nmicrobiol/info/final-submission. Refer also to any guidelines provided in this letter.

* Include a revised version of any required reporting checklist. It will be available to referees (and, potentially, statisticians) to aid in their evaluation if the manuscript goes back for peer review. A revised checklist is essential for re-review of the paper.

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-- that control panels for gels and western blots are appropriately described as loading on sample processing controls
-- all images in the paper are checked for duplication of panels and for splicing of gel lanes.

Finally, please ensure that you retain unprocessed data and metadata files after publication, ideally archiving data in perpetuity, as these may be requested during the peer review and production process or after publication if any issues arise.

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direction, we are now requesting that all authors identified as 'corresponding author' on published papers create and link their Open Researcher and Contributor Identifier (ORCID) with their account on the Manuscript Tracking System (MTS), prior to acceptance. This applies to primary research papers only. ORCID helps the scientific community achieve unambiguous attribution of all scholarly contributions. You can create and link your ORCID from the home page of the MTS by clicking on 'Modify my Springer Nature account'. For more information please visit please visit www.springernature.com/orcid.

If you wish to submit a suitably revised manuscript we would hope to receive it within 6 months. If you cannot send it within this time, please let us know. We will be happy to consider your revision, even if a similar study has been accepted for publication at Nature Microbiology or published elsewhere (up to a maximum of 6 months).

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Reviewer Comments:

Reviewer #1 (Remarks to the Author):

In this well written and interesting work Squyres and coworkers study in-depth the dynamics of the protein complex that is responsible for cell division (divisome) in the model bacterium Bacillus subtilis. The core of this complex is formed by FtsZ polymers that are dynamic, FtsZ is a tubulin homologue, and treadmill around the cell in a ring-like fashion. Previous work from the same group, and others, has shown that treadmilling affects the constriction of the division septum. FtsZ does not work alone but recruits several other conserved cell division proteins, including FtsA, SepF, ZapA and EzrA.

In the current study the effects of these additional cell division proteins on the dynamics of FtsZ treadmilling and septum synthesis have been investigated using advanced single molecule fluorescence microscopy. Interestingly, the FtsZ dynamics were, in essence, unaffected in the absence of SepF, ZapA and EzrA. However, the Z-rings formed in mutants that lacked these proteins was disturbed and did not condense into a single ring. Importantly, this lack of condensation affected the recruitment of the late cell division proteins (monitored by Pbp2B recruitment), which strongly reduced the synthesis of septal peptidoglycan syntheses. This shows that a key function of these early cell division proteins is to help recruit the late cell division proteins, by condensing FtsZ filaments.

The paper is clearly written, although I missed a detailed description of the functions of FtsA, SepF, ZapA and EzrA in the introduction. I think that a more in-depth description of the early and late cell division proteins is important to better understand the paper when it comes to non-experts. Especially since e.g. EzrA is a transmembrane domain whereas ZapA, FtsA and SepF are not, although the latter two associate with the inner leaflet of the membrane. Also, ZapA is a known crosslinker of FtsZ filaments.

These and other differences are important to know to better understand their dynamic behaviour. On several occasions throughout the manuscript such detailed information would have been useful to know, e.g. lines 127 and 157.

There are a few questions and other remarks that I have:

Are the halo-tag fusions biologically active? This is important to be sure that the lack of dynamic localization is not due to an inactive protein.

I miss a study of an ftsA knockout. Such mutant is viable in B. subtilis (Beall & Lutkenhaus J bac 1992)

The description of the condensation of the Z-ring is a bit unclear. The impression might arise that an uncondensed Z-ring is a slightly diffuse Z-ring, but that is not the case. In line 136 it is mentioned that these un-condensed structures resemble the transient FtsZ structures that occur prior to Z ring condensation. However, these transient structures are made of discrete FtsZ filaments that form multiple rings and spirals. The width of the fluorescent intensity of these discrete filaments seem comparable to a mature Z-ring. Thus the condensation can simply mean a higher concentration of FtsZ filaments in one ring (condensation of an FtsZ spiral). The reason that this issue is important, at least to me, is that in theory an FtsZ spiral should also be able to recruit Pbp2B, since it is composed of dynamic FtsZ filaments. Maybe this can be discussed (why this is not the case)?

Related to this, in the control of Figure 4E, why do you see vague FDAA bands when there is no FtsZ ring? And in the mutant, why are there no FDAA bands when you see Z spirals/uncondensed filaments? Might this have to do with age of rings?

Minor remarks:

line 123 is unclear

Figure 3F is not explained in the text.

Reviewer #2 (Remarks to the Author):

Report for Garner et al 2020

While many components of the division machinery have been identified, some many moons ago, the mechanisms by which they work together to divide the cell are poorly understood. This is highly significant work addressing functional aspects of bacterial cell division that have proved challenging. Advanced imaging methods and the combined capability of the investigative team have allowed important insights here. How DO these proteins work together is what these authors address in this manuscript.

The data supports the proposition that there are 2 types of dynamic subcomplexes in B. subtilis – a directional complex moving with cell wall synthesis and stationary ZBPs interacting with treadmilling FtsZ. The authors show that ZBPs are required for Z ring condensation at the division site, to allow septation. However, they are not required for FtsZ treadmilling or localization.

In the early part of the manuscript, at the top of p. 2, I suggest the authors mention the species (B. subtilis) as there are different members and names of division proteins in different bacterial species. For example, E. coli does not have all the same division proteins tested here.
This raises the question of whether the title should have this species name in it rather than indicating that their findings are the same (or similar) for several bacteria. This change would not take away the significance of the study in my opinion.

Some protein fusions are functional but not all – is it just the SepF fusion that is not functional? I realize that this is a challenging issue for any study with fusions, the authors need to start somewhere. How do the authors think that a second copy would affect the data?

Video 5 is impressive. Well done.

line 135 – what do the authors think 'condensing' of the Z ring means?

Some extended data Figs. don't have specific labels to all the different images eg. in Fig. 6.

The suppressor screen is excellent – ie to isolate mutations that promote lateral bundling of FtsZ filaments in cells lacking ZBPs.

Line 150 – what about the same residue (#86) in B. subtilis FtsZ? Is it likely (or shown) to be in the same position and likely to affect lateral bundling of FtsZ?

Line 164 – "Pbp2B recruitment to the Z ring decreased by 50% in ΔZBPs relative to control cells (Fig. 4E) but PBP2B still moving and active" (as judged by incorporation of substrate into CW) – the conclusion is that condensation of the Z ring is important for PBP2B recruitment.
Does PBP2B recruitment to the Z ring increase in the K86E mutant? This would provide further evidence that it is the condensation of the Z ring (due to bundling of Z filaments) and not something else (like another function of the ZBPs, that is the reason why there is decreased PBP2B localization to the Z ring.

Line 161 "we removed ZBPs" – it would be helpful to the reader to say which ones and in what way – it is in the figures but hard to follow the text without this detail.

It would be helpful and informative to know when cells were filamentous or not – did this change any data? And how long were the filaments on average? This could well prove important as more data is available on this topic.

Line 183 – there is a reference to Fig. 4F which does not appear in Fig. 4 in my copy.

There is a 50% reduction in PBP2B recruitment only – it is interesting that it is still active. What is being synthesized without Z ring constriction?

Might condensation be required to control septal synthesis rate?

Line 202- 203: Fig. 1 early-arriving proteins in red and late-arriving
proteins in blue - should be the other way around.

Lines 411-412: "cells were imaged at the point when they had filamented but were still alive." How is this being determined? I am glad that the authors ensured that they were working with live, exponentially growing cells. I assume that cells in all experiments were of similar viability?
Then again in line 444 – 'masks were refined manually to omit dead cells' – how were these identified?

The methods were very thoroughly described. I appreciate this.

Line 512: "Because ZBPs cells do not divide, Z rings will not disassemble once they are formed?" Is this really true? Are Z rings stable for this time?

What about FtsA? How does it figure in this model of two complexes in terms of its effect on FtsZ treadmilling, and condensation?

Reviewer #3 (Remarks to the Author):

Comments on Nature Microbiol 13934

This is a very interesting and thorough paper on the treadmilling of FtsZ filaments and life time of molecules in the Z-ring and whether Z-ring binding proteins (ZBP) are also treadmilling and affect the velocity of the treadmilling. The conclusion is that these ZBPs are stationary and do not affect the velocity of the Z-ring but affect the width of the ring. The peptidoglycan synthesizing proteins are not stationary, but move at the same speed as the FtsZ treadmilling velocity. In the absence of ring condensation, the number of PG synthesizing protein PBP2B is 40% reduced as well as FAAD incorporation, indicating that condensation stimulates localization of PBP2B.

Major comments

The major problem of this paper is that most of the data are in the supplementary information. Personally, I would have preferred to have the data tables in the main manuscript.

In supplementary Table 5, the width of the Z-ring of the single ZBPs is missing. Based on your supplementary figure 2, this is predominantly caused by absence of EzrA. Please add quantification of the singles to table 5. Then if EzrA is causing already lack of condensation, why do you argue that all three proteins together are needed for the condensation? The double deletion of SepFZapA does provide normal looking Z-rings (Why are they not in supplementary Table 5?). I think that you need quantified evidence for this. Deletion of EzrA increases the lifetime of FtsZ molecules in the ring (supplementary Table 4). This could mean an increase in filament length of 40%. This would be in agreement with the general idea that EzrA binds FtsZ monomers on the surface of the membrane thereby avoiding the production of extra Z-ring at the poles. Would it be more difficult for SepF and ZapA to bundle longer filaments? Assuming that FtsZ(T11A) also has longer filaments due to its longer lifetime, how do these filaments look like, nicely bundled (can you provide an image and wuantification?)? In the absence of EzrA additional polar rings are made, which reduces the available pool of FtsZ molecules to make a stable ring. Could this also affect the possibility for SepF and ZapA to condense the ring properly, i.e. not enough ring available? In deltaminC in E. coli it takes more time for a Z-ring to assemble (Coltharp, C., Buss, J., Plumer, T. M., & Xiao, J. (2016). Defining the rate-limiting processes of bacterial cytokinesis. PNAS USA, 113(8), E1044–53. http://doi.org/10.1073/pnas.1514296113).
Overproduction of EzrA also causes widening of the rings. This suggest that reduction of the available

FtsZ causes unstable Z rings that cannot be condensed by SepF and ZapA (or by EzrA), Deletion of EzrA cause polar rings, likely also reducing the amount of available FtsZ for the division ring. In the shown images the polar rings seems to be condensed whereas the central ring is wide. Is this correct or just an impression?

I agree with you that a non-condensed ring cannot function properly, but I am not convinced based on your data that the ZBP are providing this condensation. I guess that you have all data available to support your conclusion and it would be nice to have them in the manuscript or in the supplementary.

Minor comments:
"Assuming that treadmilling filaments elongate with a diffusion-limited on-rate of 5 uM-1, the concentration of free monomers in cells can be estimated to 1.3 uM." Since only 30% of the FtsZ molecules is in the ring and 5 uM FtsZ is present in the cell, the 1.3 uM of monomers does not exclude dimers and short filaments in the cytoplasm, I assume. Maybe this should be made clear in the text?

Page 6 line 7 from the bottom: on the FtsZ superstructure?

Reviewer #4 (Remarks to the Author):

I thoroughly enjoyed reading this manuscript on the dynamics of Z-ring condensation. The tidy division between periplasmic-facing, mobile components and cytoplasmic-facing, stationary components is gratifying. The remainder of the paper, focusing on quantifying the kinetics and molecule lifetimes in filaments is particularly elegant. Molecular mechanism for condensation is demonstrated via knockdown and functional complementation. The quantifications included are overall solid and rigorously support the conclusions drawn.

I have only a few comments for the authors:
Figure 2A. The way that velocity is represented is counterintuitive, since it is dx/dt and not dt/dx. Thus, at first glance it appears to decrease in the middle cartoon, just because of the way it is displayed.

It is not clear to me if the bleaching time of the dye has been corrected for when estimating binding lifetime. Reading the methods, it doesn't appear so. This should be done.

Fig. 3G. I am not entirely convinced about the widening of the ring, and I think there is information hidden by averaging the ring images. Picking just a few rings from the images in panel E and taking line profiles, I did not see such a difference in the ring widths. For example, there could be a bimodal distribution of ring widths for Delta(ZPBs) that would account for the difference in the average images. Or a wide-ring tail that dominates the average. If the intensities are normalized before averaging, dimmer and wider rings might dominate. I also think that the alignment and normalization of the noisier rings in the Delta(ZBP) case may introduce errors which could impact the averaged image. An alternative, which would reveal more such information, is to measure the FWHM for individual rings, and compare the distributions. I suggest the authors try this approach. I think it is more consistent with the Nature journals policy of showing distributions rather than just summary data. I don't think this affects the main conclusions, since there are clearly multiple bands and those are evidence for lack of bundling. These same comments apply for Fig. 4C, Extended Data Fig. 6.

For Fig. 4B, it would be interesting to also show the intensity of FtsZ as the ring condenses.

SVideo 5. This is clearly not the same imaging as displayed in SV1. Is it SIM (I think I see honeycomb)? Ah, now I see that it is mentioned in the main text. This should also be stated in the legend.

Author Rebuttal to Initial comments

Reviewer #1 (Remarks to the Author):

In this well written and interesting work Squyres and coworkers study in-depth the dynamics of the protein complex that is responsible for cell division (divisome) in the model bacterium Bacillus subtilis. The core of this complex is formed by FtsZ polymers that are dynamic, FtsZ is a tubulin homologue, and treadmill around the cell in a ring-like fashion. Previous work from the same group, and others, has shown that treadmilling affects the constriction of the division septum. FtsZ does not work alone but recruits several other conserved cell division proteins, including FtsA, SepF, ZapA and EzrA.

In the current study the effects of these additional cell division proteins on the dynamics of FtsZ treadmilling and septum synthesis have been investigated using advanced single molecule fluorescence microscopy. Interestingly, the FtsZ dynamics were, in essence, unaffected in the absence of SepF, ZapA and EzrA. However, the Z-rings formed in mutants that lacked these proteins was disturbed and did not condense into a single ring. Importantly, this lack of condensation affected the recruitment of the late cell division proteins (monitored by Pbp2B recruitment), which strongly reduced the synthesis of septal peptidoglycan syntheses. This shows that a key function of these early cell division proteins is to help recruit the late cell division proteins, by condensing FtsZ filaments.

The paper is clearly written, although I missed a detailed description of the functions of FtsA, SepF, ZapA and EzrA in the introduction. I think that a more in-depth description of the early and late cell division proteins is important to better understand the paper when it comes to non- experts. Especially since e.g. EzrA is a transmembrane domain whereas ZapA, FtsA and SepF are not, although the latter two associate with the inner leaflet of the membrane. Also, ZapA is a known crosslinker of FtsZ filaments. These and other differences are important to know to better understand their dynamic behaviour. On several occasions throughout the manuscript such detailed information would have been useful to know, e.g. lines 127 and 157.

We agree that more context about these proteins will be useful to the reader, and so we have significantly expanded the description of the ZBPs in the main text. When the proteins are first mentioned, we now clarify whether or not they are membrane associated: *"…several other FtsZ binding proteins (ZBPs): the cytoplasmic protein ZapA, the integral membrane protein EzrA, and the peripheral membrane protein SepF."*

We have also added a paragraph later on with a more thorough summary of the literature about these proteins: *"Both ZapA and SepF have been shown in vitro to promote FtsZ filament formation, stability and bundling, and to decrease FtsZ's GTPase activity. In vivo, ZapA has been shown to promote the formation of a coherent Z ring, while SepF is involved in both tethering FtsZ to the membrane and*

modulating septum morphology. EzrA, meanwhile both increases FtsZ's critical concentration and decreases filament bundling in vitro and inhibits Z ring formation and modulates the rate of Z ring recovery after photobleaching in vivo.
Thus, ZapA and SepF have both been described broadly as FtsZ stabilizing proteins, and EzrA as a FtsZ destabilizer."

There are a few questions and other remarks that I have:

Are the halo-tag fusions biologically active? This is important to be sure that the lack of dynamic localization is not due to an inactive protein.

We appreciate the opportunity to demonstrate the functionality of our fusions, and have done so in Extended Data Fig. 1. Our fusions to FtsZ, FtsA, and SepF are expressed as merodiploids; all of our other fusions are functional as sole copies.

For those proteins whose knockouts cause a critical defect in cell division, we verified that these fusions did not cause division defects by measuring cell length. For the ZBPs, whose knockouts have less dramatic phenotypes in division, we created backgrounds in which a synthetic lethal pair of the labelled protein is knocked out (Extended Data Fig. 1c). EzrA- HaloTag is able to support division in a Δ*zapA* background and ZapA-HaloTag is able to support division in a Δ*ezrA* background even though Δ*ezrA* Δ*zapA* is lethal. Additionally, although our SepF-HaloTag fusion is expressed as a second copy, this expression does not prevent the native unlabeled copy from supporting division in a Δ*ezrA* background even though Δ*ezrA* Δ*sepF* is lethal.

In conducting these experiments, we noticed a potential duplication at the ZapA locus in our ZapA-HaloTag strain. Such duplication is not present in our other HaloTag strains. We were able to produce the correct strain and have replaced all relevant data (ZapA in Fig. 1b, Fig. 2f, and Supplementary Video 2). These data are not discernable from—and support the same conclusions as—the previous data.

I miss a study of an ftsA knockout. Such mutant is viable in B. subtilis (Beall & Lutkenhaus J bac 1992)

We have added experiments with a FtsA knockout- the data is in Extended Data Fig. 11 and Supplementary Video 7. As we suspected from the previous literature, FtsA has a clear effect on FtsZ filaments and their dynamics, and a Δ*ftsA* strain shows a reduction in the proportion of filaments that are treadmilling directionally. We see defects in Z ring architecture in this strain as well, but they look quite different from the other ZBP mutants and may have more to do with the underlying defects in FtsZ filaments.

The description of the condensation of the Z-ring is a bit unclear. The impression might arise that an uncondensed Z-ring is a slightly diffuse Z-ring, but that is not the case. In line 136 it is mentioned that these un-condensed structures resemble the transient FtsZ structures that occur prior to Z ring condensation. However, these transient structures are made of discrete FtsZ filaments that form multiple rings and spirals.

The transient FtsZ structures we see early in the cell cycle do not resemble multiple Z rings or spirals, as far as we can observe. These structures, represented in Fig. 4a, more closely resemble discontinuous filaments that are only loosely organized. Similar diffuse structures during the early cell cycle in *B. subtilis* were recently observed in another study[1]. We have clarified our description of these structures and of the condensation process in the text. While previous literature has characterized the immature Z ring as an extended spiral[2], that localization pattern unfortunately was likely an artifact due to tagging with YFP, which oligomerizes. Similar spiral artifacts have been characterized for other bacterial filaments when tagged in this way[3]. Here we use instead the monomeric mNeonGreen fluorophore to tag FtsZ.

The width of the fluorescent intensity of these discrete filaments seem comparable to a mature Z- ring. Thus the condensation can simply mean a higher concentration of FtsZ filaments in one ring (condensation of an FtsZ spiral).

Due to the diffraction limit and the small size of FtsZ filaments, it is difficult to determine from signal width whether a structure is composed of one or multiple FtsZ filaments. Instead, we can use the intensity to determine whether additional FtsZ filaments are being recruited into the structure over time. Using this approach, we do find that the intensity of these loose FtsZ filaments is lower than the intensity of a mature Z ring. We have added this data to Fig. 4b
(right) showing that the maximum fluorescence intensity increases as the Z ring condenses.

The reason that this issue is important, at least to me, is that in theory an FtsZ spiral should also be able to recruit Pbp2B, since it is composed of dynamic FtsZ filaments. Maybe this can be discussed (why this is not the case)?

This is indeed a savvy question. Reviewer 2 suggested that we check whether Pbp2B recruitment was rescued when we introduce the FtsZ(K86E) mutant, which partially restores Z ring condensation. We indeed found that this mutant does not rescue Pbp2B recruitment to midcell. This suggests that the defect in Pbp2B recruitment that we see in this strain is not because FtsZ filaments are decondensed. Instead, this suggests that the ZBPs are involved in recruiting Pbp2B via another mechanism, perhaps independent of their function in Z ring condensation. In other words, the defect in Pbp2B recruitment is not just due to the lack of Z ring condensation in these strains.

Related to this, in the control of Figure 4E, why do you see vague FDAA bands when there is no FtsZ ring? And in the mutant, why are there no FDAA bands when you see Z spirals/uncondensed filaments? Might this have to do with age of rings?

This is a technical issue. There is a necessary delay between when we add FDAA label to cells and when we take the images, due to the time required to mount the sample and transfer it to the microscope. We now explain this in the methods section, as follows: *"There is in total a ~4 minute delay between FDAA labelling and imaging, and in some cases the positions of Z rings may have changed during this time. For instance, if a Z ring constricted and disassembled during this time, we would observe FDAA labelling without a Z ring, and vice versa for a newly assembled Z ring. However, we expect these events to be relatively rare because the cell cycle duration is roughly 30 minutes under these conditions."*

Minor remarks:

line 123 is unclear

We have rephrased this sentence. It now reads: *"This suggests that EzrA's roles in bundling and in filament length modulation are separate from one another".*

Figure 3F is not explained in the text.

We've added more to explain this figure (now 3g) in the main text. The sentence now reads: *"These FtsZ bands were still regularly spaced apart from one another, indicating that FtsZ was still able to localize to the division site under these conditions"*

Reviewer #2 (Remarks to the Author): Report for Garner et al 2020

While many components of the division machinery have been identified, some many moons ago, the mechanisms by which they work together to divide the cell are poorly understood.

This is highly significant work addressing functional aspects of bacterial cell division that have proved challenging. Advanced imaging methods and the combined capability of the investigative team have allowed important insights here. How DO these proteins work together is what these authors address in this manuscript.

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In the early part of the manuscript, at the top of p. 2, I suggest the authors mention the species (B. subtilis) as there are different members and names of division proteins in different bacterial species. For example, E. coli does not have all the same division proteins tested here.

We now mention *B. subtilis* much earlier in this paragraph, before the other division proteins are mentioned.

This raises the question of whether the title should have this species name in it rather than indicating that their findings are the same (or similar) for several bacteria. This change would not take away the significance of the study in my opinion.

We have added "in *B. subtilis*" to the title. Additionally, we have highlighted in the discussion that, because putative FtsZ bundlers have been identified in many diverse bacteria (and even in archaea), we think that our results here may be broadly applicable. The sentence reads: *"FtsZ bundling proteins have been identified across the bacterial tree and even in archaea, suggesting that Z ring condensation may be an important process across diverse organisms."*

Some protein fusions are functional but not all – is it just the SepF fusion that is not functional? I realize that this is a challenging issue for any study with fusions, the authors need to start somewhere. How do the authors think that a second copy would affect the data?

Indeed, the FtsZ and SepF fusions used in the paper are not fully functional, and we are uncertain about the functionality of the FtsA fusion. Regarding FtsZ, creation of a fully functional FtsZ fusion has remained elusive in *B. subtilis*; we tested the recently reported functional fusions in *E. coli* [4] are unfortunately they are temperature sensitive in *B. subtilis*. Regarding FtsA, a msfGFP fusion in the same position when expressed from pHyperSpank allows Z ring formation under depletion of the native operon (erratum to [5]), but it is not clear how this relates to our HaloTag fusion. We have thus far been unable to create sole copy fusions in SepF. However, as far as we are able to test, we do not expect that the fluorescent fusions we used will significantly affect our data. We know that each fusion does not affect cell division: we have now tested the effect of expression of each fusion in Extended Data Fig. 1, and the same tests were performed independently for the FtsA and FtsZ fusions in the erratum to [5]. Additionally, we know that overexpression at high levels of untagged FtsAZ and of SepF does not affect FtsZ dynamics (Extended Data Figs. 2e, 4), and so the more moderate expression used for the labeled constructs should similarly have no effect.

The remaining fusions, to EzrA, ZapA, DivIB, DivIC, FtsL, FtsW, and Pbp2B, are functional. We demonstrate this by measuring cell length in each of these strains in Extended Data Fig. 1, and discuss these results further in our response to reviewer 1:

"For those proteins whose knockouts cause a critical defect in cell division, we verified that these fusions did not cause division defects by measuring cell length. For the ZBPs, whose knockouts have less dramatic phenotypes in division, we created backgrounds in which a synthetic lethal pair of the labelled

protein is knocked out (Extended Data Fig. 1c). EzrA- HaloTag is able to support division in a ΔzapA background and ZapA-HaloTag is able to support division in a ΔezrA background even though ΔezrA ΔzapA is lethal.
Additionally, although our SepF-HaloTag fusion is expressed as a second copy, this expression does not prevent the native unlabeled copy from supporting division in a ΔezrA background even though ΔezrA ΔsepF is lethal."

Video 5 is impressive. Well done.

Thank you! We performed that imaging at the Advanced Imaging Center at Janelia, which has beautiful microscopes.

line 135 – what do the authors think 'condensing' of the Z ring means?

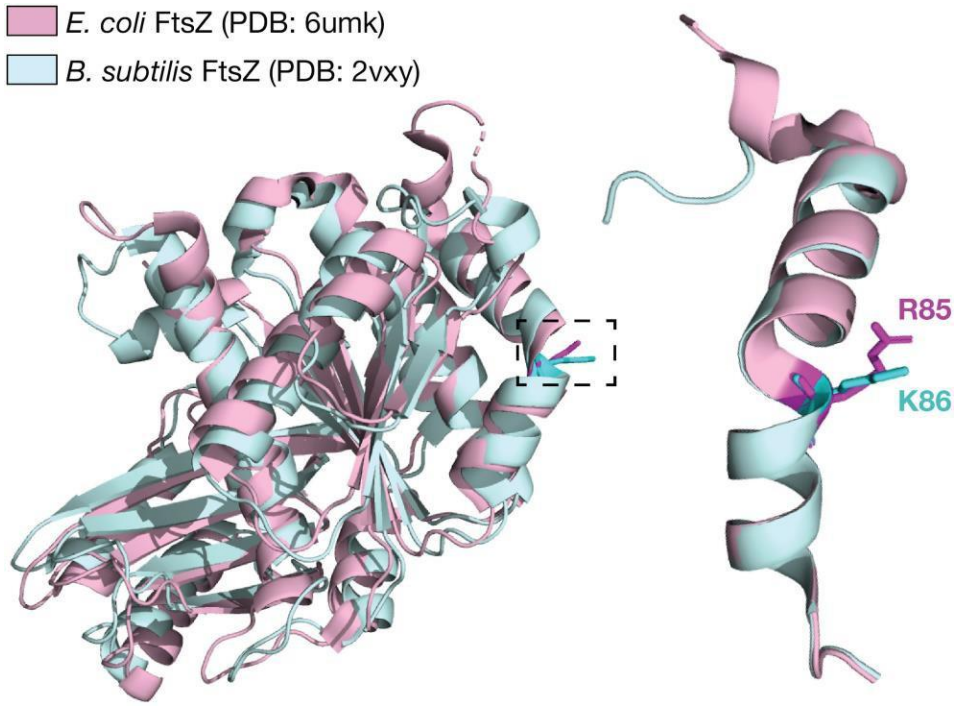
Phenomenologically, we observe that condensation of the Z ring happens as part of normal cell cycle progression, occurring predominantly in the first half of the cell cycle (Fig. 4ab).
Mechanistically, this condensation is due to lateral bunding of filaments by ZBPs, as evidenced both by *in vitro* observations of SepF and ZapA as FtsZ bundlers and by our suppressor screen, which identified a FtsZ bundling mutant. We have restored Fig. 4f to the main text, which contains a diagram of condensation that we hope communicates this more clearly. We have also defined condensation more extensively throughout the text.

Some extended data Figs. don't have specific labels to all the different images eg. in Fig. 6.
Thank you! We have corrected this.

The suppressor screen is excellent – ie to isolate mutations that promote lateral bundling of FtsZ filaments in cells lacking ZBPs.
We were very excited to encounter such a well-characterized mutant! Thank you.

Line 150 – what about the same residue (#86) in B. subtilis FtsZ? Is it likely (or shown) to be in the same position and likely to affect lateral bundling of FtsZ?

The globular domain of FtsZ is highly conserved, and point mutants that are identified in one bacterial species tend to function similarly in others; for example, we commonly use the FtsZ(D213A) GTPase mutant in our work in *B. subtilis*, which was originally identified in *E. coli*. In addition to the *E. coli* literature we mention, this residue was also identified at a putative bundling interface in *Mycobacterium tuberculosis*[6], consistent with broad conservation of this interface. Below, we show a structural alignment of *B. subtilis* and *E. coli* FtsZ. The whole globular domain is shown on the left, with the relevant residues boxed and shown as sticks, and the H3 helix specifically is aligned on the right.



Line 164 – "Pbp2B recruitment to the Z ring decreased by 50% in ΔZBPs relative to control cells (Fig. 4E) but PBP2B still moving and active" (as judged by incorporation of substrate into CW) – the conclusion is that condensation of the Z ring is important for PBP2B recruitment.

Does PBP2B recruitment to the Z ring increase in the K86E mutant? This would provide further evidence that it is the condensation of the Z ring (due to bundling of Z filaments) and not something else (like another function of the ZBPs, that is the reason why there is decreased PBP2B localization to the Z ring.

This was a great suggestion, thank you! We found that it does not! Exactly as you say, this suggests that the ZBPs have an independent function in Pbp2B recruitment. The data is included in Extended Data Fig. 9c and described in the text as follows: *"Next, to understand whether the decrease in Pbp2B recruitment was due to FtsZ's inability to condense in*
ΔZBPs cells, we asked whether the FtsZ(K86E) suppressor mutant restored Pbp2B localization to midcell. Although this mutant allowed ΔezrA ΔzapA cells to divide and partially rescued Z ring condensation, it did not rescue Pbp2B recruitment (Extended Data Fig. 9).
This indicates that the failure of ΔZBPs cells to divide is not due to defects in Pbp2B recruitment. This also suggests that the ZBPs may play a role in recruiting the late proteins to the division site that is independent of their effects on FtsZ."

Performing this experiment also involved repeating our measurements of FtsZ intensity at midcell in each strain. Although the replicates we performed for our initial submission were highly consistent, once we resumed these experiments after lockdown we saw high

variability between experiments, even for replicates of the same strain. Unlike Pbp2B intensity, which is measured using a native fluorescent fusion, FtsZ intensity measurements involve inducing expression of FtsZ-mNeonGreen and labeling with an exogenous dye. Thus, we expect that one of these steps is contributing to the variability we now observe. Nonetheless, because we are no longer able to consistently perform this measurement, we have removed FtsZ intensity from the graph in Fig. 4e.

Line 161 "we removed ZBPs" – it would be helpful to the reader to say which ones and in what way – it is in the figures but hard to follow the text without this detail.

We rephrased this to *"In ΔZBPs cells",* to clarify that we are using the same genetic background as in the previous experiments.

It would be helpful and informative to know when cells were filamentous or not – did this change any data? And how long were the filaments on average? This could well prove important as more data is available on this topic.

We have added cell length measurements for many of our strains to Extended Data Fig. 1. However, we could not readily measure cell length in some of the more severe mutants. Because *B. subtilis* grows in chains, it is necessary to use a membrane stain to measure cell length, since separate cells can chain together and appear as a single cell. These stains are typically somewhat toxic, and so staining cells with severe division defects, which are already quite sick, with a membrane stain poses too great a technical challenge. Furthermore, in each of these cases, the cell length depends on some aspect of our experimental setup, and so may or may not be informative. Specifically:

- Cells missing synthetically lethal combinations of ZBPs. In this case, cell length depends on the duration of ZBP depletion prior toimaging
- Cells overexpressing EzrA. In this case, cell length depends on the duration and amount of EzrA overexpression prior toimaging
- Δ*ftsA* cells. In this case, cell length depends on how much FtsZ we express from an ectopic locus (although they seem quite filamented even in our healthiest condition)

Line 183 – there is a reference to Fig. 4F which does not appear in Fig. 4 in my copy.
We moved this panel to the extended data initially, but we've moved it back to Fig. 4f.

There is a 50% reduction in PBP2B recruitment only – it is interesting that it is still active. What is being synthesized without Z ring constriction?

We think that there is still some diffuse cell wall synthesis, but it's too spread out to productively make a septum. You can see this in Extended Data Fig. 10b- where the decondensed Z rings are, there is some enrichment of FDAA labeling but it is quite diffuse. This is why we speculate in the discussion that FtsZ bundling might serve to localize cell wall synthesis at the septum. Interestingly enough, recent research has suggested that SepF in particular might play a role in modulating the thickness of the septal cell wall[7], although they don't investigate FtsZ bundling as a possible mechanism in that study.

Might condensation be required to control septal synthesis rate?
It's possible! If the septal synthesis enzymes are always depositing new cell wall at the same rate, FtsZ might be able to control the rate at which the septum closes by directing this cell wall synthesis into a narrower area, leading to faster invagination.

Line 202- 203: Fig. 1 early-arriving proteins in red and late-arriving proteins in blue - should be the other way around.

We've corrected this, thank you!

Lines 411-412: "cells were imaged at the point when they had filamented but were still alive." How is this being determined? I am glad that the authors ensured that they were working with live, exponentially growing cells. I assume that cells in all experiments were of similar viability? Then again in line 444 – 'masks were refined manually to omit dead cells' – how were these identified?

We added clarification about this to the methods section: *"We judged whether cells were alive based on their appearance by phase contrast microscopy and whether or not they contained fluorescent signal."* In most cases it was also possible to observe these cells continuing to grow during microscopy, but some cells were so long that their edges went out of the field of view and so we could not obtain a growth rate. For this reason, we decided to use the simpler criterion of whether or not they retained the fluorescent signal and looked un- lysed by phase contrast.

Cells with division defects have reduced viability due to an increased rate of cell lysis, so the ΔZBPs strains, the Δ*ftsA* strain, and the EzrA overexpression strain are certainly less viable (and indeed, we can observe instances of lysed cells in our imaging of these strains). We clarified that some cells have lysed at this stage by rephrasing the sentence above to *"cells were imaged at the point when they had filamented but were largely still alive".*

The methods were very thoroughly described. I appreciate this.

Thank you!

Line 512: "Because ZBPs cells do not divide, Z rings will not disassemble once they are formed?" Is this really true? Are Z rings stable for this time?

We phrased this poorly. Without longer-term imaging, we don't know whether these structures ever disappear and reappear or not (and for a structure as dynamic as this, it's hard to even define what would constitute disassembly). Rather, what we meant to say is that, because the cells never divide, the division sites remain available. In WT cells, after cytokinesis the Z rings are excluded from the former division site (new poles). In these cells, this does not happen, and so each new division site remains available for FtsZ indefinitely. We have rephrased this to "because ΔZBPs cells do not divide, these division sites remain indefinitely available for division protein localization."

What about FtsA? How does it figure in this model of two complexes in terms of its effect on FtsZ treadmilling, and condensation?

We have added a discussion of FtsA to the manuscript and included experiments in a Δ*ftsA* strain in Extended Data Fig. 11 at Reviewer 1's suggestion. In short, FtsA seems to act differently than the ZBPs, modulating FtsZ filament treadmilling directly. The text reads: *"Finally, we investigated the effects of FtsA on FtsZ filaments. FtsA is an actin homolog that serves as FtsZ's primary membrane tether, and B. subtilis ΔftsA cells are less viable and have a strong division defect and altered Z ring morphology. FtsA has been shown in vitro and in vivo to modulate FtsZ treadmilling. Indeed, ΔftsA cells showed a decrease in the fraction of directionally-treadmilling FtsZ filaments (Extended Data Fig. 11, Supplementary Video 7). Thus, unlike the ZBPs, FtsA modulates FtsZ filament treadmilling. It has also been suggested that FtsA might regulate FtsZ bundling. We observe Z ring morphology defects in the ΔftsA strain, but these defects are distinct from the condensation defect observed in the*

ΔZBPs strain (Extended Data Fig. 11). Because ΔftsA cells have severely perturbed FtsZ filaments, it will be difficult to decouple these effects from any possible higher-order effects on their bundling state."

Reviewer #3 (Remarks to the Author): Comments on Nature Microbiol 13934

This is a very interesting and thorough paper on the treadmilling of FtsZ filaments and life time of molecules in the Z-ring and whether Z-ring binding proteins (ZBP) are also treadmilling and affect the velocity of the treadmilling. The conclusion is that these ZBPs are stationary and do not affect the velocity of the Z-ring but affect the width of the ring. The peptidoglycan synthesizing proteins are not stationary, but move at the same speed as the FtsZ treadmilling velocity. In the absence of ring condensation, the number of PG synthesizing protein PBP2B is 40% reduced as well as FAAD incorporation, indicating that condensation stimulates localization of PBP2B.

Major comments

The major problem of this paper is that most of the data are in the supplementary information. Personally, I would have preferred to have the data tables in the main manuscript.

We have asked the editor for guidance on whether any of these tables can be moved into the main text.

In supplementary Table 5, the width of the Z-ring of the single ZBPs is missing. Based on your supplementary figure 2, this is predominantly caused by absence of EzrA. Please add quantification of the singles to table 5. Then if EzrA is causing already lack of condensation, why do you argue that all three proteins together are needed for the condensation?

The width of all of the single ZBP mutants has been added to Supplementary Table 5, and comparisons between the single and double/triple mutants are graphed in Extended Data Fig. 7c. It is true that removing EzrA alone contributes to an increase in Z ring width, but this increase is exacerbated upon the removal of other ZBPs. For example, Z ring width in an EzrA depletion is 390 nm; removing SepF, ZapA, or both together increases this to 510, 470, and 550 nm respectively. Similarly, Z ring width in a Δ*ezrA* strain is 490 nm and removing SepF or EzrA increases this to 590 and 610 nm, respectively. (It is necessary to compare the depletion and the knockout strains separately, since the phenotype is more moderate in the depletion strain presumably due to the presence of residual EzrA).

We therefore believe that EzrA mutant strains have a milder condensation defect, allowing these cells to retain their viability. Once the other ZBPs are removed, however, this defect is exacerbated and cells are unable to divide, hence the synthetic lethality between these proteins. Consistent with this, we find that viability can be restored under these conditions by a mutant that enhances condensation, FtsZ(K86E).

The double deletion of SepFZapA does provide normal looking Z-rings (Why are they not in supplementary Table 5?). I think that you need quantified evidence for this.

These widths are now quantified in Supplementary Table 5 and graphed in Extended Data Fig. 8. They are indeed similar to the control.

Deletion of EzrA increases the lifetime of FtsZ molecules in the ring (supplementary Table 4). This could mean an increase in filament length of 40%. This would be in agreement with the general idea that EzrA binds FtsZ monomers on the surface of the membrane thereby avoiding the production of extra Z-ring at the poles.
Would it be more difficult for SepF and ZapA to bundle longer filaments? Assuming that FtsZ(T11A) also has longer filaments due to its longer lifetime, how do these filaments look like, nicely bundled (can you provide an image and wuantification?)?

We have thought a lot about this! Without knowing more about the specific architecture of these bundles, it's not obvious what effect changing filament length would have. On one hand, longer filaments could provide more binding sites for bundling factors and thus might stabilize these bundles. On the other hand, the presence of longer filaments also means that there are fewer filaments in total, and so it might be more difficult to initiate bundling in the first place, versus the case when there are more, shorter filaments.

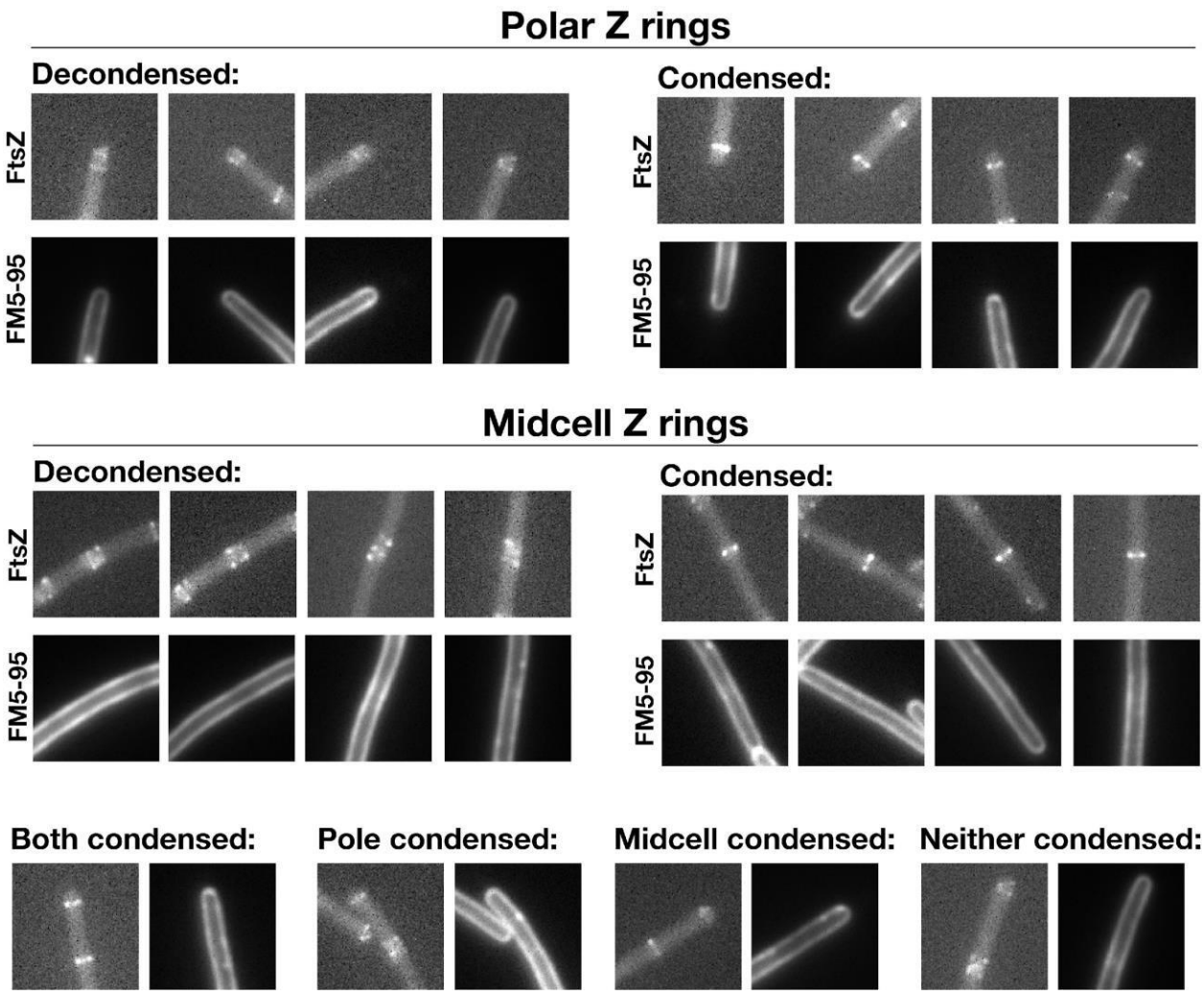
In any case, we have come to think of EzrA's impacts on bundling and on filament length as separate functions- we talk about this in more detail in Supplementary Discussion 1. Consistent with this, although there are other defects in these cells, Z rings look condensed in the FtsZ(T111A) mutant, as you suggest. This data has been added to Extended Data Fig. 3 and Supplementary Table 5.

In the absence of EzrA additional polar rings are made, which reduces the available pool of FtsZ molecules to make a stable ring. Could this also affect the possibility for SepF and ZapA to condense the ring properly, i.e. not enough ring available? In deltaminC in E. coli it takes more time for a Z-ring to assemble (Coltharp, C., Buss, J., Plumer, T. M., & Xiao, J. (2016). Defining the rate-limiting processes of bacterial cytokinesis. PNAS USA, 113(8), E1044–53. http://doi.org/10.1073/pnas.1514296113). Overproduction of EzrA also causes widening of the rings. This suggest that reduction of the available FtsZ causes unstable Z rings that cannot be condensed by SepF and ZapA (or by EzrA), Deletion of EzrA cause polar rings, likely also reducing the amount of available FtsZ for the division ring.

Yes, it is possible that issues with the amount of FtsZ available to form Z rings might exacerbate these condensation phenotypes, and that the ZBPs (especially EzrA) help counteract this. This problem might also arise more generally in cells that cannot divide, since they never form new poles and therefore these sites remain available for FtsZ. It's interesting to speculate that these ZBPs might help to retain FtsZ at midcell via their bundling activity, but we don't have any evidence for that here. One way to test this in the future might be to try overexpressing FtsZ under these conditions (although cloning such a strain will be tricky!). We were curious if we might get any FtsZ overexpression mutants in our suppressor screen as well, but we didn't find anything obvious.

In the shown images the polar rings seems to be condensed whereas the central ring is wide. Is this correct or just an impression?

In the full data set we can observe both condensed and decondensed rings at both the midcell and the poles. Shown below are some examples of each type of ring at each location in the Δ*ezrA* strain. We have also included FM5-95 membrane stained images of each cell to clarify the locations of the poles (necessary in *B. subtilis* because cells grow in chains). We also checked whether cells with condensed Z rings at the poles were more likely to have decondensed midcell rings and vice versa, as a FtsZ availability hypothesis might suggest, but we did not see such a trend- we can see cells with both polar and midcell rings condensed, neither condensed, or just one of the rings condensed. Some examples of each case are included at the bottom of the figure below.



I agree with you that a non-condensed ring cannot function properly, but I am not convinced based on your data that the ZBP are providing this condensation. I guess that you have all data available to support your conclusion and it would be nice to have them in the manuscript or in the supplementary.

We have added all of these data to the paper, and we agree that this strengthens our conclusion. Thank you very much for the suggestions!

Minor comments:

"Assuming that treadmilling filaments elongate with a diffusion-limited on-rate of 5 uM-1, the concentration of free monomers in cells can be estimated to 1.3 uM." Since only 30% of the FtsZ molecules is in the ring and 5 uM FtsZ is present in the cell, the 1.3 uM of monomers does not exclude dimers and short filaments in the cytoplasm, I assume. Maybe this should be made clear in the text?

In our TIRF microscopy videos, we see that there are FtsZ filaments that are moving outside of the Z ring,

as well. Our interpretation is therefore that of the 5 μM of FtsZ in the cell, 1.3 μM (25%) is monomeric, 30% is FtsZ filaments in the Z ring, and the remaining 45% consists of these FtsZ filaments outside of the Z ring, as well as the dimers and short filaments in the cytoplasm that you mention.

Page 6 line 7 from the bottom: on the FtsZ superstructure?

Yes, we had a word in the wrong place. Thank you!

Reviewer #4 (Remarks to the Author):

I thoroughly enjoyed reading this manuscript on the dynamics of Z-ring condensation. The tidy division between periplasmic-facing, mobile components and cytoplasmic-facing, stationary components is gratifying. The remainder of the paper, focusing on quantifying the kinetics and molecule lifetimes in filaments is particularly elegant. Molecular mechanism for condensation is demonstrated via knockdown and functional complementation. The quantifications included are overall solid and rigorously support the conclusions drawn.

I have only a few comments for the authors:

Figure 2A. The way that velocity is represented is counterintuitive, since it is dx/dt and not dt/dx. Thus, at first glance it appears to decrease in the middle cartoon, just because of the way it is displayed.

We chose this representation because it replicates the way that kymographs have typically been displayed in this field, with time on the vertical axis (for example, Figs. 1c, 2b, 4d). In particular, the lifetimes shown in this panel correspond to those in the kymographs in the next panel, 2b center. We believe this will ultimately be more intuitive in the context of our paper, especially for those in the field.

It is not clear to me if the bleaching time of the dye has been corrected for when estimating binding lifetime. Reading the methods, it doesn't appear so. This should be done.

We have clarified our approach to photobleaching in the methods section. Because our experiments are conducted *in vivo*, the simplest approach of immobilizing dye particles under the same buffer conditions and measuring their photobleaching properties directly was not possible. Instead, we followed the approach described in [8], in which the photobleaching constant can be estimated by repeating the experiment at multiple imaging intervals. In our case we found that the lifetimes we measured were invariant with imaging time (Extended Data Fig. 2d), leading us to conclude that photobleaching is negligible under our imaging conditions on the timescale of these single molecule lifetimes ($k_b = 0$). We have commented on this in the methods section: *"We measured the contribution of photobleaching to our lifetimes by repeating the experiment at 1 second imaging intervals rather than 500 ms intervals without changing the exposure time; the measured lifetime did not change, indicating that the photobleaching contribution was negligible."*

Consistent with this, we have data from cells treated with PC190723, which severely inhibits FtsZ dynamics. In these cells, we are readily able to observe single fluorescent molecules with minutes-long lifetimes (kymographs shown below). Of course, this alone is not a photobleaching control, but it is consistent with our observation above that this dye is very photostable on the seconds time scale under our imaging conditions.

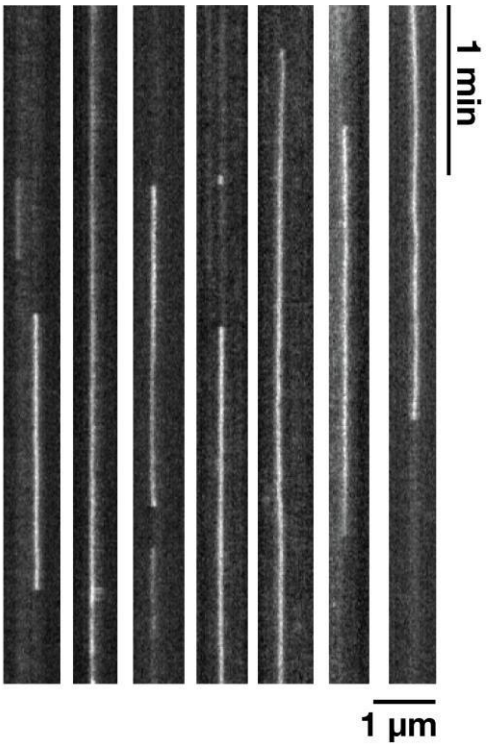


Fig. 3G. I am not entirely convinced about the widening of the ring, and I think there is information hidden by averaging the ring images. Picking just a few rings from the images in panel E and taking line profiles, I did not see such a difference in the ring widths. For example, there could be a bimodal distribution of ring widths for Delta(ZPBs) that would account for the difference in the average images. Or a wide-ring tail that dominates the average. If the intensities are normalized before averaging, dimmer and wider rings might dominate. I also think that the alignment and normalization of the noisier rings in the Delta(ZBP) case may introduce errors which could impact the averaged image. An alternative, which would reveal more such information, is to measure the FWHM for individual rings, and compare the distributions. I suggest the authors try this approach. I think it is more consistent with the Nature journals policy of showing distributions rather than just summary data. I don't think this affects the main conclusions, since there are clearly multiple bands and those are evidence for lack of bundling. These same comments apply for Fig. 4C, Extended Data Fig. 6.

This is a great point. We have made the suggested change, and now show the full distributions for each case.

For Fig. 4B, it would be interesting to also show the intensity of FtsZ as the ring condenses.

Agreed! This data is now included in Fig. 4b.

SVideo 5. This is clearly not the same imaging as displayed in SV1. Is it SIM (I think I see honeycomb)? Ah, now I see that it is mentioned in the main text. This should also be stated in the legend.

Thank you! The first sentence of the legend now reads: "EzrA overexpression decreases FtsZ filament length, visualized by SIM-TIRF microscopy."

Decision Letter, first revision:

Dear Ethan,

Thank you for submitting your revised manuscript "Dynamics of bacterial cell division: Z ring condensation is essential to divide B. subtilis" (NMICROBIOL-20072190A). It has now been seen by the original referees and their comments are below. The reviewers find that the paper has improved in revision, and therefore we'll be happy in principle to publish it in Nature Microbiology, pending minor revisions to satisfy the referees' final requests and to comply with our editorial and formatting guidelines.

We are now performing detailed checks on your paper and will send you a checklist detailing our editorial and formatting requirements in about a week. Please do not upload the final materials and make any revisions until you receive this additional information from us.

Thank you again for your interest in Nature Microbiology and please do not hesitate to contact me if you have any questions.

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{REDACTED}

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Reviewer #1 (Remarks to the Author):

I am happy with the reply of the authors and the additional experiments/controls they have performed.

****
Reviewer #2 (Remarks to the Author):

I am satisfied that the referee comments have been addressed adequately. This is a comprehensive and rigorous report on the dynamics of the divisome of B. subtilis.

Elizabeth Harry

****
Reviewer #3 (Remarks to the Author):

This is a very interesting and thorough paper. I think that all the isssues were resolved, which made the paper clearer and more complete.

Tanneke den Blaauwen

****

Reviewer #4 (Remarks to the Author):

All of my questions and comments have been addressed.

Decision Letter, first revision:

Dear Dr. Garner,

Thank you for your patience as we've prepared the guidelines for final submission of your Nature Microbiology manuscript, "Dynamics of bacterial cell division: Z ring condensation is essential to divide B. subtilis" (NMICROBIOL-20072190A). Please carefully follow the step-by-step instructions provided in the personalised checklist attached, to ensure that your revised manuscript can be swiftly handed over to our production team.

We need to receive your revised paper, with all of the requested files and forms, within 5 business days, by 9th February 2021. Owing to strict production deadlines, failure to submit by this date will result in a delay in formal acceptance and publication. Please get in contact with us immediately if you anticipate delays, and provide us with an estimate regarding when you will submit these files.

When you upload your final materials, please include a point-by-point response to any remaining reviewer comments.

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In recognition of the time and expertise our reviewers provide to Nature Microbiology's editorial process, we would like to formally acknowledge their contribution to the external peer review of your manuscript entitled "Dynamics of bacterial cell division: Z ring condensation is essential to divide B. subtilis". On a trial basis for those reviewers who give their assent, we will be publishing their names alongside the published article. We will not be publishing any of the submitted peer review comments.

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Tanneke den Blaauwen

****

Reviewer #4 (Remarks to the Author):

All of my questions and comments have been addressed.

Final Decision Letter:

Dear Ethan,

I am delighted to accept your Letter "Single-molecule imaging reveals that Z ring condensation is essential for cell division in Bacillus subtilis" for publication in Nature Microbiology. Thank you for having chosen to submit your work to us and many congratulations.

Before your manuscript is typeset, we will edit the text to ensure it is intelligible to our wide readership and conforms to house style. We look particularly carefully at the titles of all papers to ensure that they are relatively brief and understandable.

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Congratulations once again to you and your co-authors for putting together such a nice story, I look forward to seeing it published.