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Actin turnover protects the cytokinetic contractile ring from structural instability

Zachary McDargh, Tianyi Zhu, Hongkang Zhu and Ben O'Shaughnessy
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MS TITLE: Actin turnover protects the cytokinetic contractile ring from structural instability

AUTHORS: Zachary McDargh, Tianyi Zhu, Hongkang Zhu, and Ben O'Shaughnessy
ARTICLE TYPE: Research Article

We have now reached a decision on the above manuscript.

To see the reviewers' reports and a copy of this decision letter, please go to: https://submit-jcs.biologists.organd click on the 'Manuscripts with Decisions' queue in the Author Area. (Corresponding author only has access to reviews.)

As you will see, your study was well received. Nevertheless, the reviewers still raise some points and questions that prevent me from accepting the paper at this stage. They suggest, however, that a revised version might prove acceptable, if you can address their concerns, including some rearrangements of the manuscript (reviewer 2's major comment). If you think that you can deal satisfactorily with the criticisms on revision, I would be pleased to see a revised manuscript.

Please ensure that you clearly highlight all changes made in the revised manuscript. Please avoid using 'Tracked changes' in Word files as these are lost in PDF conversion.

I should be grateful if you would also provide a point-by-point response detailing how you have dealt with the points raised by the reviewers in the 'Response to Reviewers' box. Please attend to all of the reviewers' comments. If you do not agree with any of their criticisms or suggestions please explain clearly why this is so.

Reviewer 1

Advance summary and potential significance to field

This is a very nice study of the role of cofilin in constriction of the contractile ring in fission yeast. The authors use their previously developed and calibrated model to examine what happens when there is less severing of long actin filaments.

They find that the bridging instability ensues in this case - there is a greater tension on long filaments, and because these filaments sling to the curved wall, they peel off. This is a cool physical phenomenon, and several experimental observations support it. there is a nice discussion of biological implications.

The paper is well written, novel and significant.

Comments for the author

Here are several critical comments:
I am not quite sure what can be concluded from fig 5b. What i see is just an anecdotal evidence for cyclic behavior, both in the experiment, and in simulations - 2-3 irregular peaks hardly prove anything. To make this point seriously, one needs something like power spectrum from a longer simulation and experiment.
This sentence:
"To examine the role of cofilin we adapted a molecularly explicit model of the fission yeast contractile ring we developed previously (McDargh et al., 2021), severely constrained by extensive data making the fission yeast contractile ring presently the most amenable to realistic mathematical modeling."
is unclear; also "severely" is not a proper word here
Something is missing from the model description: what are the kinetics of corsslinkers' and myosins' association/dissociation with actin filaments?
What is the reason for appearance for an extremely bent filament in the bottom image of fig 2a?
tens of pN tensile force per filament seems awfully great... would it not break the filament, or rip it off crosslinkers and/or formins? In the model, are dissociation rates depending on forces, as they should?

Reviewer 2

Advance summary and potential significance to field

In this manuscript, McDargh et al. simulated the contractile ring constriction during fission yeast cytokinesis using their computation model to recapitulate the unique “bridging” phenotype. Their detailed model (published in a preprint by the same group), based upon experimentally determined parameters, largely reproduced the cytokinetic defects, including peeling off of actomyosin bundles during the ring constriction. Such “bridging” phenotype was first discovered in the Pollard lab through the study of cofilin mutants that sever actin filaments very slowly (Chen and Pollard 2011 JCB). It was later confirmed by the Chen lab (Malla et al. 2021 MBoC) and the Balasubramanian lab (Cheffings et al. 2019 MBoC). Further, the later found that “bridging” depend on both type II and type V myosins and the frequency of such events anticorrelates with the density of myo2 in the ring. Nevertheless, it remained unclear how such actin filament bundles detach off from the contractile ring and why. The results of this study strongly support the hypothesis that severing of actin filaments by cofilin modulates the tension in the contractile ring. Without effective severing, the elevated tension in the actin filaments result in their breaking off from the plasma membrane through the pulling force of type II myosins. The model also explains the critical role of Myo2 in “bridging”.
The key findings of this study are novel and exciting. This computation model which has been published in another preprint (McDargh et al. 2021), largely recapitulates the phenotype of two cytokinetic mutants adf1-M3 and myo2-E1 for the first time. The unique strength of the model lies in its incorporation of many experimentally determined parameters including the actin filament length the number of various molecules in the cytokinetic nodes and etc.. The study clearly demonstrated the previously underappreciated importance of cofilin in the maintenance of the ring tension. This adds to the known role of cofilin in the contractile ring assembly. The study shall be of great interest to cell biologists who are interested in either cytokinesis or actin dynamics in general.

Comments for the author

The only major concern of the reviewer is that the format of the manuscript. The manuscript can be significantly improved through reshuffling of its various parts. For example, the details of the computation model described in the 1st section of the Results shall be moved to the Methods section. Another example the Results contain many interpretations that can be moved to Discussion.
Specifically, such statement “These observations in live cells and in simulations are consistent with …” in the next to the last section of the Results is more suitable for the Discussion. Overall, the reviewer suggests the authors to substantially reorganize the text and considering shortening the manuscript to a report.

Minor concerns,
1) Please add line numbers to the manuscript. It will help the reviewers to track the changes.
2) Please add a definition of “bridging” in the abstract.
3) The figures generally require more detailed explanation in the legends.
4) Many panels miss the statistical analyses including Fig. 4B and 5A.
5) Fig. 1A: please explain the cartoon (next to the main diagram) depicting the node. Additionally, it is unclear why Rng2 is included in this model. Its role in cytokinesis has not been examined in this study.
6) Fig. 1B: it is unclear how the force is generated by the crosslinking of actin filaments in the first panel from the top.
7) Fig. 1C: Please indicate whether this model includes the turnover of Myo2 from the plasma membrane.
8) Fig. 2: Please add the color legends for actin, myosins and etc in the diagram.
9) Fig. 2B: It will make more sense to switch the positions of the 2nd and 3rd panels. The 2nd panel represent the combination of both myo2 and myp2 for their contribution to the ring tension, while the 1st and 3rd represent the individual role played by these two myosins respectively. The current arrangement is confusing.
10) Fig. 3A: The reviewer would suggest move the majority of this part of figure legend to the Results.
11) Fig. 4C: Shall the X-axis be the ring circumference? Are the differences between WT and the cofilin mutant significant?
12) Fig. 4D: Please mark the average filament length of wild type and adf1-M3 in this plot. This will help illustrate the differences between their barbed end tensions.
13) Fig. 5A: Please provide data to support that the difference in the node density between the bridging and non-bridging sections is significant.
14) Fig. 5B: Please explain why the model fails to recapitulate the large number of bridging events before the contractile ring constriction and at the constriction onset (-10 to 0 min).
Overall, the reviewer finds the findings from the manuscript quite exciting, but it needs some revisions to streamline the presentation before it can be accepted. The reviewer will be happy to review the revised manuscript.

Reviewer 3

Advance summary and potential significance to field

The article ‘Actin turnover protects the cytokinetic contractile ring from structural instability’ by Zachary McDargh, Tianyi Zhu, Hongkang Zhu and Ben O’Shaughnessy uses a very detailed, molecularly precise model to simulate the cytokinetic ring contraction in S. pombe and how control of actin length helps to ascertain successful ring contraction. The work addresses an interesting and important question, the importance of actin length control during cytokinetic ring contraction and how mutations affecting this length control, such as a cofilin mutant, leads to the formation of straight actin bundles bridging across the cell body and disturb ring constriction. The simulations reveal that increases in actin bundle tension due to the increased length of actin filaments lead to the detachment of actin from the membrane and straightening of actin filaments forming actin bridges. Equally, decrease of myosin 2 attachment to actin leads to actin bridge formation as it was reported experimentally in myosin 2 mutants. Hence, the present work nicely highlights how

mutations that affect very different parts of the cytokinetic ring lead to similar phenotypes due to the underlying physics of the actomyosin ring constriction.
The work builds up on another work by the first and last author together with others (McDargh, Z., Wang, S., Chin, H.F., Thiyagarajan, S., Karatekin, E., Pollard, T.D., and O'Shaughnessy, B. (2021). Myosins generate contractile force and maintain organization in the cytokinetic contractile ring. bioRxiv) that is currently a preprint on Biorxiv and describes in detail the computational model employed in the present work. The entire code is available freely on the programming code sharing platform Github. Though the cited work is not reviewed yet or under review, it is difficult to judge the correctness of the entire, elaborate simulation, but given the track record of the O' Shaughnessy group in the simulation of the cytokinetic ring machinery together with the information provided (i.e. the modelling parameters) in the present work as well as in the McDargh (2021) preprint there is no reason to not trust the modelling.

To conclude, I think that the present work addresses an interesting question with rigorous molecular simulation and provides new insights into the detailed workings of the cytokinetic actomyosin ring. Hence, it warrants publication in the Journal of Cell Science.

Comments for the author

Clarifications/ remarks:
In addition to the figures, it would be great if the authors could provide some movie depicting the dynamics of the actomyosin ring during constriction as this would allow the reader to evaluate how closely the simulation comes to the dynamics observed in cells.
Page 6: The end of the following phrase doesn't seem right to me: 'Accordingly we expect …and (ii) with artificially elevated ring tensions *T* by using unphysiologically higher values of f (4-fold increase), the forces exerted by Myo2 clusters (see Material and Methods).'
Figure 5 B, C: the x-axis in the 5B starts at 0 min with the first maximum at ~ 5 min, while the one of 5C starts at -20 min with a first max at – 4 min. The different scaling of the x axis makes the two histograms appear similarly shaped. Could the authors, please, justify the different scaling (e.g. different definitions of t = 0 min)?
Simulation parameters: can the authors please clarify how the myo2 node density of 18 per micron (node to node distance of 55 nm) is compatible with the excluded volume cut-off distance of r^(myo2-myo2) = 132 nm?
In the discussion, I think the energy gain of straightening an actin filament is unnecessarily overestimated. When straightening a curved actin filament of 1 micron length with 1 micron^(-1) curvature, the end-to-end distance changes from 860 nm to 1000 nm, releasing ~ 500 kT. That's still a lot and convincing enough for the energetic argument the authors do.
The reference of McDargh et al. has a slight typo. It is 10.1101/2021.05.02.442363

First revision

Author response to reviewers' comments

Detailed responses to reviewers

Descriptions of changes to the manuscript are indicated in italics below. In the revised manuscript, all substantial changes are highlighted yellow.

Reviewer 1

This is a very nice study of the role of cofilin in constriction of the contractile ring in fission yeast. The authors use their previously developed and calibrated model to examine what happens when there is less severing of long actin filaments. They find that the bridging instability ensues in this case - there is a greater tension on long filaments, and because these filaments sling to the curved wall, they peel off. This is a cool physical phenomenon, and several experimental observations support it. there is a nice discussion of biological implications. The paper is well written, novel and significant.

We thank the reviewer for these positive comments.

I am not quite sure what can be concluded from fig 5b. What i see is just an anecdotal evidence for cyclic behavior, both in the experiment, and in simulations - 2-3 irregular peaks hardly prove anything. To make this point seriously, one needs something like power spectrum from a longer simulation and experiment.

We thank the reviewer for raising this issue, and we agree that a statistically rational fitting of the data in Fig. 5b is needed. This figure shows the distribution of bridging times during a simulated constriction episode, i.e. those instants in time when a bridging instability was triggered, averaged over 30 simulations each lasting 18 min. The right panel shows the same distribution measured experimentally over 29 constrictions (and includes an earlier maturation phase preceding constriction). Please note that the duration of each simulation and each experimental observation cannot be increased, since fission yeast constriction lasts ~ 25 min.

Remarkably, simulations and experiment both showed a mean of 3 bridging events per constriction (90 events in 30 simulations, 89 events in 29 experimental constrictions). To make a far more quantitative comparison, in the revised manuscript we fit the simulated data to a sum of Gaussians using the identical statistical procedure to that used in the experimental study by Cheffings et al., 2019. The number of Gaussians, their means, their widths and amplitudes are fitting parameters. The best fit to our simulations yielded 3 Gaussians, centred at 3 times separated by ~ 4.5 min. (We used the Gaussian Mixture Model with non-linear least squares fitting, and the Akaike Information Criterion to compare fits with different numbers of Gaussians.) This quantitively supports the hypotheses that: bridging is cyclic, occurring once every ~ 4.5 min, with 3 bridging events per constriction; the bridging events occur at about the same times for every constriction, with a certain random variation; in the limit (infinite number of simulations) the distribution of bridging times tends to a sum of 3 Gaussians centred at the 3 mean bridging times.

The experimental best fit also yielded 3 Gaussians, centred at 3 times separated by ~ 7 min. Though the time interval is slightly greater than in simulations, we do believe that the ability of simulations to reproduce the observed cyclic bridging with 3 bridges per constriction is quite remarkable, and suggests simulations capture the essential bridging phenomenon in live cells.

In the revised manuscript we added the results of this statistical analysis to Fig. 5B, showing the best fit and indicating the Gaussian means. To describe this analysis and the comparison with experiment, we added a few sentences to the main text (lines 235-244) and we updated the caption to Fig. 5B.

This sentence: "To examine the role of cofilin we adapted a molecularly explicit model of the fission yeast contractile ring we developed previously (McDargh et al., 2021), severely constrained by extensive data making the fission yeast contractile ring presently the most amenable to realistic mathematical modeling."is unclear; also "severely" is not a proper word here

By this sentence, we meant to indicate that a realistic model of the fission yeast ring is highly constrained by the large amount of experimental data gathered for contractile rings of this organism. Examples include: the amounts of myosin II (Myo2 and Myp2), actin, formin Cdc12, α-actinin and many other key components throughout constriction; the turnover times of these components; the organization and spatial distributions of Myo2, formin Cdc12 and other components in nodes; the distance of Myp2 from the membrane; the load-free velocity of Myo2 on actin; the velocity distribution of nodes parallel to the membrane. This has no parallel for any other organism. All of the above data was incorporated into our model.

We reworded the text to more clearly state how experimental data constrains our model (lines 104-109).

Something is missing from the model description: what are the kinetics of corsslinkers' and myosins' association/dissociation with actin filaments?

The heads of the ~ 8 Myo2 dimers belonging to a node are represented in a coarse-grained fashion by an ellipsoid (one ellipsoid for each node) with dimensions equal to those measured by FPALM super- resolution microscopy (Laplante et al., 2016). An actin filament intersecting a Myo2 ellipsoid experiences a force perpendicular to the filament that binds it to the Myo2 cluster. Subsequently, if at any time the filament experiences a greater force in the opposite direction it will become unbound; this is the unbinding force threshold. Similar binding/unbinding kinetics apply to Myp2 clusters.

The kinetics for binding of actin filaments by α-actinin crosslinkers are as follows. Actin filament subunits within range (30 nm) stochastically become bound by an α-actinin crosslinker with a probability per unit time chosen to yield the observed density of α-actinin along the ring. Crosslinks unbind spontaneously after a mean time (taken from experiment) or when overstretched (> 50 nm length).

We have considerably expanded the Materials and Methods section to include a more detailed description of the model, including the above binding/unbinding kinetics (lines 337-350).

What is the reason for appearance for an extremely bent filament in the bottom image of fig 2a?

Typical myosin forces in the ring can easily bend a filament that separates from the main bundle. The force to substantially bend a filament of length " (i.e., to maintain a radius of curvature of order its length) is roughly $f \sim l_p KT/l^2$ where l_p = 10 μm is the actin filament persistence length. For a typical (wild type) length at constriction onset, l = 2 μm, this yields f ~.01 pN, far smaller the Myo2 and Myp2 forces per node-attached cluster, of order 10 pN.

Thus, unbundled single filaments can easily be bent into extreme shapes. This occurs, for example, if a formin-nucleated filament fails to get quickly captured by a Myo2 or Myp2 cluster, so the filament grows inwards, away from the ring. Its pointed end may subsequently be captured by myosin at some other ring location. This was the fate of the highly bent filament mentioned by the reviewer, during the interval ~ 340-420 sec after constriction onset (see simulation images below; arrows indicate the filament [NOTE: We have removed a figure which was provided for the referees in confidence.]). After the filament is nucleated at its barbed end, the pointed end is captured at ~ 360 sec. Subsequently, pronounced bending occurs due to elongation of the filament while its ends are almost pinned.

tens of pN tensile force per filament seems awfully great... would it not break the filament, or rip it off crosslinkers and/or formins?

This is an excellent question. Experiment demonstrates that filaments survive tensions of tens of pN, since measured ring tensions of ~ 600 pN are shared by ~ 50 filaments in the cross-section (the number is known from the measured total length of actin in the ring, and a number of this order is consistent with electron microscopy measurements). All of this data is built into our simulations. We are not aware of fracture measurements for fission yeast actin, but single actin filament fracture tensions of ~ 100 pN measured in rabbit skeletal muscle (Kishino and Yanagida, 1988 are consistent with actin withstanding ~ 10 pN loads in contractile rings.

Our simulations assume these forces do not pull actin filaments away from the formins that anchor them to the nodes. We agree with the reviewer that these are large forces (which reach a maximum at the formin-capped barbed ends) and one might worry they could dissociate the filaments. However, formin binding to actin filament barbed ends appears very strong: dissociation constants K_D = 20 nM for budding yeast formin Bni1p (Pruyne et al., 2002), and K_D ~3 nM for mouse formins FRLα and mDia1 (Harris et al., 2004) were reported. Taking K_D ~ 10 nM as a representative typical value gives an estimated binding energy $\varepsilon \approx -KT \ln K_D\ b^3 \sim 16\ KT$ after estimating the microscopic length scale b~2 nm. This yields a crude estimate for the dissociation force of 16 kt/b ~30 − 35 pN. Thus, the formin-actin bonds appear strong enough to survive these forces. We speculate that other ring components such as Cdc15 may also help to stabilize formin-actin binding.

Regarding *α*-actinin, in our simulations these forces do indeed easily dissociate α-actinin crosslinkers: force-induced *α*-actinin dissociation is by far the most common dissociation

pathway (we find these crosslinkers have a minimal effect on the ring).

We added comments about the strength of actin filaments and formin-actin barbed end binding, in relation to actin filament tensions realized in the ring simulations (lines 297-302).

In the model, are dissociation rates depending on forces, as they should?

Effectively, the simulated *α*-actinin dissociation rate depends on the applied force, since we use a length criterion (dissociation occurs above a certain length) which can be translated to a force criterion. Actin filaments unbind from Myo2 or Myp2 clusters following a simple force-dependent rule: unbinding occurs if the applied force exceeds the unbinding threshold. These features are described in the new and more detailed model description in Materials and Methods.

Reviewer 2

The key findings of this study are novel and exciting. This computation model, which has been published in another preprint (McDargh et al. 2021), largely recapitulates the phenotype of two cytokinetic mutants adf1-M3 and myo2-E1 for the first time. The unique strength of the model lies in its incorporation of many experimentally determined parameters including the actin filament length, the number of various molecules in the cytokinetic nodes and etc.. The study clearly demonstrated the previously underappreciated importance of cofilin in the maintenance of the ring tension. This adds to the known role of cofilin in the contractile ring assembly. The study shall be of great interest to cell biologists who are interested in either cytokinesis or actin dynamics in general.

We thank the reviewer for these positive comments.

The only major concern of the reviewer is that the format of the manuscript. The manuscript can be significantly improved through reshuffling of its various parts. For example, the details of the computation model described in the 1st section of the Results shall be moved to the Methods section. Another example, the Results contain many interpretations that can be moved to Discussion.
Specifically, such statement "These observations in live cells and in simulations are consistent with …" in the next to the last section of the Results is more suitable for the Discussion.
Overall, the reviewer suggests the authors to substantially reorganize the text and considering shortening the manuscript to a report.

We thank the reviewer for these suggestions. We have rearranged some materials for a more organized and logical presentation, as below. As the revised manuscript is much longer than the limit for a short report, we do believe it best to maintain its status as a research article to clearly convey the content to readers.

We completely rewrote Materials and Methods, which now features a more detailed description of our model. This enabled us to shift some model details from the first (model) section of Results to Materials and Methods. The model section of Results retains a summarized description of the model which we feel is essential, so that the remainder of the manuscript is meaningful to readers.

We moved the first paragraph of "Contractile rings in adf1-M3 mutants with reduced actin severing have longer actin filaments and higher ring tension" to Discussion (lines 252-261). This paragraph discusses different cofilin mutants. To begin the "Contractile rings in adf1-M3…" section we replaced this paragraph with 3 introductory sentences introducing the mutant simulations (lines 191-196).

As specifically suggested by the reviewer, we moved the paragraph beginning with "These observations in live cells and in simulations are consistent with.." *to Discussion (the shifted paragraph, lines 289-296, now begins* "Consistent with this conclusion.."*) This paragraph was the last of the section "Bridge formation is negatively correlated with Myo2 concentration," and discussed why lower Myo2 density sections should be more susceptible to bridging. The shifted*

paragraph was replaced with a single sentence conclusion, the final sentence of the section (lines 230-232). The new opening sentence of this section (lines 223-224) was shifted from its former location in that section.

Minor concerns

1) Please add line numbers to the manuscript. It will help the reviewers to track the changes.

We added line numbers.

2) Please add a definition of "bridging" in the abstract.

A more detailed definition of bridging was added to the abstract (lines 18-20).

3) The figures generally require more detailed explanation in the legends.

Figure 1: More detailed descriptions were added of the node schematic, of the coarse-grained representation of the node in our model, and of turnover of components in the ring. Figure 2: Clarification about the representations for ring components and other features of the figure were added to the caption. Figures 4 and 5: Results of statistical analyses were added. For Figure 5, we added details and results of the scheme used to fit the bridging simulation data and to compare it to the experimental bridging data.

4) Many panels miss the statistical analyses including Fig. 4B and 5A.

Figures 4B, 4C and 5A: descriptions of statistical analysis were added to the captions, and statements of statistical significance are now incorporated into the figures.

5) Fig. 1A: please explain the cartoon (next to the main diagram) depicting the node. Additionally, it is unclear why Rng2 is included in this model. Its role in cytokinesis has not been examined in this study.

We revised the caption to Fig. 1A to explain the picture depicting the coarse-grained representation of the node used by our model, consisting of an ellipsoid representing the heads of 8 Myo2 dimers and a sphere of 70 nm diameter centred 40 nm from the membrane representing other node components Rng2p and Cdc15p. As the reviewer states, Rng2 is not explored in our study. The significance of this sphere for simulations is only as a spacer, positioning the Myo2 ellipsoid 94 nm from the plasma membrane (as measured with FPALM microscopy, Laplante et al., 2016).

6) Fig. 1B: it is unclear how the force is generated by the crosslinking of actin filaments in the first panel from the top.

In our simulations *a*-actinin crosslinkers are represented as springs of rest length 30 nm and spring constant 25 pN/μm taken from experiment (Claessens et al., 2006; Meyer and Aebi, 1990). These are highly dynamic crosslinkers: as filaments move, linkers tend to get stretched so the springs exert forces on the filaments to which they are bound. A linker stretched to 50 nm dissociates from its host filaments, corresponding to a dissociation force of 0.5 pN. Time averaged, these dynamic crosslinkers generate an effective viscous interaction among the filaments (i.e., the time-averaged force increases with increasing relative filament velocities).
The origin of the ;-actinin forces is now explicitly described in Materials and Methods (lines 354-359).

7) Fig. 1C: Please indicate whether this model includes the turnover of Myo2 from the plasma membrane.

For simplicity the model assumes a whole node turnover scheme, in which Myo2 binds and dissociates from the ring as a cluster of 8 Myo2 dimers together with its host node. The node dissociation time is 41 s, consistent with turnover times of node components which were

experimentally measured. The rate of binding of nodes per unit length of ring is chosen to yield the experimentally determined density of nodes along the ring.

The model turnover scheme, including Myo2 and Myp2 turnover, is now briefly alluded to in the model section of Results (lines 137-139) and described in more detail in Materials and Methods (lines 364-380).

8) Fig. 2: Please add the color legends for actin, myosins and etc in the diagram.

In Figure 2, the colour code for representations of nodes, Myo2, Myp2 and actin filaments is identical to that in Figure 1. *This is now stated in the caption of Figure 2.*

9) Fig. 2B: It will make more sense to switch the positions of the 2nd and 3rd panels. The 2nd panel represent the combination of both myo2 and myp2 for their contribution to the ring tension, while the 1st and 3rd represent the individual role played by these two myosins respectively. The current arrangement is confusing.

We switched the 2nd and 3rd panels of Figure 2B and updated the caption accordingly.

10) Fig. 3A: The reviewer would suggest move the majority of this part of figure legend to the Results.

The caption of Fig. 3A was rewritten to be more concise. Fig. 3A is in fact already cited and described in several places in Results (lines 174-184).

11) Fig. 4C: Shall the X-axis be the ring circumference? Are the differences between WT and the cofilin mutant significant?

Yes, Fig. 4C shows ring tension at different stages as the ring constricts and its circumference decreases. We added an analysis of the differences between tensions of wild type and *adf1-M3* rings at each ring circumference exceeding 8 μm, which showed the differences are statistically significant.

Results of the test are now indicated in Fig. 4C and the test is described in the caption.

12) Fig. 4D: Please mark the average filament length of wild type and adf1-M3 in this plot. This will help illustrate the differences between their barbed end tensions.

We indicated the average filament lengths of wild-type and adf1-M3 rings in Fig. 4D and updated the figure caption.

13) Fig. 5A: Please provide data to support that the difference in the node density between the bridging and non-bridging sections is significant.

We performed a paired *t* test on the difference in node densities in the bridging and non-bridging regions which showed the difference to be statistically significant. *The statistical significance was added to Fig. 5A and the caption was updated.*

14) Fig. 5B: Please explain why the model fails to recapitulate the large number of bridging events before the contractile ring constriction and at the constriction onset (-10 to 0 min).

We thank the reviewer for pointing out this feature of the experimental data, which we had failed to comment on. Following assembly, the fission yeast ring undergoes a ~ 25 min maturation episode before constriction onset, during which Myp2 and other components are recruited. Our modeling study does not address this maturation episode: all simulations describe constriction only, i.e from time 0 onwards, and though very interesting maturation is beyond the scope of the present study. The experimental data shows that rings in the cofilin mutant suffer bridging even during maturation (primarily in the 10 min before constriction onset), suggesting ring tension is already substantial.

The fact that the experimental measurements of bridging begin during maturation, before

constriction onset, is now mentioned in Results (lines 242-244) and in the caption of Fig. 5B.

Reviewer 3

.. I think that the present work addresses an interesting question with rigorous molecular simulation and provides new insights into the detailed workings of the cytokinetic actomyosin ring. Hence, it warrants publication in the Journal of Cell Science.

We thank the reviewer for these comments.

In addition to the figures, it would be great if the authors could provide some movie depicting the dynamics of the actomyosin ring during constriction as this would allow the reader to evaluate how closely the simulation comes to the dynamics observed in cells.

We created two movies: Movies S1 and S2, showing simulations of constricting wild type and adf1-M3 rings, respectively. In the latter, 3 instances of bridging occur and are indicated (arrows). The movies are referenced in the main text (lines 148, 215, 229, 239).

Page 6: The end of the following phrase doesn't seem right to me: 'Accordingly we expect …and (ii) with artificially elevated ring tensions " by using unphysiologically higher values of f (4-fold increase), the forces exerted by Myo2 clusters (see Material and Methods).'

We reworded this text (lines 178-184) to provide a clearer explanation of why we anticipated bridging (i) in myo2-E1 mutants with weaker myosin-actin binding, and (ii) in a hypothetical mutant in which the myosin II exerts higher forces on actin filaments so that the net ring tension is higher than wild type.

Figure 5 B, C: the x-axis in the 5B starts at 0 min with the first maximum at ~ 5 min, while the one of 5C starts at -20 min with a first max at – 4 min. The different scaling of the x axis makes the two histograms appear similarly shaped. Could the authors, please, justify the different scaling (e.g. different definitions of t = 0 min)?

We thank the reviewer for pointing out this feature of the experimental data, which we had failed to comment on. Following assembly, the fission yeast ring undergoes a ~ 25 min maturation episode before constriction onset, during which Myp2 and other components are recruited. For both the simulation and experimental data of Fig. 5B, time = 0 is defined as the onset of constriction. Our modeling study does not address the maturation episode: all simulations describe constriction only. Though very interesting, maturation is beyond the scope of the present study. However the experimental measurements begin shortly after assembly and include maturation, showing that rings in cofilin mutants suffer bridging even during maturation (primarily during the 10 min before constriction onset). This suggests ring tension is already substantial during maturation.

Figure 5B shows the distribution of bridging times during a simulated constriction episode, i.e. those instants in time when a bridging instability was triggered, averaged over 30 simulations each lasting 18 min. The right panel shows the same distribution measured experimentally over 29 constrictions (and includes an earlier maturation phase preceding constriction). Remarkably, simulations and experiment both showed a mean of 3 bridging events per constriction (90 events in 30 simulations, 89 events in 29 experimental constrictions). To make a far more quantitative comparison, in the revised manuscript we fit the simulated data to a sum of Gaussians using the identical statistical procedure to that used in the experimental study by Cheffings et al., 2019. The number of Gaussians, their means, their widths and amplitudes are fitting parameters. The best fit to our simulations yielded 3 Gaussians, centred at 3 times separated by ~ 4.5 min. (We used the Gaussian Mixture Model with non-linear least squares fitting, and the Akaike Information Criterion to compare fits with different numbers of Gaussians.) This quantitively supports the hypotheses that: bridging is cyclic, occurring once every ~ 4.5 min, with 3 bridging events per constriction; the bridging events occur at about the same times for every constriction, with a certain random variation; in the limit (infinite number of simulations) the distribution of bridging times tends to a sum of 3 Gaussians centred at the 3 mean bridging times.

The experimental best fit also yielded 3 Gaussians, centred at 3 times separated by ~ 7 min. Though the time interval is slightly greater than in simulations, we do believe that the ability of simulations to reproduce the observed cyclic bridging with 3 bridges per constriction is quite remarkable, and suggests simulations capture the essential bridging phenomenon in live cells.

In the revised manuscript we added the results of this statistical analysis to Fig. 5B, showing the best fit and indicating the mean bridging times of the Gaussians. To describe this analysis and the comparison with experiment, and to point out that the experimental data includes maturation, we added a few sentences to Results (lines 235-244) and we updated the caption of Fig. 5B.

Simulation parameters: can the authors please clarify how the myo2 node density of 18 per micron (node to node distance of 55 nm) is compatible with the excluded volume cut-off distance of r^(myo2-myo2) = 132 nm?

In simulations the excluded volume interaction between nodes is a soft potential with a cut-off scale of 132 nm taken from FPALM measurements of the spatial distribution of Myo2 in a node (Laplante et al., 2016). Given the mean ~ 50 nm node separation, this means the Myo2 of adjacent nodes overlaps considerably, and nodes moving in opposite directions interfere with one another as they move past one another. We found this was a necessary contribution to the drag force on a node, in order to reproduce the experimental node velocity distribution (Laplante et al., 2016).

We added a statement of the explicit excluded volume interactions between nodes to Materials and Methods, as well as a brief mention of how as a result of this interaction nodes overlap and exert an effective mutual frictional resistance to relative node motions in the ring (lines 356-361).

In the discussion, I think the energy gain of straightening an actin filament is unnecessarily overestimated. When straightening a curved actin filament of 1 micron length with 1 micron^(-1) curvature, the end-to-end distance changes from 860 nm to 1000 nm, releasing ~ 500 kT. That's still a lot and convincing enough for the energetic argument the authors do.

We thank the reviewer for pointing this out, and we agree ~ 4000 kT was an overestimate. To be more systematic, we used the data of Fig. 4B, D for the filament length and tension in this estimate, which now yields about 750 kT. We took a typical actin filament length ~ 2 μm and tension ~ 15 pN at 500 sec after constriction onset (Figs. 4B,D), noting that the mean filament tension is one half the barbed end values shown in Fig. 4D. With the ring radius ~ 1.3 μm, a straightened filament increases its end-to-end distance by ~ 0.2 μm. Roughly, this leads to a free energy release of ~ 750 kT . *We updated the estimated energy advantage in Discussion (lines 272-276).*

The reference of McDargh et al. has a slight typo. It is 10.1101/2021.05.02.442363

We thank the reviewer for pointing this out. *The error was fixed.*

Second decision letter

MS ID#: JOCES/2022/259969

MS TITLE: Actin turnover protects the cytokinetic contractile ring from structural instability

AUTHORS: Zachary McDargh, Tianyi Zhu, Hongkang Zhu, and Ben O'Shaughnessy
ARTICLE TYPE: Research Article

I am happy to tell you that your manuscript has been accepted for publication in Journal of Cell Science, pending standard ethics checks.