

Spatial Variation of Microtubule Depolymerization in Large Asters

Keisuke Ishihara, Franziska Decker, Paulo Caldas, James Pelletier, Martin Loose, Jan Bruges, and Timothy Mitchison

Corresponding author(s): Keisuke Ishihara, Max Planck Institute of Molecular Cell Biology and Genetics

Review Timeline:	Submission Date:	2020-11-17
	Editorial Decision:	2020-11-20
	Revision Received:	2020-12-28
	Accepted:	2021-01-04

Editor-in-Chief: Matthew Welch

Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

RE: Manuscript #E20-11-0723

TITLE: Spatial Variation of Microtubule Depolymerization in Large Asters Suggests Regulation by MAP Depletion

Dear Dr. Ishihara:

After reading your manuscript and your response to the reviewers' comments, I am pleased to say that MBoC is potentially interested in publishing your work and invites you to submit a revised manuscript.

Your study presents the interesting observation of spatially varying microtubule depolymerization rates (and spatially invariable polymerization rates) in large microtubule asters in *Xenopus* egg extract, applying a method for speed determination to dense microtubule networks that was previously applied to bacterial filament networks. The authors propose a limiting component model as an explanation for this observation.

The authors satisfactorily addressed most of the concerns of the reviewers in their rebuttal letter. In preparing the revised manuscript as outlined in the rebuttal letter, major issues worth paying attention are:

(1) The figure provided on page 2 of the rebuttal letter seems to suggest that only 2 out of 3 experiments support the main finding of the study, namely that the depolymerization rate of microtubules in the aster "periphery" is slower than in the "interior". In 1 out of the 3 experiments the depolymerization rate in the "periphery" is equal to that in the "interior". This raises questions concerning the robustness of the main claim made in this manuscript and requires additional experimental support, testing whether indeed the difference in depolymerization rates depends on "periphery" versus "interior".

(2) The authors nucleate asters artificially from Aurora A beads. It seems appropriate to include a statement/discussion about the possibility of a gradient of phosphorylated proteins being the cause for the spatial variation in depolymerization rates. It may also be worth considering to remove the ultimately hypothetical proposal of the limiting factor model from the title of the manuscript, unless experimental support can be provided.

Sincerely,

Thomas Surrey
Monitoring Editor
Molecular Biology of the Cell

Dear Dr. Ishihara,

The review of your manuscript, referenced above, is now complete. The Monitoring Editor has decided that your manuscript is not acceptable for publication at this time, but may be deemed acceptable after specific revisions are made, as described in the Monitoring Editor's decision letter

above.

A reminder: Please do not contact the Monitoring Editor directly regarding your manuscript. If you have any questions regarding the review process or the decision, please contact the MBoC Editorial Office (mboc@ascb.org).

When submitting your revision include a rebuttal letter that details, point-by-point, how the Monitoring Editor's and reviewers' comments have been addressed. (The file type for this letter must be "rebuttal letter"; do not include your response to the Monitoring Editor and reviewers in a "cover letter.") Please bear in mind that your rebuttal letter will be published with your paper if it is accepted, unless you have opted out of publishing the review history.

Authors are allowed 180 days to submit a revision. If this time period is inadequate, please contact us at mboc@ascb.org.

Revised manuscripts are assigned to the original Monitoring Editor whenever possible. However, special circumstances may preclude this. Also, revised manuscripts are often sent out for re-review, usually to the original reviewers when possible. The Monitoring Editor may solicit additional reviews if it is deemed necessary to render a completely informed decision.

In preparing your revised manuscript, please follow the instruction in the Information for Authors (www.molbiolcell.org/info-for-authors). In particular, to prepare for the possible acceptance of your revised manuscript, submit final, publication-quality figures with your revision as described.

To submit the rebuttal letter, revised manuscript, and figures, use this link: [Link Not Available](#)

Please contact us with any questions at mboc@ascb.org.

Thank you for submitting your manuscript to Molecular Biology of the Cell. We look forward to receiving your revised paper.

Sincerely,

Eric Baker
Journal Production Manager
MBoC Editorial Office
mbc@ascb.org

Dear Dr. Thomas Surrey, MBoC monitoring editor,

We appreciate your critical feedback on our manuscript (#E20-11-0723) and the invitation to submit a revision.

We have incorporated the following changes as outlined in our initial response to Review Commons:

- Updated Fig. 2B, vertical lines indicate mean values of the velocity distributions
- Supplemental Fig. 1 (related to Fig. 2), velocity distributions for individual fields of view.
- Supplemental Fig. 2 (related to Fig. 3), laser ablation-induced depolymerization velocity as a function of distance from the center.
- Supplemental Fig. 3 (related to Fig. 4), Tau-mCherry showing depletion based on fluorescence intensity.
- Supplemental Fig. 4 (related to Fig. 4), 10 kDa Dextran showing no spatial change in fluorescence intensity as a control for the method.
- Supplementary Table 1 (related to Table 1) Expanded list of MAPs in the frog egg with their reported activity on microtubule dynamics parameters. The length of this list explains why it is difficult to deplete MAPs one by one to test their effect on polymerization dynamics.

In addition, we provide a point-by-point response to your specific comments below. We believe the changes that we have made in the text and figures address the concerns.

If the revised manuscript meets the editorial standards and will be published in MBoC, we would like to suggest this article to be included in your next special issue on Quantitative Cell Biology.

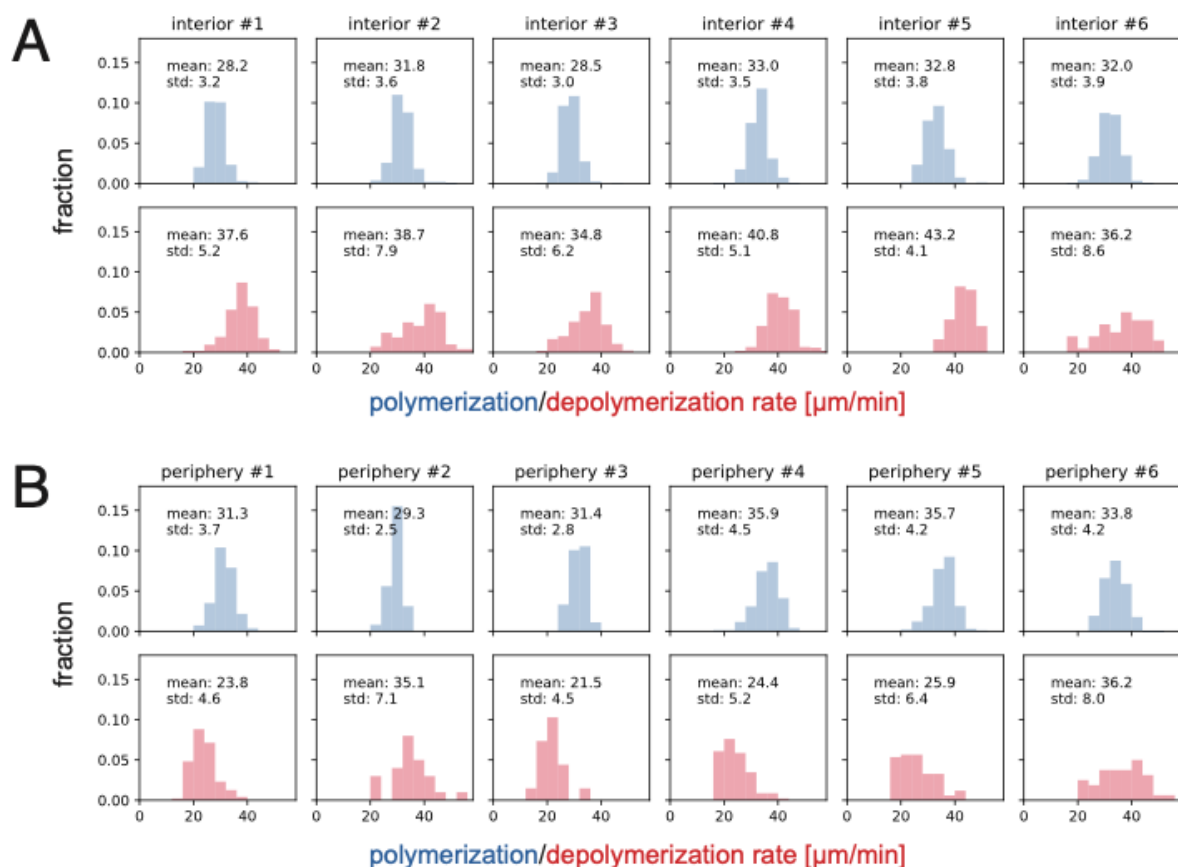
Sincerely,

Keisuke Ishihara and Timothy Mitchison
On behalf of other co-authors

RESPONSE TO MONITORING EDITOR

(1) The figure provided on page 2 of the rebuttal letter seems to suggest that only 2 out of 3 experiments support the main finding of the study, namely that the depolymerization rate of microtubules in the aster "periphery" is slower than in the "interior". In 1 out of the 3 experiments the depolymerization rate in the "periphery" is equal to that in the "interior". This raises questions concerning the robustness of the main claim made in this manuscript and requires additional experimental support, testing whether indeed the difference in depolymerization rates depends on "periphery" versus "interior".

In response, we revised Supplemental Figure 2 to include all the primary data. This figure displays 12 measurements made on 12 field of views from 2 independent extracts.



Four out of the six measurements in the periphery show the slow depolymerization rate (panel B, bottom distributions). Variability of polymerization/depolymerization rates may originate from the biological variability of eggs, extract preparations, and/or our quantification method. However, the relatively small variation in polymerization rate ($\sim 10\%$ around the mean) across multiple fields of view provides some guarantee that these potential issues are small.

To better articulate our current concerns with the variable results of the depolymerization rate across multiple fields of view, we have included the following sentence in the discussion:

...the TIRF method is limited to a small 80x80 μm field of view, which must be fixed in one position for 2 min of the time lapse acquisition before moving to another position. During this time, the peripheral region is moving outward due to aster growth. Thus, our definition of the periphery in the TIRF method is less precise, possibly explaining the high position-to-position variability in the peripheral depolymerization rate (Supplemental Figure 1) ...

We also think that the large extract batch variability in depolymerization rates we report using the laser cutting method is significant. We added the following section to the result where we go through figure 3:

“The average depolymerization rate was surprisingly variable between asters and extract batches (Fig 3D). This variability suggests that plus end depolymerization rate is not governed by microtubule structure alone, which should be constant, but also by the precise concentration of MAPs and motors that regulate depolymerization, which are likely to vary between extract batches. “

(2) The authors nucleate asters artificially from Aurora A beads. It seems appropriate to include a statement/discussion about the possibility of a gradient of phosphorylated proteins being the cause for the spatial variation in depolymerization rates. It may also be worth considering to remove the ultimately hypothetical proposal of the limiting factor model from the title of the manuscript, unless experimental support can be provided.

To explicitly raise the possible alternative mechanisms for the regulation of depolymerization, we have added the following highlighted section in the discussion:

Multiple hypotheses could be considered to account for the observed spatial regulation of depolymerization rates, including chemical activity gradients or timer mechanisms coupled to the nucleotide state in the microtubule lattice (Roostalu et al. (2020) eLife, Bollinger et al. (2020) Scientific Reports).

Regarding the title, we have decided to follow the suggestion and remove the speculative conclusion about the limiting factor model. We hope our paper will increase interest and discussion of depolymerization rates, and perhaps invention of new probes that are specific to depolymerization.

RE: Manuscript #E20-11-0723R

TITLE: "Spatial Variation of Microtubule Depolymerization in Large Asters"

Dear Dr. Ishihara:

I am pleased to accept your manuscript for publication in *Molecular Biology of the Cell*. Your revisions and point-by-point response have satisfactorily addressed the reviewers' concerns. Your technically advanced study provides interesting information on the spatial regulation of microtubule depolymerization rates in large *Xenopus* egg extract asters and proposes a plausible model for a molecular mechanism. Your suggestion to include your article in the next special issue on *Quantitative Cell Biology* will be considered.

Sincerely,
Thomas Surrey
Monitoring Editor
Molecular Biology of the Cell

Dear Dr. Ishihara:

Congratulations on the acceptance of your manuscript.

A PDF of your manuscript will be published on MBoC in Press, an early release version of the journal, within 10 days. The date your manuscript appears at www.molbiolcell.org/toc/mboc/0/0 is the official publication date. Your manuscript will also be scheduled for publication in the next available issue of MBoC.

Within approximately four weeks you will receive a PDF page proof of your article.

Would you like to see an image related to your accepted manuscript on the cover of MBoC? Please contact the MBoC Editorial Office at mboc@ascb.org to learn how to submit an image.

Authors of Articles and Brief Communications are encouraged to create a short video abstract to accompany their article when it is published. These video abstracts, known as Science Sketches, are up to 2 minutes long and will be published on YouTube and then embedded in the article abstract. Science Sketch Editors on the MBoC Editorial Board will provide guidance as you prepare your video. Information about how to prepare and submit a video abstract is available at www.molbiolcell.org/science-sketches. Please contact mboc@ascb.org if you are interested in creating a Science Sketch.

We are pleased that you chose to publish your work in MBoC.

Sincerely,

Eric Baker
Journal Production Manager

MBoC Editorial Office
mbc@ascb.org
