Mff oligomerization is required for Drp1 activation and synergy with actin filaments during mitochondrial division

Henry Higgs, Ao Liu, and Frieda Kage

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Submission Date:	2021-05-02
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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 #E21-04-0224
TITLE: Mff oligomerization is required for Drp1 activation and synergy with actin filaments during mitochondrial division

Dear Harry,

Two experts in the field reviewed your manuscript. You will see that both reviewers found your data on Mff oligomerization interesting. However, you will see that they suggest some major revisions that you need to address before publication of your manuscript in MBoC. Reviewer#1 concerns can be addressed by adding a description of the statistical tests in different figures and by adding a discussion on how oligomerization of Mff could cause a change in Mff distribution.
Reviewer#2 has a concern about the role of the Drp1 interactions with actin. A possible additional experiment has been suggested by this reviewer. If possible, this experiment should be tested.

Sincerely,

Laurent Blanchoin
Monitoring Editor
Molecular Biology of the Cell

Dear Dr. Higgs,

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 and the reviewer comments below.

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 haveopted 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

Reviewer #1 (Remarks to the Author):

Mitochondrial fission is a critical event in the life of a cell and defects in mitochondrial fission are related to disease states. The mechanism of mitochondrial fission in mammalian cells remains unclear mainly owing the lack of understanding at the biochemical levels of the proteins involved. The Mff protein bound to the outer mitochondrial membrane recruits Drp1 in a mechanism thought to require the oligomerization of Mff. Furthermore, the actin cytoskeleton has been shown to support the recruitment of Drp1 but the mechanism is unclear. Here, Liu et al. show biochemical evidence for the mechanism of activation of Drp1 by a critical oligomerization step of the Mff protein. The data shown support that Mff forms a trimer via a coiled-coil region adjacent to the transmembrane domain. Oligomerization of Mff supports Drp1 protein interaction and this interaction is strengthened by the presence of actin filaments.

Overall, the data shown by the authors support their conclusions and clarifies missing information and conflicting results in the field. However, the biochemical experiments appear discontinuous with some of the cell work performed by the authors specifically regarding how the oligomerization of Mff relates to the distribution (uniform vs punctate) of Mff on mitochondria.

Major comments:
1- The punctate versus uniform distribution of Mff is interesting but it is unclear why this distribution is essential for function. How does the oligomerization state of Mff regulate its distribution on mitochondria? What is the biological relevance of punctate distribution of Mff/Drp1 on mitochondria? If this information is known, can you provide more biological background in your introduction. I suspect however, that the biological relevance of this localization pattern remains unclear. If this is the case, please specular how your results on the oligomerization of Mff could cause a change in Mff distribution on mitochondria and how the actin cytoskeleton could participate in this event.

2- Please define the statistical tests used in Figures 7B-D, S5C, S6B-C, S7C.

3- In Table S1, please define whether the numbers reported are means ± standard deviations.

Minor comments:
1- The color scheme used for labeling different populations on certain graphs are hard to distinguish.
For example, is nearly impossible to distinguish the purple from the blue used to label Mff-L2P and Mff-ΔTM in figure 1D and F, figure 3D and figure 5A.
Same for the different shades of red used to label the curves of Figures 2B and 3C.
Same for the shades of blue used to label the punctate versus uniform populations in Figures S5B and C.

2- Figure 1F: What do the red data points label?

3- Figure S2C: The font size for the table is illegible. Please increase the font size.

4- Many graphs illustrating % as a function of variables have their Y-axis ending at 120%. I suspect the authors ended their axis beyond 100 to have extra space for labeling their curves. However, since values >100% are not possible, please end the scale at 100%. These graphs are in Figures 2A-D, 3C, 3D, S2A, S2B, S5C.

Reviewer #2 (Remarks to the Author):

Liu et al. investigated biochemical interactions between Mff, Drp1 and actin. They show that increasing concentrations of Mff stimulate Drp1 oligomerization and GTP hydrolysis. Mff also oligomerizes by itself in vitro and this oligomerization is a requirement for stimulating GTP hydrolysis by Drp1. Interestingly, actin synergistically enhances the effects of Mff on Drp1 activation at a specific actin concentration. Mff does not appear to interact directly with actin. Instead, it activates Drp1 that is bound to actin filaments. The authors also show that Mff oligomerization is required for mitochondrial and peroxisomal fission. The experiments in this manuscript are solid.

The authors suggests that actin filaments and Mff act synergistically to activate Drp1 during fission. They must do so in parallel, because Mff and actin do not interact with each other. This conclusion is surprising, because mitochondrial constriction is also promoted by myosin as previously shown by these authors. The authors thus propose that actin filaments play two sequential roles in constriction. The first role would be the previously described initial constrictions mediated by actomyosin contraction. The second role is to stimulate further constriction by Drp1.

There is a bit of a problem with the main conclusion, because Drp1 interactions with actin were previously also suggested to act as a cytosolic reservoir of Drp1 instead of directly promoting fission. The authors should determine whether actin binding, as determined here with in vitro experiments, reflects a direct role in fission in vivo as proposed here or acts as a reservoir for future fission events as previously proposed. A possible precedent for a direct interaction between actin filaments and Drp1 is suggested by mutations in the stalk region of yeast Vps1, which affect actin binding and the progression of vesicle constriction (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4386032/). This could be sorted out with transfections experiments using mutations in Drp1 that affect actin binding but not Mff induced oligomerization. Although this may seem a tall order, it is the title of the article and so it should be

tested in vivo.

Minor points:

The Y-axis of Fig. 1F seems to be mislabeled.

The panels with enlargements of peroxisomes in Fig. 7 were cut off in the PDF.

RE: Manuscript #E21-04-0224R
TITLE: "Mff oligomerization is required for Drp1 activation and synergy with actin filaments during mitochondrial division"

Dear Harry,

Your revised manuscript is now suitable for publication. However, reviewer#2 suggested to edit the new paragraph in the discussion.

Therefore, I give you the opportunity to edit your manuscript before the final decision on your very interesting study.

Sincerely,

Laurent Blanchoin
Monitoring Editor
Molecular Biology of the Cell

Dear Dr. Higgs,

The review of your manuscript, referenced above, is now complete. The Monitoring Editor has decided that your manuscript requires minor revisions before it can be published in Molecular Biology of the Cell, as described in the Monitoring Editor's decision letter above and the reviewer comments (if any) below.

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.

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To submit the rebuttal letter, revised version, and figures, please use this link (please enable

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Thank you for submitting your manuscript to Molecular Biology of the Cell. Please do not hesitate to contact this office if you have any questions.

Sincerely,

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

Reviewer #1 (Remarks to the Author):

The authors have adequately addressed all the comments from this reviewer. I have no further requests.

Reviewer #2 (Remarks to the Author):

The replies to the reviewers comments and the additions to the discussion and methods are sufficient. The authors do need to edit the part that they added to the discussion and fix the references in that part.

Dear Dr. Blanchoin,

We have up-loaded manuscript and figures in individual files.

Best,
Henry Higgs

RE: Manuscript #E21-04-0224RR
TITLE: "Mff oligomerization is required for Drp1 activation and synergy with actin filaments during mitochondrial division"

Dear Harry

I am pleased to accept your manuscript for publication in Molecular Biology of the Cell.

Sincerely,
Laurent Blanchoin
Monitoring Editor
Molecular Biology of the Cell

Dear Dr. Higgs:

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.

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

Sincerely,

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