Roles of Mso1 and the SM protein Sec1 in efficient vesicle fusion during fission yeast cytokinesis

Kenneth Gerien, Sha Zhang, Alexandra Russell, Yi-hua Zhu, Vedud Purde, and Jian-Qiu Wu

Corresponding author(s): Jian-Qiu Wu, The Ohio State University

Review Timeline:

Submission Date:	2020-01-23
Editorial Decision:	2020-03-03
Revision Received:	2020-05-07
Accepted:	2020-05-13

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 #E20-01-0067
TITLE: Roles of Mso1 and the SM protein Sec1 in efficient vesicle fusion during fission yeast cytokinesis

Monitoring Editor (Remarks to Author):

Dear Dr. Wu,

Your paper entitled "Roles of Mso1 and the SM protein Sec1 in efficient vesicle fusion during fission yeast cytokinesis" has now been examined by two expert reviewers. As you will see both reviewers thought the work was interesting, well done and the topic appropriate for MBoC, but while reviewer #2 only suggested minor changes the 1st reviewer had a more extended list of concerns and suggestions for improvement.

While some of the suggestions may be beyond the scope of this work, I would encourage you to address them as well as you can within the coming months. I think this work will be a valuable addition to both the exocytosis and cytokinesis fields. I look forward to receiving a revised version with a cover letter that addresses each of the reviewers' comments with a point by point response.

Sincerely,

Patrick Brennwald
Associate Editor

Dear Prof. Wu,

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.

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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):

The manuscript by Gerien, et al. presents the first examination of the function of the two proteins Mso1p and Sec1p in fission yeast. Studies using baker's yeast have previously characterized Mso1p and Sec1p, and placed them functionally into the context of yeast exocytosis.
The authors suggest that in fission yeast Mso1p binds to Sec1p and may be required for Sec1p localization to the site of cell division. Furthermore, the authors suggest that both Mso1p and Sec1p are required for proper vesicle fusion and thereby ring constriction during cytokinesis. Although the proposed findings are generally intriguing, several issues should be addressed.

Please see attachment

Reviewer #2 (Remarks to the Author):

This is an excellent study from the group of Jianqiu Wu. The authors do an excellent job of studying the role of the secretory pathway proteins Mso1 and Sec1 in cytokinesis in fission yeast. Although the secretory pathway is extremely well characterized in budding yeast and mammalian cells, how the secretory pathway and vesicle trafficking impact on cytokinesis is less well understood and fission yeast in turn has developed into an excellent organism to address this question. The quality of data, as is usual from the Wu lab, is first rate. The paper has several interesting observations and I recommend publications. I have a few minor points that will make the paper stronger.

1. In figure 1, the description of the timing Mso1 assembly is not convincing. It may be better to compare the timing of Rlc1-GFP with Mso1-citrine in figure 1 D, in addition to Mso1 with the SPB.

2. Figure 2H. This is one of the most exciting parts of the paper that has not received the attention I would have expected. IT may be possible to better resolve the link between the two daughters. Can the authors use a marker such as ain1, which does not so intensely label the ring and look at fixed cells for the size distribution of contracting rings, and if they become stuck at a certain size. Using axial plane view will help. Does this phenotype mean that the Mso1 is involved in abscission? This should atleast be discussed.

3. The sec1-M2 cells in figure 5D look a bit strange and I am not sure of the phenotype shown is a result of starved and already dead cells. The cells in 5C look healthier, despite the fluorescent tag.

Reviewer #1

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The authors suggest that in fission yeast Mso1p binds to Sec1p and may be required for Sec1p localization to the site of cell division. Furthermore, the authors suggest that both Mso1p and Sec1p are required for proper vesicle fusion and thereby ring constriction during cytokinesis. Although the proposed findings are generally intriguing, several issues should be addressed.

Thanks the reviewer for your interest in our study.

- In many quantifications error bars are missing or there is no description about what the presented error bar is (standard deviation or SEM). Furthermore, information about how many cells per culture were examined and which statistical test was used is missing. These missing points are very relevant for the reader and have to be added to each quantification and corresponding figure legend.

We have added error bars to our *mso1Δ* septation quantification in Figure 2. We have changed the text to indicate standard deviations were used in all quantifications. We have also indicated how many measurements were taken and what type of statistical tests were performed.

- Mso1p and Sec1p are not only linked to each other functionally, but also to SNARE proteins, small GTPases and the exocyst complex. For that reason, protein levels for relevant proteins, especially the SNAREs, in the Δmso1 and Sec1-M2 mutant strain should be examined.

We have quantified the protein levels of SNARE proteins in both mutants using the global intensity of fluorescently tagged proteins (Figure S3). We found that the v-SNARE Syb1 and the t-SNARE Psy1 did have significantly higher levels in the *mso1*Δ mutant than wild type cells.

- In Figure 1A you propose that S. pompe Mso1p possesses a Sec1p binding area similar to S. cerevisiae. To strengthen that claim you should test different N and C-terminal truncation mutants of Mso1p. Additionally, in Fig5A it is unclear if the mutation in Sec1p affects Mso1p interaction or possibly e.g. Sec1p protein stability or SNARE interactions in general. That critical point should be addressed by for example co-immunoprecipitation.

We created and purified recombinant N and C-terminal fragments of Mso1 and tested both to see if they can interact with purified Sec1 by an *in vitro* binding assay. This assay showed that full length Mso1 and the N-terminal portion of Mso1 can interact with Sec1, but not the C-terminal portion. We have included this data in Figure 4D as further evidence of the Mso1-Sec1 interaction.

We believe determining the stability Sec1-M2 and how this mutation effects the interaction between Sec1 and Mso1 would be a great experiments to run. Unfortunately, we are currently unable to perform these experiments because the laboratories are shut down due to COVID-19.

- Figure 3A: It is very difficult to see any significant secretion differences between the strains and temperatures. Especially, since there is also less secretion in the wild type strain at elevated temperature. This result has to be re-examined or the result part should be toned down.

We have toned down the text as the following: "Similarly, *mso1*Δ cells secreted less acid phosphatase at both 25 and 36°C (Figure 3A). This suggests that exocytosis may be compromised in *mso1*Δ cells."

- Figure 4D and 5E: What are the Sec1p puncta seen in the Δmso1 and Sec1-M2 strains? Are they accumulated secretory vesicles or protein aggregates? Please perform co-staining with relevant cell organelle markers.

To determine if Sec1 puncta are accumulated vesicles in the *mso1Δ* cells, we imaged Sec1 with v-SNARE Syb1 which marks vesicles. We found that these Sec1 did not colocalize with vesicles (Figure S4B). We also found that these puncta do not colocalize with Atg8 (Figure S4B), an autophagy marker. This leads us to believe these puncta are protein aggregates.

- Mso1p was originally identified as a multicopy suppressor of Sec1p. Please examine if the growth defect of Sec1-M2 or Δmso1 mutant strains can be rescued by overexpression of Mso1p or Sec1p respectively?

We performed the requested overexpression tests using the *nmt1* promoter, which is regulated by thiamine, to determine if Mso1 could rescue *sec1-M2* or if Sec1 could rescue *mso1Δ*. We did not see any obvious rescue for any of these strains at different temperatures or overexpression levels.

Minor comments:

- A systematic use of colour code for the different strains in the quantifications would be appreciated.

We changed color code for some of the quantifications.

- Figure 1D: it would be nice for the reader to add arrows to indicate the Mso1p spots and present the actual distance between the two Sad1p spots in each example picture.

We have added these to the image to help increase the readability of the figure.

- Figure 1E: it might be easier understandable for the reader if either different colours are used or the two groups are shown in separate columns.

We have made the two categories different colors to make the figure easier to understand.

- Is there a growth defect of the Δmso1 strain at the permissive temperature (figure 2A)?

No, we do not see any growth defects for *mso1Δ* at the permissive temperature of 25˚C both on plate and liquid culture. The deletion strain grows comparably to WT at this temperature.

Reviewer #2

This is an excellent study from the group of Jianqiu Wu. The authors do an excellent job of studying the role of the secretory pathway proteins Mso1 and Sec1 in cytokinesis in fission yeast. Although the secretory pathway is extremely well characterized in budding yeast and mammalian cells, how the secretory pathway and vesicle trafficking impact on cytokinesis is less well understood and fission yeast in turn has developed into an excellent organism to address this question. The quality of data, as is usual from the Wu lab, is first rate. The paper has several interesting observations and I recommend publications. I have a few minor points that will make the paper stronger.

Thanks the reviewer for recognizing the quality of our study.

1. In figure 1, the description of the timing Mso1 assembly is not convincing. It may be better to compare the timing of Rlc1-GFP with Mso1-citrine in figure 1 D, in addition to Mso1 with the SPB.

We imaged Mso1-mNeonGreen with Rlc1-tdTomato to determine when Mso1 arrives relative to the contractile ring. We found that Mso1 arrives at the division site on average about 4 minutes before the cytokinesis nodes finish condensing into a compact contractile ring. This timing agrees with our analysis with the SPBs, as the nodes finish condensing approximately 10 minutes after SPB separation. We added this data to Result section as the following: "Consistently, Mso1 appeared at the division site when Rlc1 nodes coalesced into a compact contractile ring (Supplemental Figure S1B), which happens at the start of anaphase (Wu et al., 2003)."

2. Figure 2H. This is one of the most exciting parts of the paper that has not received the attention I would have expected. IT may be possible to better resolve the link between the two daughters. Can the authors use a marker such as ain1, which does not so intensely label the ring and look at fixed cells for the size distribution of contracting rings, and if they become stuck at a certain size. Using axial plane view will help. Does this phenotype mean that the Mso1 is involved in abscission? This should at least be discussed.

We imaged Ain1-mEGFP in *mso1Δ* to observe the ring at the end of constriction. We do not see contractile rings that are stuck at a certain size, although we do see that the constriction and disassembly of the ring takes longer than in WT, similar to what we observe for the myosin marker during disassembly. We have also discussed these results in more detail in the text: "These ring disassembly defects suggested some problems in contractile-ring constriction and/or plasma-membrane closure in *mso1*Δ cells. First we observed ring constriction using Ain1-mEGFP, whose concentration in the ring is constant during constriction (Wu and Pollard, 2005), to see if the rings stalled during constriction. We confirmed that the constriction took much longer in *mso1*Δ than WT cells, but the ring did not appear to pause or stall at any point (Figure 2H)."

3. The sec1-M2 cells in figure 5D look a bit strange and I am not sure of the phenotype shown is a result of starved and already dead cells. The cells in 5C look healthier, despite the fluorescent tag.

The difference in the appearance of the cells is due to the cells being grown at 36˚C for different amounts of time. The cells in 5D were grown at 36˚C for 4 hours while those in 5C were grown at 36˚C for 2 hours.

RE: Manuscript #E20-01-0067R
TITLE: "Roles of Mso1 and the SM protein Sec1 in efficient vesicle fusion during fission yeast cytokinesis"

Dear Prof. Wu:

I have looked carefully over your revised manuscript entitled "Roles of Mso1 and the SM protein Sec1 in efficient vesicle fusion during fission yeast cytokinesis". I think you have done an outstanding job of addressing all of the reviewers' major concerns and therefore I am pleased to tell you that I am recommending that the revised paper be accepted for publication in its current form. I also concur with the reviewers suggestion that the paper be featured in Highlights for the ASCB newsletter and MBoC Table of Contents. Congratulations on this impressive piece of work!

Sincerely,
Patrick Brennwald
Monitoring Editor
Molecular Biology of the Cell

Dear Prof. Wu:

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.

Your paper is among those chosen by the Editorial Board for Highlights from MBoC. Hightlights from MBoC appears in the ASCB Newsletter and highlights the important articles from the most recent issue of MBoC.

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