Subunit exchange among endolysosomal tethering complexes is linked to contact site formation at the vacuole

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(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 #E21-05-0227
TITLE: Subunit exchange among endolysosomal tethering complexes is linked to contact site formation at the vacuole

Dear Prof. Ungermann:

Thank you for submitting this brief report to MBoC. As you can see below, the two reviewers indicated generally high quality of the data, and overall interest in the study. However, there was substantial disagreement as to the novelty of the findings, and whether any new information was learned.

Therefore, I would be willing to consider a revised manuscript that addresses these concerns specifically, and if possible, the experiment suggested by reviewer #1.

Sincerely,
-Mary

Mary Munson
Monitoring Editor
Molecular Biology of the Cell

Dear Prof. Ungermann,

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).

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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):

Previous studies showed that Vps39 is involved both in the HOPS as well as the vCLAMP complex. The current study suggest that this subunit can dynamically exchange between these two protein complexes. Furthermore, the HOPS complex can exchange Vps39 with the CORVET subunit Vps3, resulting in the formation of a tethering complex with potentially new functions in endo-lysosomal trafficking. Although the physiological role for this subunit competition between the 3 protein complexes is not clear, the study adds a new level of complexity to the role of these tethers. The experiments are well designed/controlled and the data are of high quality. Therefore, I support the publication of this manuscript.
One idea that could be tested: is HOPS function a prerequisite for the formation of vCLAMP (or: is the Vps39 subunit of vCLAMP delivered by HOPS)? To test this idea, a vps41 deletion could be tested by co-IP for the formation of the vCLAMP complex.

Reviewer #2 (Remarks to the Author):

Gonzales Montoro et al report that Vps39 has at least two distinct functions: one as a component of the HOPS tethering complex and one as part of the vCLAMPs tether. They went on to show that the CORVET component Vps3 can take the place of Vps39 in the HOPS complex, presumably resulting in hybrid complex.

While the experiments are generally well performed, with the exception of the growth assays that are substandard, the data are all confirmatory of previous knowledge published the Ungermann lab and other laboratories and do not go beyond.

Functions of HOPS complex components outside/independent of HOPS:
Vps39: vCLAMP (papers by the Ungermann and Schuldiner labs, confirmed by many other labs working in yeast and mammalian cells)
VPS41: cargo release from myosinV (Wong et al., Curr Biol. 2020)
Regulated secretion (Asensio et al., Dev Cell 2013, and a number of subsequent studies)
TGN to late endosome transport (Pols et al., Nat Commun. 2013)

The Ungermann lab has also shown previously (Peplowska et al., Dev Cell 2007) that HOPS and CORVET can interconvert and identified two additional complexes iHOPS (Vps8/Vamp6) and iCORVET (Vps3/Vps41). Thus, they already knew back then that the assembly can change and the complexes are dynamic in their subunit composition.

Therefore, neither the moonlighting function nor the complex composition is novel.

We would like to thank all the reviewers for their constructive feedback. Below, you will find our specific answers to your questions.

Reviewer #1 (Remarks to the Author):

Previous studies showed that Vps39 is involved both in the HOPS as well as the vCLAMP complex. The current study suggest that this subunit can dynamically exchange between these two protein complexes. Furthermore, the HOPS complex can exchange Vps39 with the CORVET subunit Vps3, resulting in the formation of a tethering complex with potentially new functions in endo-lysosomal trafficking. Although the physiological role for this subunit competition between the 3 protein complexes is not clear, the study adds a new level of complexity to the role of these tethers. The experiments are well designed/controlled and the data are of high quality. Therefore, I support the publication of this manuscript.

We would like to thank the reviewer for their positive evaluation.

One idea that could be tested: is HOPS function a prerequisite for the formation of vCLAMP (or: is the Vps39 subunit of vCLAMP delivered by HOPS)? To test this idea, a vps41 deletion could be tested by co-IP for the formation of the vCLAMP complex.

This is a very good idea. Our previous experiments suggested that it is Vps39 rather than HOPS that is required for vCLAMP formation (Gonzalez Montoro et al., 2018). However, the reviewer is right in that we could not exclude that HOPS formation per se is required to form the contact site. We therefore tested the interaction of Vps39 with Tom40 in wild-type, *vps41*Δ and *vps11*Δ cells by co-immunoprecipitation, and we have included this experiment as Figure2D. Deletion of the other HOPS subunits strongly impairs the interaction of Vps39 with Tom40, although a small amount of Tom40 is still co-purified. There are several explanations for this, including the one suggested by the reviewer, and we have discussed this in the text.

Reviewer #2 (Remarks to the Author):

Gonzales Montoro et al report that Vps39 has at least two distinct functions: one as a component of the HOPS tethering complex and one as part of the vCLAMPs tether. They went on to show that the CORVET component Vps3 can take the place of Vps39 in the HOPS complex, presumably resulting in hybrid complex.

While the experiments are generally well performed, with the exception of the growth assays that are substandard, the data are all confirmatory of previous knowledge published the Ungermann lab and other laboratories and do not go beyond.

We thank the reviewer for their assessment of our work. Regarding the criticisms, we respectfully disagree. As for the growth assays in Figure 1B, we analyze here subtle differences in growth on $ZnCl_2$ due to the vCLAMP formation. These are certainly less striking than comparing for instance *vps39*Δ mutants and wild-type cells, but in agreement with the overall behavior of many contact site proteins, which have an overall mild phenotype, presumably due to many compensatory mechanisms. Our point here is simply

that an artificial vacuole-mitochondrial contact does not substitute for the specific vCLAMPs formed by Vps39 and Tom40, and we believe that this point is shown by the growth tests in all assays.

Functions of HOPS complex components outside/independent of HOPS:
Vps39: vCLAMP (papers by the Ungermann and Schuldiner labs, confirmed by many other labs working in yeast and mammalian cells)
VPS41: cargo release from myosinV (Wong et al., Curr Biol. 2020)
Regulated secretion (Asensio et al., Dev Cell 2013, and a number of subsequent studies)
TGN to late endosome transport (Pols et al., Nat Commun. 2013)

The reviewer is right in that several studies identified HOPS subunits with additional roles outside of the complex. However, many previous studies relied primarily on overexpression of subunits or deletions (Asensio et al., 2013; Pols et al., 2013, Gonzalez Montoro et al. 2018 and Elbaz-Alon et al., 2014), including our own work. Here, we analyzed for the first time the dynamics of the HOPS and CORVET subunits at endogenous levels to determine the possible fraction of the subunits engaged in other functions. Our data thus reveal that vCLAMPs and HOPS rely on the same pool of Vps39. This is particularly obvious, when we overexpress Vps11, and find that vCLAMP formation is strongly affected (Figure 2B,C). We believe that this study thus provides a first important advance in appreciating the dynamic exchange of HOPS and CORVET subunits that is taking place in cells, yet are aware of the limitations of such analyses.

The Ungermann lab has also shown previously (Peplowska et al., Dev Cell 2007) that HOPS and CORVET can interconvert and identified two additional complexes iHOPS (Vps8/Vamp6) and iCORVET (Vps3/Vps41). Thus, they already knew back then that the assembly can change and the complexes are dynamic in their subunit composition.

The initial work of our group on CORVET indeed also showed that an intermediate complex of the HOPS subunit Vps41 and CORVET Vps3 can form (Peplowska et al., 2008), which we later showed also in several deletion mutants (Ostrowicz et al., 2010). We also postulated yet another intermediate complex (Vps8-Vps39-Class C), but did not find evidence later (Ostrowicz et al., 2010). In the Peplowska work, we observed this complex after overexpression of Vps3, and only after hours, so we were not sure about its relevance under physiological conditions. Here, we clearly show by mass spectrometry that Vps3 copurifies Vps41 with the remaining Class C proteins and Vps21 (Figure 3 E), and show that Vps41 and Vps3 colocalize in specific dots (Figure 3C,D). This shows that this complex a) exists in cells, and b) can form a compensatory complex if Vps39 is missing. We speculate that this is due to the other functions of Vps39, for instance in vCLAMPs (which were not known back in 2008). We consider this an important advance and a prerequisite to understand complex dynamics of tethering complexes, a so far poorly addressed and technically challenging issue in the entire trafficking field.

Therefore, neither the moonlighting function nor the complex composition is novel.

While we agree with the statement, this is not the point of this study as outlined in our response letter.

We show here that

- Vps39 function in vCLAMPs is specific and cannot be replaced by a simple bridging (unlike what has been shown for other tethers of MCSs).

- individual pools of Vps39 and Vps41 exist outside of their HOPS association (using endogenously expressed proteins), in agreement with their functions in vCLAMPs or in myosinV release (also a yet poorly understood process).

- Vps41 is part of a Vps3 complex *in vivo* as shown by mass spectrometry, which can explain the stabilization of the HOPS subcomplex once Vps39 partitions into vCLAMPs.

RE: Manuscript #E21-05-0227R
TITLE: "Subunit exchange among endolysosomal tethering complexes is linked to contact site formation at the vacuole"

Dear Prof. Ungermann:

I am pleased to say that I have looked over your revised manuscript, and your response to the reviewers. These changes look quite reasonable to me, and I am therefore accepting your manuscript, with one minor revision, below. Thank you for sending this interesting manuscript to MBoC; it provides new important insights into cross-talk between membrane tethers for fusion, and those used at membrane contact sites.

Minor revision: Figure 4C is not called out in the text. Also, I think its conclusion is a bit overstated, and should be moderated (or caveats indicated). The conclusion in 4C that the intermediate compartment is actually on-pathway is only suggested by the data. Clearly the intermediate is altered by overexpression or deletion of subunits, but direct dynamic exchange and directionality of the endogenous subunits is inferred.

Best,
-Mary

Mary Munson
Monitoring Editor
Molecular Biology of the Cell

Dear Prof. Ungermann,

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.

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To submit the rebuttal letter, revised version, and figures, please use this link (please enable cookies, or cut and paste URL):
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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

Response to reviewer

Minor revision: Figure 4C is not called out in the text. Also, I think its conclusion is a bit overstated, and should be moderated (or caveats indicated). The conclusion in 4C that the intermediate compartment is actually on-pathway is only suggested by the data. Clearly the intermediate is altered by overexpression or deletion of subunits, but direct dynamic exchange and directionality of the endogenous subunits is inferred.

We thank the reviewer for pointing this out. Figure 4C has been mentioned in the text. We agree, however, that this is model indeed a working model that requires further analyses. We therefore adjusted the text accordingly, both in the main part and in the figure legend. The main text now reads:

"…..We conclude that the association of CORVET and HOPS subunits in a heterohexameric Class-C-Vps3-Vps41 complex can explain Vps39 engagement in vCLAMPs (Figure 4C). This working model is based on our deletion and overexpression analyses and requires further validation. Future studies need to address how Vps39 can partition between both functions, as a subunit of HOPS and vCLAMPs."

Further small adjustments are visible in the track changes file associated with the submission.

RE: Manuscript #E21-05-0227RR
TITLE: "Subunit exchange among endolysosomal tethering complexes is linked to contact site formation at the vacuole"

Dear Christian and Ayelén:

Thank you for the minor changes, I am now pleased to accept your manuscript for publication in Molecular Biology of the Cell.

Best,
-Mary

Mary Munson
Monitoring Editor
Molecular Biology of the Cell

Dear Prof. Ungermann:

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.

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