Fusion with wild-type SNARE domains is controlled by juxtamembrane domains, transmembrane anchors, and Sec17

Amy Orr, Hongki Song, and William Wickner

Corresponding author(s): William Wickner, Geisel School of Medicine at Dartmouth

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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 #E21-11-0583
TITLE: Fusion with wild-type SNARE domains is controlled by juxtamembrane domains, transmembrane anchors, and Sec17

Dear Bill,

Comments have been received from two reviewers, and both of them are quite positive. They offer a number of suggestions for improving the clarity of the presentation, for considering some additional experiments that should be straightforward, and for enriching the interpretations of the Jx swap result and some of the other data.

Reviewer #1 is willing to take another look at a revised manuscript, and hopefully it will be a quick turnaround. I look forward to seeing the resubmission.

Sincerely,

Benjamin Glick
Monitoring Editor
Molecular Biology of the Cell

Dear Dr. Wickner,

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):

Juxtamembrane (Jx) domains are short sequences sandwiched between the SNARE domain and the transmembrane anchor of most SNARE proteins. The past two decades have seen a dozen or more published studies of Jx domains but have not led to a complete understanding of their mechanistic role(s). This manuscript takes advantage of the Wickner lab's HOPS-dependent proteoliposome fusion assay to take another run at this problem. The most striking finding is that swapping the juxtamembrane domains of the R- and Qa-SNAREs abolishes fusion. This is a thought-provoking result, since at first glance the two juxtamembrane domains are similar in length and might be expected to be functional in either configuration.

Main Comments

1. The loss of membrane fusion activity caused by Jx swapping relies on two additional 'insults' to the system: leaving out Sec17/18 and severing the Qb-SNARE from its transmembrane membrane anchor. Thus, the Jx swap is an artificial situation superimposed on two additional artificial situations. (Yet another insult, truncation of the Qc-SNARE to render full zippering impossible, is tolerated in combination with the Jx swap so long as Sec17/18 are present. Thus Sec17 and 18, in addition to their classical roles as SNARE disassembly chaperones, are capable of compensating for a truly impressive range of insults.)

2. Returning to the loss-of-function phenotype of the Jx swap, what could be responsible? My guess, which seems reasonably consistent with the recent paper by Hu et al. (2021) and also with the only available high-resolution structural information about Jx domains (Stein et al., 2009), is that the α-helicity of the SNARE domains propagates into the Jx domains. In this case the R- and Qa-SNARE Jx domains lie next to each other. However, their relative orientation, both positional and rotational (around the helical axis), is totally different in the Jx swap. Having the R- and Qa-SNARE Jx domains related by a 90-degree clockwise rotation around the four-fold axis of the coiled coil is completely different than having them related by a 90-degree counterclockwise rotation around that same axis. And if, as seems reasonable, helical continuity between each SNARE domain and its Jx domain is maintained, then the Jx residues that point toward one another would be completely different in the swapped and unswapped configurations.

3. What does it mean for Qa and Qb to assemble by themselves? Do the authors think that this is a 1:1 complex? If so I find that surprising; I'm not aware of another instance in which SNAREs have been found to form dimers. Alternatively, is the Qa:Qb complex 2:2, or 3:1, or 1:3? And, if so, aren't off-pathway intermediates like these expected to diminish SNARE assembly? Could this be related to the observation that "there is more fusion in proportion to the Qb:Qa complex with sQb than with wild-type, membrane-anchored Qb"?

Other Comments

5. The authors spend a good deal of effort on "models for reconstitution of vacuolar Qb function", pointing out that each "has its limitations". What about assessing the function of the Jx swap using the full system with preassembled SNAREs, HOPS, Sec17/18, and ATP? To prevent Sec17/18 from simply complementing the Jx swap, might one use Sec17 lacking the hydrophobic loop?

6. In recent work, the authors have found that other 'insults' are much more efficiently rescued when Sec18 and ATPgammaS are added in addition to Sec17. That seems to be much less true in the current work. What do they think is going on?

7. Bar graphs might fruitfully be employed in quantifying SNARE association - it's difficult to infer this from bands on a gel.

8. Finally, a request on behalf of reviewers everywhere: please, please don't submit manuscripts that lack page numbers!

Reviewer #2 (Remarks to the Author):

Summary:

The authors examine to the role of the juxtamembrane (Jx) region of vacuolar SNAREs in membrane fusion, and in so doing establish (rather surprisingly) that this portion of the R-SNARE (Nyv1p) is essential for membrane fusion. Moreover, the authors show that the Jx region is unique to the the R- and Qa-SNAREs, as a reciprocal swap of the Jx region between Nyv1p and Vam3p (the Qa-SNARE) blocks membrane fusion. They further show that the fusion defect apparent with SNAREs harboring swapped Jx regions can be bypassed by either the addition of Sec17p or by "anchoring" the Qb-SNARE (Vti1p) to the Qa-SNARE. Finally, they show that the block in membrane fusion brought about by the Jx swap is inherent to the SNAREs themselves as a synthetic "tether" (comprised of [GST-PX]2) cannot rescue the block in membrane fusion.

Critic:

The manuscript reports the results of a series of well-crafted experiments in which the authors' use their established in vitro assay, measuring membrane fusion with purified components, to great effect. The manuscript is clearly written, and the figures

are easy to navigate. My only comment / suggestion for improvement would be to clarify the statements that the R-SNARE contains a pair of conserved tryptophans (WW) - particularly as these residues are proposed to play a critical SNARE-specific role in the Jx. Whilst this is the case for the R-SNARE Snc2p, it is not the case for Sec22p, Snc1p (MW) or Ykt6p (MFY). Are the authors referring to conservation amongst Nyv1ps / VAMP7s?

"Rebuttal letter"
Manuscript #E21-11-0583

January 24, 2022

Editorial Office
Molecular Biology of the Cell

Dear Colleagues,

This is our "Rebuttal" Letter. It's not really a rebuttal, but how we've used reviewers' comments to improve the paper! Our comments are shown in **bold** below. Many thanks for all your kind work on this study.

Sincerely,

Bill Wickner

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Reviewer #1 (Remarks to the Author):

Juxtamembrane (Jx) domains are short sequences sandwiched between the SNARE domain and the transmembrane anchor of most SNARE proteins. The past two decades have seen a dozen or more published studies of Jx domains but have not led to a complete understanding of their mechanistic role(s). This manuscript takes advantage of the Wickner lab's HOPS-dependent proteoliposome fusion assay to take another run at this problem. The most striking finding is that swapping the juxtamembrane domains of the R- and Qa-SNAREs abolishes fusion. This is a thought- provoking result, since at first glance the two juxtamembrane domains are similar in length and might be expected to be functional in either configuration.

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2. Returning to the loss-of-function phenotype of the Jx swap, what could be responsible? My guess, which seems reasonably consistent with the recent paper by Hu et al. (2021) and also with the only available high-resolution structural information about Jx domains (Stein et al., 2009), is that the α-helicity of the SNARE domains propagates into the Jx domains. In this case the R- and Qa-SNARE Jx domains lie next to each other. However, their relative orientation, both positional and rotational (around the helical axis), is totally different in the Jx swap. Having the R- and Qa-SNARE Jx domains related by a 90-degree clockwise rotation around the four-fold axis of the coiled coil is completely different than having them related by a 90-degree counterclockwise rotation around that same axis. And if, as seems reasonable, helical continuity between each SNARE domain and its Jx domain is maintained, then the Jx residues that point toward one another would be completely different in the swapped and unswapped configurations. **This is an excellent, important point! We agree, and have added this into the Discussion, near the end of the 2nd paragraph (lines 441-447). Thanks!**

3. What does it mean for Qa and Qb to assemble by themselves? Do the authors think that this is a 1:1 complex? If so I find that surprising; I'm not aware of another instance in which SNAREs have been found to form dimers. **We published in eLife just such an analysis (below), and saw complex between Qa and Qb (lane 7 of part A in this figure). Of course we can't know the relative stability or stoichiometry of this complex, though it's stable enough to withstand repeated washes during its isolation and there's only a modest diminution in Qb associated with Qa alone (lane 7) compared to the amount seen in the 4-SNARE complex (lane 2).**

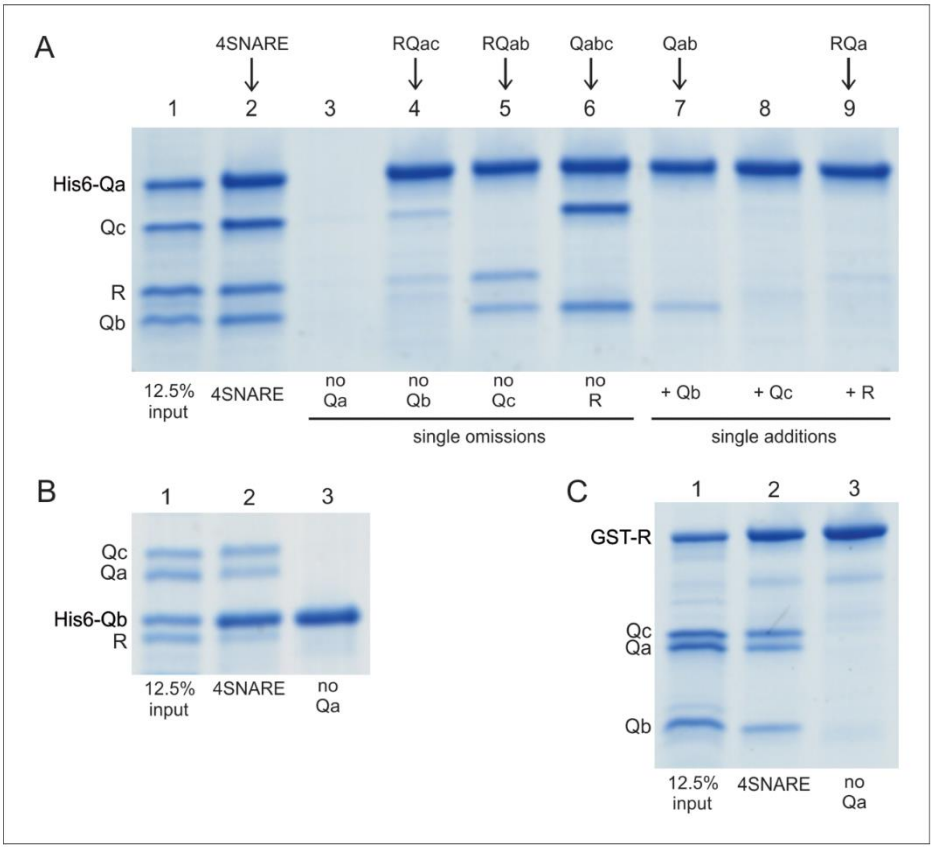

Figure 7. Spontaneous formation of SNARE complexes in detergent. His_6-tagged Qa SNARE (**A**), his_6-tagged Qb SNARE (**B**) or GST-tagged R SNARE (**C**) were mixed at 4 µM with 4 µM of the other indicated full-length SNAREs, in a total volume of 50 µl in pulldown buffer (20 mM HEPES-NaOH, pH 7.4, 150 mM NaCl, 10% glycerol, 100 mM ß-octylglucoside), plus 20 mM imidazoleCl, pH 7.0 for incubations with a his_6-tagged SNARE. After nutation for 1 hr at 4 ˚C, a portion (40 µl) was transferred to tubes containing either 20 µl of a 50% slurry of (**A, B**) nickel-NTA agarose (Qiagen, Hilden, Germany) or (**C**) glutathione agarose 4B (Genesee Scientific, San Diego, CA). Each was nutated at 4 ˚C for 1 hr, diluted with 0.5mls of pulldown buffer (**C**) or pulldown buffer plus imidazole (**A, B**), and centrifuged (500xg, 6 min, 4 ˚C). Supernatants were removed, and the beads were washed three more times with 0.5 ml portions of the same buffer. Proteins were eluted with 50 µl of SDS sample buffer with *ß*-mercaptoethanol by heating (95˚C, 5 min). Eluates were analyzed by Coomassie-stained gel. The substantial increase in molecular weight for his_6-Qa and his_6-Qb is caused by the presence of a 36 amino acyl linker between the his_6 tag and the N-terminus of each of these SNAREs (***Izawa et al., 2012***).

Alternatively, is the Qa:Qb complex 2:2, or 3:1, or 1:3? And, if so, aren't off-pathway intermediates like these expected to diminish SNARE assembly? Could this be related to the observation that "there is more fusion in proportion to the Qb:Qa complex with sQb than with wild-type, membrane-anchored Qb"? **If the wild-type Qb formed complexes with such off-pathway stoichiometries, while sQb does not, this could indeed relate to the greater "fusion in proportion to the Qb:Qa**

complex with sQb than with wild-type, membrane-anchored Qb ", as you suggest. It is noteworthy though that Ypt7/R and Ypt7/QaQb proteoliposomes which are incubated with HOPS form rapid-fusion intermediates (Harner and Wickner, 2018; Song et al., 2020), indicating that substantial Qa and Qb on these Ypt7/QaQb proteoliposomes are in a functional state rather than being tied-up in dead-end complexes. We now have added these points to the text (lines 301-305).

Other Comments

5. The authors spend a good deal of effort on "models for reconstitution of vacuolar Qb function", pointing out that each "has its limitations". What about assessing the function of the Jx swap using the full system with preassembled SNAREs, HOPS, Sec17/18, and ATP? To prevent Sec17/18 from simply complementing the Jx swap, might one use Sec17 lacking the hydrophobic loop? **A great suggestion. We've now done this experiment and it's the new Fig. 10. It shows, in the presence of Sec18 and ATP, that Jx swap with R/Y and R/Qa proteoliposomes (plus HOPS, sQb, and Qc) suppresses fusion, which is restored by adding Sec17 (but not by Sec17F22SM23S), showing that the bypass requires the Sec17 apolar loop. We also see that with Y/R and YQaQb, there is less loss of fusion upon Jx swap, and no specific**

restoration by Sec17 or effect of whether its N-loop is apolar. Thank you, this was a useful addition to the paper!

6. In recent work, the authors have found that other 'insults' are much more efficiently rescued when Sec18 and ATPgammaS are added in addition to Sec17. That seems to be much less true in the current work. What do they think is going on? **As reported earlier (Schwartz et al., 2017), and shown in Figure 4, low levels of Sec17 (e.g. 100nM) need Sec18 and ATPγS for restoration of fusion, while high levels of Sec17 (e.g. 500nM) can restore fusion without Sec18. We've not emphasized this, as it's not central to our current study.**

7. Bar graphs might fruitfully be employed in quantifying SNARE association - it's difficult to infer this from bands on a gel. **We now provide bar graphs.**

8. Finally, a request on behalf of reviewers everywhere: please, please don't submit manuscripts that lack page numbers! **Sorry, now done!**

Thank you for the insightful, and helpful, review!

Reviewer #2 (Remarks to the Author): Summary:

The authors examine to the role of the juxtamembrane (Jx) region of vacuolar SNAREs in membrane fusion, and in so doing establish (rather surprisingly) that this porYon of the R-SNARE (Nyv1p) is essenYal for membrane fusion. Moreover, the authors show that the Jx region is unique to the the R- and Qa-SNAREs, as a reciprocal swap of the Jx region between Nyv1p and Vam3p (the Qa-SNARE) blocks membrane fusion. They further show that the fusion defect apparent with SNAREs harboring swapped Jx regions can be bypassed by either the addiYon of Sec17p or by "anchoring" the Qb-SNARE (VY1p) to the Qa-SNARE. Finally, they show that the block in membrane fusion brought about by the Jx swap is inherent to the SNAREs themselves as a syntheYc

"tether" (comprised of [GST-PX]2) cannot rescue the block in membrane fusion.

Critic:

The manuscript reports the results of a series of well-crafted experiments in which the authors' use their established in vitro assay, measuring membrane fusion with purified components, to great effect. The manuscript is clearly written, and the figures are easy to navigate. My only comment / suggestion for improvement would be to clarify the statements that the R-SNARE contains a pair of conserved tryptophans (WW) - particularly as these residues are proposed to play a critical SNARE-specific role in the Jx. Whilst this is the case for the R-SNARE Snc2p, it is not the case for Sec22p, Snc1p (MW) or Ykt6p (MFY). Are the authors referring to conservation amongst Nyv1ps / VAMP7s?
We've now toned this down to note that the two tryptophans are also seen in Snc2p, not (fallaciously) claiming they're conserved in every R-SNARE. Thanks!

RE: Manuscript #E21-11-0583R
TITLE: "Fusion with wild-type SNARE domains is controlled by juxtamembrane domains, transmembrane anchors, and Sec17"

Dear Bill,

The reviewer is satisfied with your changes, but suggests two minor corrections. Please address these points and submit the final revised manuscript.

Sincerely,
Benjamin Glick
Monitoring Editor
Molecular Biology of the Cell

Dear Dr. Wickner,

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).

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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 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

Reviewer #1 (Remarks to the Author):

The authors have done an excellent job of addressing my concerns with new experiments and revised text. I have two small suggestions:

1) In Fig. 1A, I believe that the Jx domain of Qb should be shown as similar in length to those of R and Qa.

2) The authors might consider inserting a paragraph break at line 409.

"Rebuttal", for E21-11-0583R

Thank you for these comments. We've done exactly as requested, adjusting the length of the Qb Jx domain in Figure 1A and adding the indicated paragraph break.

Again, many thanks!

Best regards,

Amy Orr, Hongki Song, and Bill Wickner

RE: Manuscript #E21-11-0583RR
TITLE: "Fusion with wild-type SNARE domains is controlled by juxtamembrane domains, transmembrane anchors, and Sec17"

Dear Bill,

Thanks for making those final changes, and in general, for sending so much nice work to MBoC. I am pleased to accept your manuscript for publication in Molecular Biology of the Cell.

Sincerely,
Benjamin Glick
Monitoring Editor
Molecular Biology of the Cell

Dear Dr. Wickner:

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

Sincerely,

Eric Baker
Journal Production Manager
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