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Requirement of phosphatidic acid binding for distribution of the bacterial protein Lpg1137 targeting syntaxin 17

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Original submission

First decision letter

MS ID#: JOCES/2021/259538

MS TITLE: Requirement of phosphatidic acid binding for distribution of bacterial protein targeting syntaxin 17

AUTHORS: Misaki Murata, Riku Kanamori, Tomoe Kitao, Tomoko Kubori, Hiroki Nagai, Mitsuo Tagaya, and Kohei Arasaki
ARTICLE TYPE: Research Article

We have now reached a decision on the above manuscript.

To see the reviewers' reports and a copy of this decision letter, please go to: https://submit-jcs.biologists.org and click on the 'Manuscripts with Decisions' queue in the Author Area. (Corresponding author only has access to reviews.)

As you will see, the reviewers raise several substantial criticisms that prevent me from accepting the paper at this stage. However, a revised version might prove acceptable, if you can address their concerns. If you think that you can deal satisfactorily with the criticisms on revision, I would be pleased to see a revised manuscript. We would then return it to the reviewers.

We are aware that you may be experiencing disruption to the normal running of your lab that makes experimental revisions challenging. If it would be helpful, we encourage you to contact us to discuss your revision in greater detail. Please send us a point-by-point response indicating where you are able to address concerns raised (either experimentally or by changes to the text) and where you will not be able to do so within the normal timeframe of a revision. We will then provide further guidance. Please also note that we are happy to extend revision timeframes as necessary.

Please ensure that you clearly highlight all changes made in the revised manuscript. Please avoid using 'Tracked changes' in Word files as these are lost in PDF conversion.

I should be grateful if you would also provide a point-by-point response detailing how you have dealt with the points raised by the reviewers in the 'Response to Reviewers' box. Please attend to

all of the reviewers' comments. If you do not agree with any of their criticisms or suggestions please explain clearly why this is so.

Reviewer 1

Advance summary and potential significance to field

The authors' group previously reported that the Legionella effector Lpg1137 localizes to the ER, mitochondria, and the MAM in host cells and degrades the SNARE STX17. In the present study, the authors show that this specific localization of Lpg1137 is achieved by the interaction with phosphatidic acid (PA), depending on K266 and R276 in its C-terminal region. Accordingly, the Lpg1137 K266A/R276A mutant fails to localize to membranes and degrade STX17.
Overall, the data are mostly convincing, and the manuscript is clearly written.
This study provides a novel mechanism as to how bacterial effector proteins affect host cell functions. I do not find major problems with this paper and have only one relatively minor question.

Comments for the author

Fig. 3B: The immunoprecipitation data shows that Lpg1137 K266A/R276A does not interact with STX17. This is a bit confusing. Even if this mutant mislocalizes, it can associate with STX17 in cell lysates after solubilization with Triton X-100.
How do these mutations affect Lpg1137-STX17 interaction in the lysates? This appears to be inconsistent with the authors' observation that the Lpg1137 K266A/R276A mutant retains STX17 degradation activity, which indicates that this mutant can recognize STX17.

Reviewer 2

Advance summary and potential significance to field

Murata and co-workers report that the Legionella effector Lpg1137, which is known to proteolytically degrade Stx17, contains a phosphatidic acid (PA)-binding motif that is important for its intracellular localization and function. The authors find, by lipid blot and liposome sedimentation assays, that Lpg1137 binds specifically to PA, and they identify a double mutant that lacks PA binding. The double mutant showed aberrant intracellular localization and lacked the ability of wild-type Lpg1137 to degrade Stx17 and to suppress autophagy. Overall the main conclusions are well supported by the data, although some amendments need to be performed (see Suggestions). The manuscript would perhaps be best suited for a journal devoted to pathogen-host interactions but should also be of some interest to cell biologists.

Comments for the author

1. Whereas the biochemical data look convincing, the fluorescence micrographs are less compelling. For instance, it is difficult to conclude from Fig. 3D whether wild-type or mutant Lpg1137 co-localizes with Stx17, and whether Stx17 co-localizes with bacteria in cells expressing wild-type or mutant Lpg1137. I might help to show highly enlarged insets of the areas of interest.
2. The authors claim that PA is found on ER/mitochondra-associated membranes but they do not show any specific markers for neither ER nor mitochondria. Such markers should be included.
3. The authors fail to cite Figs. 4 and 5 properly in the text. They need to discuss what is shown in the different panels.

First revision

Author response to reviewers' comments

Responses to the reviewer's comments

Reviewer #1
Comment: The immunoprecipitation data shows that Lpg1137 K266A/R276A does not interact with STX17. This is a bit confusing. Even if this mutant mislocalizes, it can associate with STX17 in cell lysates after solubilization with Triton X-100. How do these mutations affect Lpg1137-STX17 interaction in the lysates? This appears to be inconsistent with the authors' observation that the Lpg1137 K266A/R276A mutant retains STX17 degradation activity, which indicates that this mutant can recognize STX17.

Reply to the comment - We believe that this is due to the difference in experimental conditions. In the case of the *in vitro* experiment, perhaps the protein concentrations are high enough for recombinant Stx17 and Lpg1137 to interact with each other, whereas in cell lysates their concentrations may not be high enough. We confirmed using in situ proximity ligation assay that Lpg1137$_{K266A/R276A}$ mutant is not inproximity to Stx17 (Fig S2B). A similar result was previously reported for the *Legionella* deAMPylase SidD (Chen et al, PLoS Pathogens 2013; Tascon et al, PLoSPathogens 2020): elimination of the membrane localization domain of SidD causes failure to recognize a target substrate in the cell, although the enzymatic activity is detectable in *in vitro* assay using a recombinant protein that lacks membrane localization domain.

Reviewer #2
Comment 1: Whereas the biochemical data look convincing, the fluorescencemicrographs are less compelling. For instance, it is difficult to conclude from Fig. 3D whether wild-type or mutant Lpg1137 co-localizes with Stx17, and whether Stx17 co-localizes with bacteria in cells expressing wild-type or mutant Lpg1137. I might help toshow highly enlarged insets of the areas of interest.

Reply to the comment - Thank you very much for your suggestion. According to this suggestion, we have compared the co-localization ratio between Stx17 and Lpg1137^{S68A} (a protease-dead mutant) or Lpg1137$^{S68A+K266/R276A}$, and the result of which shows that the co-localization ratio between Lpg1137 and Stx17 is markedly reduced by K266/R276A mutation in Lpg1137. This result is presented as Fig S2A. It should be noted that expression of wild-type Lpg1137 in cells causes Stx17 degradation, which makes it impossible to compare the localization of wild-type Lpg1137 with Stx17.

Comment 2: The authors claim that PA is found on ER/mitochondria-associated membranes, but they do not show any specific markers for neither ER nor mitochondria. Such markers should be included.

We are not certain whether the reviewer requests us to show the co-localization of a PA marker with the ER and/or mitochondria or to show the co-localizatin of Lpg1137 with the ER and/or mitochondria. Unfortunately, there is no good PA marker available at the present moment. However, the presence of PA in the ER- mitochondria interface is firmly established (Kameoka et al. (2018) Phosphatidic Acid and Cardiolipin Coordinate Mitochondrial Dynamics. Trends Cell Biol 28. 67- 76). Sesaki and colleagues demonstrated that the mitochondria fission factor Drp1, which functions on the ER-mitochondria interface as well as mitochondria directly binds to PA (Adachi et al, Mol Cell 2016; Adachi et al, Mol Cell 2020). Regarding the latter question, we showed that Lpg1137$_{S68A}$ co-localizes to Stx17 (Fig S2), which has been shown to localizes to the ER-mitochondria interface (Arasaki et al. Dev Cell 2015, Nat Commun 2017).

Comment 3: The authors fail to cite Figs. 4 and 5 properly in the text. They need to discusswhat is shown in the different panels.

Reply to the comment – Thank you very much for pointing out our lack of explanation. Following your comment, we have re-written the results section relatedto Figs 4 and 5 (please see pages 7, bottom, and 8).

Second decision letter

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ARTICLE TYPE: Research Article

I am happy to tell you that your manuscript has been accepted for publication in Journal of Cell Science, pending standard ethics checks.

Reviewer 1

Advance summary and potential significance to field

The authors appropriately address my previous concerns.

Comments for the author

I have no further concerns.

Reviewer 2

Advance summary and potential significance to field

The authors have successfully addressed the points I raised, and I am happy to recommend publication of their revised manuscript.

Comments for the author

No revision needed.