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En bloc TGN recruitment of *Aspergillus* TRAPPII reveals TRAPP maturation as unlikely to drive RAB1-to-RAB11 transition

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MS TITLE: En bloc recruitment of Aspergillus TRAPPII to the TGN reveals that TRAPP maturation is unlikely to drive the transition between RAB1 and RAB11

AUTHORS: Mario Pinar and Miguel A Penalva
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.)

First of all I would like to apologise for the amount of time it has taken to gather these reviews. We had to approach quite unusual number of reviewers but I wished to retain the rigour of our processes. As you will see, the reviewers raise a number of substantial criticisms that prevent me from accepting the paper at this stage. They suggest, however, that 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.

There is one specific issue where new data might lead to an improved manuscript - based around the use of your destabilising mutants described in your recent paper. I do not consider this to be an absolutely essential addition but would like you to consider this possibility as I do think it would add to the usefulness of the data set.

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 manuscript of Pinar and Penalva addresses the question if the GEF-complex TRAPPII gets assembled by association of its specific subunits onto a pre-localized TRAPPI core-complex. This process could present a basis for the Rab1-to-Rab11 conversion on the membrane of the Golgi.

The study presents a systematic way of investigation the localization of TRAPPI / II / III - complex-specific subunits in time resolved in vivo microscopy using A. nildulans as a model. The data nicely supports the statements the authors make.

Comments for the author

There are a few important issues that the authors need to address:

1. There seems to be no evidence that TRAPP I exists in vivo (Thomas et al., 2018), as the authors also state in their introduction. Considering that TRAPPI might exist only in low abundance (Pinar et al., submitted 2019) raises the question, if it is detectable in fluorescence microscopy and therefore, if the here applied techniques are sufficient to answer the raised question. I find it important that the authors comment in their manuscript more explicitly on this issue.

2. The authors should test the mentioned TRAPPII destabilizing mutants (Pinar et al., submitted 2019) on the colocalization between Trs120 and Bet3. Does destabilization of TRAPPII change the colocalization and thus uncover a possible TRAPPI core? Of course, there may be artificial changes in the localization, which may not answer the overall question.

3. The statement at the end of the discussion that “the signals that TRAPPII would recognize to be recruited to TGN cisternae represent a target for future investigations” is needs to be adjusted. Thomas et al. (2016) clearly showed that TRAPP II recruitment and activity depends on anionic lipids and Arf1. It would be better to state that future investigations will rather focus on the regulation of these processes.

4. Minor issue: I am aware that TRAPPIII is not their direct focus here. However, as a control it would be good to show the colocalization of Trs120 and Trs85 over time to compare TRAPP II and III in their assay. To exclude that Trs85 tagging interferes with function, the authors should then also include a growth test (similar to Figure 1C) of their tagged constructs.

Reviewer 2

Advance summary and potential significance to field

In this manuscript the authors test a model in which the TRAPPII complex is assembled from a previous TRAPPI membrane-localized core. Using time-lapse imaging of Aspergillus, the authors find no evidence to support the model. The work is of high quality and in general the data support the conclusions.

Comments for the author

The work is of high quality, nicely presented and quantified and the conclusions are generally well supported.

The data are dependent on fluorescence microscopy and therefore on the detection limits of this method. Further comment on the possibility of the existence of TRAPPI even at low levels is required.

The TRAPP destabilising mutants described in the 2019 paper by the same authors provide a potential route to probe the localization dependence in a little more detail. For example, does

destabilisation affect Trs80 localization? These would seem to be simple experiments from which additional conclusions might be drawn although the authors might wish to comment on how definitive such experiments might be.

I do still question the final conclusion that the data refute the TRAP conversion model. There are two issues here -
i) is this indeed the case but only in Aspergillus?
ii) could the possibility of separate complexes existing on the same cisternae not be further studies using immunogold EM and/or protein interaction analysis e.g. affinity-purification proteomics?

Lastly, there are some minor typos including in the summary statement that should be corrected.

First revision

Author response to reviewers' comments

Reviewer 1 Advance Summary and Potential Significance to Field:
The manuscript of Pinar and Penalva addresses the question if the GEF-complex TRAPPII gets assembled by association of its specific subunits onto a pre-localized TRAPPI core-complex. This process could present a basis for the Rab1-to-Rab11 conversion on the membrane of the Golgi. The study presents a systematic way of investigation the localization of TRAPPI / II / III - complex- specific subunits in time resolved in vivo microscopy using A. nildulans as a model. The data nicely supports the statements the authors make.
Reviewer 1 Comments for the Author:
There are a few important issues that the authors need to address:

1. There seems to be no evidence that TRAPP I exists in vivo (Thomas et al., 2018), as the authors also state in their introduction. Considering that TRAPPI might exist only in low abundance (Pinar et al., submitted 2019) raises the question, if it is detectable in fluorescence microscopy and therefore, if the here applied techniques are sufficient to answer the raised question. I find it important that the authors comment in their manuscript more explicitly on this issue.
Thank you for raising this point, which led us to realize that our wording with regard to the existence of TRAPPI in vivo was confusing through the MS. We believe that, like in budding yeast, TRAPPI might not even exist, and that the low amounts of this complex detected in cell extracts proceeds from disassembly of other TRAPPs due to in vitro manipulations (Pinar et al. 2019, now published in PLoS Genet 15(12): e1008557. https://doi.org/10.1371/journal.pgen.1008557). Thus, we have introduced the following sentence in the introduction, just after discussing on p3, at the end of the first paragraph, that TRAPPI does not seem to exist in vivo

Indeed work in yeast led to the conclusion that TRAPPIII, rather than TRAPPI is the TRAPP physiologically activating RAB1 in the secretory pathway (Thomas *et al.*, 2018). In spite of this fact, we will use in some cases the expression TRAPPI/TRAPPIII (rather than just TRAPPIII) to facilitate reference to previous literature and to acknowledge the unlikely possibility that TRAPPI has a physiological role.

Thus, TRAPP maturation would involve TRAPPIII >TRAPPII conversion (not TRAPPI > TRAPPII). To clarify this we have made minor changes across the MS, including the abstract and discussion (1st and 2nd paragraph, p12). In addition, following your suggestion below (comment #2), we have now included an experiment in which we ablate TRAPPII, which results in a major artificial increase in TRAPPI (Pinar et al. 2019). This augmented pool of TRAPPI is cytosolic suggesting that by itself it does not have any Golgi localization (Results, 2nd paragraph, p9, on to p10 and new Fig 6 and Fig S6). Thus, we believe that TRAPPI, even if it would exist, wouldn't have the capacity to mature into TRAPPII on Golgi membranes as they progress from RAB1 to RAB11. If there is maturation it should rather be TRAPPIII maturing into TRAPPII, but

substantial evidence beyond microscopy studies reported here (summarized on discussion, 2nd paragraph, p12 on to p13, and answer to your point #2) argues against this possibility. Lastly, we have introduced new text in the discussion spelling out the potential limitations of the techniques that we used here (last sentence on p13, 1st paragraph and first sentence in 1st complete paragraph on p12),

2. The authors should test the mentioned TRAPPII destabilizing mutants (Pinar et al., submitted 2019) on the colocalization between Trs120 and Bet3. Does destabilization of TRAPPII change the colocalization and thus uncover a possible TRAPPI core? Of course, there may be artificial changes in the localization, which may not answer the overall question.
This was a very sound suggestion that we have addressed thoroughly. Our MS mentioned by the referee is now published (PLoS Genet 15(12): e1008557. https://doi.org/10.1371/journal.pgen.1008557). We have carried out additional experiments, summarized in new Fig 6, in which we follow the localization of two core complex subunits in the trs120Δ TRAPPII-destabilizing mutant (Results, 2nd paragraph on p9, on p10, and new Fig 6 and Fig S6). This mutation displaces Bet3 and Trs23 from TGN puncta to a cytosolic haze, further confirming that TRAPPII is the major, if not the only, complex detected in TGN puncta. Therefore, even though we removed TRAPPII, we could not detect TRAPPIII (which also contains Bet3 and Trs23) in the TGN. This argues against the possibility that the high abundance of TRAPPII in the TGN obscures the presence, in a lower abundance, of TRAPPIII (p12, last 10 lines, on to p13). Thirdly, as trs120Δ artificially increases the abundance of TRAPPI at the expense of TRAPPII (Pinar et al, 2019, Fig 3H), these experiments indicate that TRAPPI by itself does not localize to Golgi cisternae (2nd paragraph, p12). Additional experiments investigating the fate of Trs85-TRAPPIII in *trs120*Δ cells are summarized below, in response to a query raised by referee 2

3. The statement at the end of the discussion that "the signals that TRAPPII would recognize to be recruited to TGN cisternae represent a target for future investigations" is needs to be adjusted.
Thomas et al. (2016) clearly showed that TRAPP II recruitment and activity depends on anionic lipids and Arf1. It would be better to state that future investigations will rather focus on the regulation of these processes.
The referee is quite right. We have corrected his unfortunate sentence at the end of the discussion.

4. Minor issue: I am aware that TRAPPIII is not their direct focus here. However, as a control it would be good to show the colocalization of Trs120 and Trs85 over time to compare TRAPP II and III in their assay.
Thank you for this comment. We agree that this would provide new insight into the regulation of TRAPPs, but regretfully we have not yet developed the technology to perform this experiment. TRAPPII reporters are generally bright and stand 3D/4D acquisition reasonably well. In addition, TGN puncta are relatively large. In contrast, Trs85-GFPX3 fluorescence is much weaker, in part because a significant proportion of the signal is cytosolic (like in the budding yeast, Thomas et al, 2018), and in part because the reporter is distributed over numerous small puncta, as opposed to the less numerous and larger TRAPPII/TGN puncta. As Trs85-GFPX3 puncta are much fainter and need to be resolved from the cytosolic haze, the excitation conditions that their visualization requires are incompatible with 3D/4D acquisition. Moreover, the low brightness of TRAPPIII puncta precludes co-filming with TRAPPII using a beam splitter, which generally requires 'equilibrated' signals in both channels. We note, however, that TRAPPIII puncta are clearly different from TRAPPII puncta in that the former are smaller and conspicuously more numerous, strongly suggesting that either the two sets of puncta are different structures or TRAPPIII small puncta represent domains of TRAPPII-containing cisternae. In the discussion (middle of 3rd paragraph on p11), we favor the possibility that small TRAPPIII puncta reflect the well-established role of TRAPPIII in early secretory compartments ('ER/early Golgi interface) although we also acknowledge the second ('domains') possibility (end of 1st paragraph on p13). Addressing the latter would possibly require super-resolution microscopy, whereas the co-filming of TRAPPII with TRAPPIII would require the development and validation of brighter TRAPPIII reporters, using other fluorescent tags, a task that, we believe, is beyond the timescale of this manuscript's revision and that, as the referee indicates, is not the direct focus here.

In any case, all the above explanations highlighting the differences between TRAPPII and TRAPPIII/Trs85 localization were not clearly explained in the former version. In view of this comment, we have taken advantage of the need to revise the text on Trs85 to introduce the control requested by the referee (see the point immediately below) to spell them out more clearly (last paragraph on p5, on to p6)

To exclude that Trs85 tagging interferes with function, the authors should then also include a growth test (similar to Figure 1C) of their tagged constructs.
This experiment is now shown on revised Fig S1 and appropriately commented in the text. (Results section 'Revisiting the localization of *A. nidulans* Trs85/TRAPPIII'

Reviewer 2 Advance Summary and Potential Significance to Field:
In this manuscript the authors test a model in which the TRAPPII complex is assembled from a previous TRAPPI membrane-localized core. Using time-lapse imaging of Aspergillus, the authors find no evidence to support the model. The work is of high quality and in general the data support the conclusions. Reviewer 2 Comments for the Author:
1- The work is of high quality, nicely presented and quantified and the conclusions are generally well supported. The data are dependent on fluorescence microscopy and therefore on the detection limits of this method. Further comment on the possibility of the existence of TRAPPI even at low levels is required.
This concern has been answered in our response to referee 1's comment 1, please kindly refer to it.

2- The TRAPP destabilising mutants described in the 2019 paper by the same authors provide a potential route to probe the localization dependence in a little more detail. For example, does destabilisation affect Trs80 localization? These would seem to be simple experiments from which additional conclusions might be drawn although the authors might wish to comment on how definitive such experiments might be.

The referee surely refers to Trs85 localization in TRAPPII-destabilizing mutants, which is a sound experiment that we have now performed (new Fig 6D and last paragraph of the results). TRAPPII disorganization is complete in a trs120Δ mutant (our paper Pinar et al. 2019 that was cited as 'submitted' is now published (PLoS Genet 15(12): e1008557. https://doi.org/10.1371/journal.pgen.1008557). Trs85/TRAPPIII localization is not affected by trs120Δ and thus TRAPPIII localization is TRAPPII-independent, implying that TRAPPII and III do not compete for the core TRAPP. These findings are now considered in several places in the discussion. See also point #2 by referee 1.

3- I do still question the final conclusion that the data refute the TRAP conversion model. There are two issues here -
i) is this indeed the case but only in Aspergillus? and ii) could the possibility of separate complexes existing on the same cisternae not be further studies using immunogold EM and/or protein interaction analysis e.g. affinity-purification proteomics?

We address these two comments together given that they are related.
'Only in Aspergillus': This might be possible; given that TRAPPI is very minor, if at all exists, and that TRAPPIII activates RAB1 in the secretory pathway, TRAPP maturation would involve the conversion of TRAPPIII into TRAPPII. In yeast, a detectable proportion of TRAPPIII and its substrate, RAB1, localizes to the TGN, and this pool of TRAPPIII could potentially mature to TRAPPII on the same cisterna.
Separate complexes: In Aspergillus, the above-mentioned experiments in which TRAPPII was ablated (new Fig 6 and associated text, and discussion, middle of 3rd paragraph, p12) failed to reveal the presence of TRAPPIII in the TGN, which appears to be a difference with yeast. Thus, although formally possible, there is no evidence that two separate complexes exist on the same cisterna. Moreover, in kymographs core Bet3 and Trs120 appear and dissipate at the same time in every TGN cisterna, indicating that most if not all core TRAPP is accounted for by TRAPPII.

The possibility that Aspergillus and yeast are different with regard to TRAPP maturation is always open until similar studies to those reported here are carried out in yeast; if there were a

difference, it might be related to the fact that Aspergillus TRAPPII/RAB11-mediated endocytic recycling plays an essential role in maintaining polarized hyphal growth (last paragraph of the discussion).

Lastly, there are some minor typos including in the summary statement that should be corrected. Corrected, thank you

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Second decision letter

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MS TITLE: En bloc TGN recruitment of Aspergillus TRAPPII reveals TRAPP maturation as unlikely to drive RAB1-to-RAB11 transition

AUTHORS: Mario Pinar and Miguel A Penalva
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. I did not find it necessary to return the revised manuscript to the reviewers and I would like to thank you for the comprehensive job you have done in revising this work.