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The lipid kinase PI4KIIIβ preserves lysosomal identity

Sunandini Sridhar, Bindi Patel, David Aphkhazava, Fernando Macian, Laura Santambrogio, Dennis Shields and Ana Maria Cuervo

Corresponding author: Ana Maria Cuervo, Albert Einstein College of Medicine

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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.)

1st Editorial Decision 30 November 2012

Thank you for submitting your revised manuscript for consideration by the EMBO Journal. It has now been seen by two advisors whose comments are enclosed. As discussed, these advisors were asked to judge the work in its present state from publication, taking into account the previous referee reports. As you will see, they both express interest in your manuscript and they are broadly in favour of publication, pending satisfactory textural revision.

I would therefore like to invite you to submit a revised version of the manuscript, addressing the comments of the two advisors, as well as those of the previous referees in accordance with the referee response already provided to us.

Regarding the comment from referee 2 to remove the data on lysosome reformation, we have decided that it should be retained ion the manuscript, but the issue should be discussed in detail in the paper. In particular the possible role of membrane fission.

We also encourage you to include the double loss of function experiment currently in progress in revision as it may link the dataset in your paper more directly to the Rong et al. study (Nat Cell Biol. 2012 Sep;14(9):924-34).

We may still obtain comments from a third advisor and would forward these if they arrive before the resubmission, expecting that any additional key issues be addressed.

Please note that we would like to encourage a rapid revision so that a timely publication can be achieved.

When preparing your letter of response to the advisors' comments, please bear in mind that this will form part of the Review Process File, and will therefore be available online to the community. For more details on our Transparent Editorial Process, please visit our website:
http://www.nature.com/emboj/about/process.html

Thank you for the opportunity to consider your work for publication. I look forward to your revision in the very near future.

Yours sincerely,

Editor
The EMBO Journal

REFEREE REPORTS

Advisor #1

This manuscript demonstrates the requirement for PI4P production by PI4KIIIβ to maintain the normal lysosome homeostasis. Lysosome homeostasis includes the reformation of lysosomes at steady state and under conditions of increased flux through the lysosomes (ie during nutrient-deprivation induced autophagy). The authors show that of loss of PI4KIIIβ from the lysosome causes hypertubulation of the lysosome and loss of the fidelity of lysosome reformation after prolonged increased flux from increased autophagy (ALR: autophagic lysosome reformation). The cause of this loss of fidelity is likely to be due to a dysregulation of clathrin-coat formation. This manuscript complements the recent study of Rong et al in NCB who demonstrate PI4P is on the lysosome, and that generation of PI4,5P2 is required for ARL.

The data in the manuscript uses both live imaging and fixed cells, along with extensive immunolocalization, biochemical analysis including subcellular fractionation to support the model to explain the phenotype observed after loss of PI4KIIIβ. They demonstrate a unique subpopulation of PI4KIIIβ is present on lysosomes which distinguishes it from the Golgi-localized pool, and may explain the lysosome-specific activity. Loss of this subpopulation (as well as presumably the rest of the population) causes hypertubulation but this is nicely separated from the well characterized role of PI4KIIIβ at the Golgi. Using a temperature block they further show a selectivity of cargo sorting controlled by PI4KIIIβ at the Golgi (VSV G) versus LAMP1 at the lysosome. The manuscript would be improved by focusing on the key points, and reducing the text which is at times repetitive. In addition, some points whch are not directly relevant could be removed for example in the Introduction the paradigm of PI3P could be eliminated, references to MHC as they have removed the data relating to this, and the discussion could be more focused as some ideas have been introduced for example the role of PI4P in Golgi.

Minor points:
1. The first 4 sentences of the Introduction are not very clear, and in particular the first is confusing.
2. Page 4 second paragraph, the text develops an agreement about the lack of knowledge of the role of PI4P which is not necessary and very artificial.
3. Page 10, it is confusing to read (Cathespin D(pepstatinA)) please clarify earlier that pepstatin A binds and inhibits Cathespin D if this is the case.
4. The model is informative but it is not clear what the purple clathrin adaptor represents (the blue clathrin adaptor corresponds to AP2).

Advisor #2

It is very interesting as it reports for the first time the involvement of PI4KIIIβ in lysosome function. In fact I see very little overlap with the published report. Indeed, I would suggest to focus more on the lysosomal effect of PI4KIIIβ depletion than on autophagy-induced lysosomal reformation, as this would elminate any possible overlap with the NCB paper and also because it would be easier to interpret, because possibly the two lysosomal phenotypes (at the steady state and upon autophagy induction) are not necessarily connected.

It is puzzling, especially for the hypothesized inhibtory effect of PI4P on tubule formation and for the hypothesis of a random and increased assembly of coat in the absence of PI4P. A more reasonable model that would fit the data could envisage a role for PI4KIIIβ in membrane fission: this would explain the longer tubules (the increase in the length rather than in the number of tubules is more impressive), the larger and reduced number "donor" compartment in PI4KIIIβ KD. The lack of fission would associate with the missorting caused by PI4KIIIβ KD and this would induce to hypothesize that PI4KIIIβ couples sorting and fission and that in the absence of PI4KIIIβ both processes are impaired. It remains to be established whether the derangement of the two processes are connected or just coincident and both independently caused by the lack of PI4KIIIβ activity. You might want to suggest to introduce these considerations.

Overall the mechanistic insight both upstream and downstream PI4KIIIβ, are very limited, but still the novelty of the findings would be enough to justify their publication.

In summary, if the answer has to be yes or no, I would say yes but there is space for further improvement of the manuscript.

1st Revision - authors' response 08 December 2012

(Please see next page.)

Point-by-point answer to EMBO J reviewers' comments

Referee #1

This manuscript demonstrates the requirement for PI4P production by PI4KIIIβ to maintain the normal lysosome homeostasis. Lysosome homeostasis includes the reformation of lysosomes at steady state and under conditions of increased flux through the lysosomes (ie during nutrient-deprivation induced autophagy). The authors show that of loss of PI4KIIIβ from the lysosome causes hypertubulation of the lysosome and loss of the fidelity of lysosome reformation after prolonged increased flux from increased autophagy (ALR: autophagic lysosome reformation). The cause of this loss of fidelity is likely to be due to a dysregulation of clathrin-coat formation.

This manuscript complements the recent study of Rong et al in NCB who demonstrate PI4P is on the lysosome, and that generation of PI4,5P2 is required for ARL.

The data in the manuscript uses both live imaging and fixed cells, along with extensive immunolocalization, biochemical analysis including subcellular fractionation to support the model to explain the phenotype observed after loss of PI4KIIIβ. They demonstrate a unique subpopulation of PI4KIIIβ is present on lysosomes which distinguishes it from the Golgi-localized pool, and may explain the lysosome-specific activity. Loss of this subpopulation (as well as presumably the rest of the population) causes hypertubulation but this is nicely separated from the well characterized role of PI4KIIIβ at the Golgi. Using a temperature block they further show a selectivity of cargo sorting controlled by PI4KIIIβ at the Golgi (VSV G) versus LAMP1 at the lysosome.

The manuscript would be improved by focusing on the key points, and reducing the text which is at times repetitive.

We have shortened the manuscript and eliminated repetitive paragraphs and statements. After these changes the text was reduced to 53,926 characters without space. Note that the current final count reflects the next text added to describe and discuss the results of the new studies requested to further clarify the interplay between lysosomal *PI4KIIIβ* and PIP5Ks.

In addition, some points which are not directly relevant could be removed for example in the Introduction the paradigm of PI3P could be eliminated, references to MHC as they have removed the data relating to this, and the discussion could be more focused as some ideas have been introduced for example the role of PI4P in Golgi.

We have eliminated the points considered unnecessary by this reviewer as stated above.

Minor points:

1. The first 4 sentences of the Introduction are not very clear, and in particular the first is confusing.

We have re-written the first part of the introduction and reorganized to make it more concise.

2. Page 4 second paragraph, the text develops an agreement about the lack of knowledge of the role of PI4P which is not necessary and very artificial.

We have eliminated part of that paragraph.

3. Page 10, it is confusing to read (Cathespin D(pepstatinA)) please clarify earlier that pepstatin A binds and inhibits Cathespin D if this is the case.

We have clarified now the use of fluorescent pepstatin.

4. The model is informative but it is not clear what the purple clathrin adaptor represents (the blue clathrin adaptor corresponds to AP2).

We have modified the model (now in Fig. 8) to improve the labeling and changed all adaptor molecules to the same color and to incorporate the possible contribution of alterations to fission.

Referee #2

It is very interesting as it reports for the first time the involvement of PI4KIIIbeta in lysosome function. In fact I see very little overlap with the published report. Indeed, I would suggest to focus more on the lysosomal effect of PI4KIII beta depletion than on autophagy-induced lysosomal reformation, as this would elminate any possible overlap with the NCB paper and also because it would be easier to interpret, because possibly the two lysosomal phenotypes (at the steady state and upon autophagy induction) are not necessarily connected.

We have introduced now changes in the text in the Results and in the Discussion sections to emphasize, as pointed by this advisor, that basal and inducible processes of lysosomal efflux and the role of PI4KIIIβ in each of them could be different. Following editorial recommendations, we have kept the studies on lysosomal regeneration induced by starvation and have expanded them to directly address the functional interaction between lysosomal *PI4KIIIβ* and the recently described lysosomal PIP5Ks, PIP5K1A and PIP5K1B. Our new studies reveal that *PI4KIIIβ* contributes to the function of those kinasesion modulation of vesicular budding (PIP5K1B) and vesicle scission at the tip of the tubule (PIP5K1A) by providing the PI(4)P needed for the generation of $PI(4,5)P_2$ by these enzymes. But in addition, we demonstrate that *PI4KIIIβ* is required for proper sorting of lysosomal materials during efflux and that this function is independent of PIP5Ks. These results are now shown in Figure 8, Supplementary Figure 8 and Movie 8, and are described in pages 16-18 and discussed in pages 20-22.

It is puzzling, especially for the hypothesized inhibtory effect of PI4P on tubule formation and for the hypothesis of a random and increased assembly of coat in the absence of PI4P. A more reasonable model that would fit the data could envisage a role for PI4KIIIb in membrane fission: this would explain the longer tubules (the increase in the length rather than in the number of tubules is more impressive), the larger and reduced number "donor" compartment in PI4KIIIbeta KD. The lack of fission would associate with the missorting caused by PI4KIIIb KD and this would induce to hypothesize that PI4KIIIb couples sorting and fission and that in the absence of PI4KIIIb both processes are impaired. It remains to be established whether the derangement of the two processes are connected or just coincident and both independently caused by the lack of PI4KIIIb activity. You might want to suggest to introduce these considerations.

We are in complete agreement with this alternative mechanism proposed by the reviewer and in fact, our initial comments about the problems with the recruitment of the scission machinery, although less clearly than this advisor comments, intended to explain a problem in the fission of the vesicles. We did not emphasize more this point in the earlier version of this manuscript because our proteomic analysis did not reveal the presence of well known scission proteins. Since instead, we found marked changes in motors and clathrin, we favored that transition from vesicle to tubule occurred because of the abnormal assembly and stretching of tubules. However, as pointed by this advisor, it is reasonable to propose that yet-to-identify scission proteins fail to be recruited to the normal sites of vesicle formation and that these vesicles become tubules instead. We have now given more relevance to this explanation in the discussion and incorporate this option in the revised model (Fig. 9) in the form of “scission” molecules.

Overall the mechanistic insight both upstream and downstream PI4KIIIbeta, are very limited, but still the novelty of the findings would be enough to justify their publication.

Referee #3

I had a careful look at the manuscript and I believe that it is acceptable as it is. The inhibitory role of PI4P for lysosomal tubulation is interesting, somewhat against the simple model, whereby PI4P is a precursor for PI(4,5)P2 synthesis, which in turn mediates clathrin-mediating budding through PIP2-binding adaptors. That scenario would be more consistent with the model proposed by Rong et al in Nature Cell Biology, although they did not really address the role of PI4P synthesis in that paper.

We appreciate very much the thoughtful reasoning of this advisor regarding the interplay between the two kinases. Following editorial recommendations we have now performed studies in double knock-down cells to better elucidate the functional interaction between lysosomal *PI4KIIIβ* and the recently described lysosomal PIP5Ks, PIP5K1A and PIP5K1B. Our new studies reveal that *PI4KIIIβ* contributes to the function of those kinases on modulation of vesicular budding (PIP5K1B) and vesicles scission at the tip of the tubule (PI5KA) by providing the PI(4)P needed for the generation of $PI(4,5)P_2$ by these enzymes. But in addition, we demonstrate that *PI4KIIIβ* is required for proper sorting of lysosomal materials during efflux and that this function is independent of PIP5Ks. These results are now shown in Figure 8, Supplementary Figure 8 and Movie 8, and are described in pages 16-18 and discussed in pages 20-22.

The concerns raised by the referees on the fractionation experiments have been well addressed. Obviously, I would love to know the consequence of the PI4K knockdown on PI4P and PI(4,5)P2 levels in lysosomes, but these experiments are not trivial, as only a handful of labs can do that and the amount of material may be too low for such analyses. Therefore, I don't feel it is right to reject this manuscript based on these missing experiments, which may not be feasible. They could also use the fluorescent PI4P probes, but I am not sure that they would necessarily recognize the lysosomal pools of PI4P, considering they tend to bind proteins as well and these proteins may not be present on lysosomes.

We thank this advisor for the realistic evaluation of the potential for further experiments. As pointed out by the advisor, measurement of those lipid species in lysosomes is not trivial. We have tried the fluorescent PI(4)P probes and show some studies in isolated lysosomes (Fig. S6F) but the binding is weak (probably because of the reasons anticipated by this advisor), and once in the intact cell the results are of difficult interpretation.

The paper is very strong as it is, and I think that it would be a great match for EMBO. So my personal verdict is : ACCEPT.

Summary of the changes incorporated in response to comments from prior peer-review

- Inclusion of results with a second knock-down for PI4KIIIβ of lower efficiency (Fig. S1E, F and G)
- Provide future evidence of role of PIK4IIIβ in cargo sorting by studying the effect of the knock-down of the kinase in starvation-induced lysosomal regeneration (Fig.7 and movie S7)
- Added experiments to support the independence of the Golgi and lysosomal functions of PIK4IIIβ (Fig. S5A, S5B) we have included now a more complete analysis of kinetics of trafficking for LAMPs from Golgi to lysosomes and of the secretory protein VSG (in addition of the in bulk secretion already included in the original version)
- Include a better characterization of the lysosomal compartment analyzed in this study (Fig. 5A).
- Replace movies and panels that were not considered of sufficient quality or it was recommended to present in a different manner (new figures 2F, 5A, B, 7 A, B,C, E and Movies S2, S3)
- Reorganized some parts of the manuscript in which reviewers' recommended to place more emphasis on the biochemical characterization
- Re-written the last part of this work, where we analyze the role of PIK4IIIβ on starvation-induced lysosomal regeneration, to place our findings in the context of the recent publication by Yu's group (as the data nicely complement each other and one of the reviewers asked for this explanation).
- Clarified the novel aspect of our study that include:
    - Identification of PIK4IIIβ in lysosomes
    - Demonstration that Lys- PIK4IIIβ is required to preserve lysosomal homeostasis (stable composition of lysosomal constituents under basal conditions)
    - Identification of the mechanism by which PIK4IIIβ controls basal efflux of lysosomal material 1) at the level of vesicle formation (size/shape) and 2) at the level of cargo sorting/retention (we show loss of lysosomal components and demonstrate presence of LAMPs and PI4KIIIβ in the same exiting vesicles and direct interaction between both proteins and adaptor molecules)
    - Identification of the need for PI4KIIIβ not only for basal lysosomal efflux but also under conditions requiring augmented lysosomal recycling such as the pathway of starvation-induced lysosomal regeneration. We demonstrate that Lys-PI4KIIIβ is required for proper sorting under those conditions.
    - Demonstration that Lys-PIK4IIIβ exerts its role in lysosomal efflux in part in a coordinate manner with the novel Lys-PIP5Ks recently described by providing the substrate for these kinases, but it also acts independent of these kinases in its contribution to cargo sorting.