

A three-organelle complex made by wrappER contacts with peroxisome and mitochondria responds to liver lipid flux changes

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Original submission

First decision letter

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MS TITLE: A three-organelle complex formed by wrappER contacts with peroxisome and mitochondria responds to changes in hepatic lipid flux

AUTHORS: Nicolo Ilacqua, Irene Anastasia, Andrea Raimondi, Philippe Lemieux, Thomas Q de Aguiar Vallim, Katalin Toth, Eugene V Koonin, and Luca Pellegrini

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 gave favourable reports and indicate the importance of your study. The reviewers raised some points that will require amendments to your manuscript. I hope that you will be able to carry these out because I would like to be able to accept your paper, following its revision and submission of a rebuttal addressing the reviewers comments.

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

This original paper makes use of the state-of-the-art protocols to describe the formation of a novel complex between ER, mitochondria and peroxisomes according to cell metabolism. It does offer an advanced understanding of the interplay between intracellular anatomy and FA accumulation/elimination.

Comments for the author

This is a beautifully crafted manuscript which mirrors unique technical competence. Data are compelling and obtained by using the most advanced methodologies to assess ultrastructural homeostasis of mammalian cells. Data provided are fully supported both qualitatively and quantitatively. Publication of the work will impact the community by advancing the current understanding on the role played by contacts in the redesign of cellular physiopathology. There are nonetheless minor outstanding points -listed below- on which attention by the authors should be called. Addressing those will further complete an already outstanding manuscript in which structure is mechanistically linked with metabolism.

- 1) How linear is the correlation between lipid flux and the complex formation? In other words which other factors could come into play, and which is the threshold point? This should be further discussed and contextualised in the available literature.
- 2) It would be informative exploring whether this three-organelle complex formed by wrappER affects the degree of the recently discovered NAM (Nucleus Associated Mitochondria): a quick re-assessment of the available data should quickly provide the answer. NAM results in modifications of cellular genetic and therefore likely involved in the hierarchy here presented between lipid metabolism and organelles remodelling.

Reviewer 2

Advance summary and potential significance to field

A recent study from the author's group employed serial section electron tomography (SSET) and 3D reconstruction analyses to study organelle architecture in mouse liver (PMID: 33730569). This study revealed the presence of mitochondria wrapped by rough ER (wrappER) and demonstrated a role in the dynamic regulation of lipid homeostasis.

The current manuscript builds upon this discovery and approach. Employing similar advanced imaging methods, the authors describe a three-way organelle complex formed by wrappER that organizes ER, peroxisomes, and mitochondria. Interestingly, this tripartite organelle complex is influenced by the metabolic state - increasing in response to fasting-feeding transitions and in response to disruptions in VLDL synthesis. Proteomics suggests that there may be an increase in fatty acid oxidation, but this conclusion is more speculative.

Overall, this manuscript is rigorous and makes an important discovery that will be of broad interest to the cell biology and metabolism communities.

Comments for the author

Comment 1: It is stated that loss of MTP results in lipid droplet accumulation but no evidence is included to support this statement. Images would be useful.

In addition, it would be useful to comment on the spatial distribution of lipid droplets and their proximity to these PEWM complexes. Are they nearby?

Presumably since these complexes are proposed to be sites of lipid oxidation lipid droplets would need to transfer fatty acids to these organelles during periods of lipolysis (or lipophagy)?

Comment 2: The conclusion that the formation of PEWM complexes is associated with β -oxidation activation either needs to be supported by functional data or needs to be toned down to be more speculative. Currently, no functional data related to fatty acid oxidation or flux is included. The proteomic increase in factors associated with β -oxidation is interesting and suggestive, but does not necessarily indicate a functional change or output.

Comment 3: What is the evidence that these cells are hepatocytes and not some other type of liver cell (stellate etc.)?

Minor comment 1: “wrapper” should be “wrappER” (line 160 page 8).

First revision

Author response to reviewers' comments

Reviewer 1

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This original paper makes use of the state-of-the-art protocols to describe the formation of a novel complex between ER, mitochondria and peroxisomes according to cell metabolism. It does offer an advanced understanding of the interplay between intracellular anatomy and FA accumulation/elimination.

This is a beautifully crafted manuscript which mirrors unique technical competence. Data are compelling and obtained by using the most advanced methodologies to assess ultrastructural homeostasis of mammalian cells. Data provided are fully supported both qualitatively and quantitatively. Publication of the work will impact the community by advancing the current understanding on the role played by contacts in the redesign of cellular physiopathology. There are nonetheless minor outstanding points -listed below- on which attention by the authors should be called. Addressing those will further complete an already outstanding manuscript in which structure is mechanistically linked with metabolism.

We thank the Reviewer for sharing their time and expertise with us. We are grateful for the positive comments on the novelty and quality of our study.

1) How linear is the correlation between lipid flux and the complex formation? In other words which other factors could come into play, and which is the threshold point? This should be further discussed and contextualised in the available literature.

We agree that this is an important point to discuss and thank the Reviewer for suggesting it. In the revised manuscript we added the following paragraph at the end of the Discussion.

“In this study, we showed that the number of PEWM complex in the liver is highest when the hepatic cell switch from pyruvate to fatty acid respiration. Thus, other factors could come into play in regulating the assembly and disassembly of this cellular structure, such as changes in insulin levels, the amount of dietary cholesterol, a ketogenic diet, and, in general, any other factors known to regulate liver energy metabolism (Puchalska and Crawford, 2017; Yki-Järvinen et al., 2021). We anticipate, therefore, that studies addressing the role of PEWM complex dynamics will help understand the etiology of liver metabolic syndromes and diseases”.

2) It would be informative exploring whether this three-organelle complex formed by wrappER affects the degree of the recently discovered NAM (Nucleus Associated Mitochondria): a quick re-assessment of the available data should quickly provide the answer. NAM results in modifications of cellular genetic and therefore likely involved in the hierarchy here presented between lipid

metabolism and organelles remodelling.

We agree that this would be a very interesting question to investigate. However, we would like to point out that, contrary to what the Reviewer wrote, this is not a quick study to do. In fact, it would require *ad hoc* electron tomography and electron microscopy data; the ones we have are not suitable to address such a focused question that, therefore, needs to be addressed in a stand-alone study.

Reviewer 2

Advance Summary and Potential Significance to Field:

A recent study from the author's group employed serial section electron tomography (SSET) and 3D reconstruction analyses to study organelle architecture in mouse liver (PMID: 33730569). This study revealed the presence of mitochondria wapped by rough ER (wrappER) and demonstrated a role in the dynamic regulation of lipid homeostasis.

The current manuscript builds upon this discovery and approach. Employing similar advanced imaging methods, the authors describe a three-way organelle complex formed by wrappER that organizes ER, peroxisomes, and mitochondria. Interestingly, this tripartite organelle complex is influenced by the metabolic state - increasing in response to fasting-feeding transitions and in response to disruptions in VLDL synthesis. Proteomics suggests that there may be an increase in fatty acid oxidation, but this conclusion is more speculative.

Overall, this manuscript is rigorous and makes an important discovery that will be of broad interest to the cell biology and metabolism communities.

We thank the Reviewer for sharing their time and expertise with us. We are grateful for the positive comments on the novelty and quality of our study.

Comments for the Author:

Comment 1: It is stated that loss of MTP results in lipid droplet accumulation, but no evidence is included to support this statement. Images would be useful.

This might be a misunderstanding. We have published the data regarding the accumulation of LD in the *Mttp*^{-/-} liver in our *Cell Reports* paper (Fig. 7F; Anastasia et al., 2021) and cited it when we discussed this point. Noteworthy, our data confirms those originally published by Stephen Young (Fig. 6A-B of Raabe et al., JCI, 1999); therefore, in this revised manuscript we have added this reference too. The revised manuscript now reads:

(line 149) "... and LD accumulation is quite pronounced due to the interruption of FA flux in the form of VLDL (Anastasia et al., 2021; Raabe et al., 1999)."

In addition, it would be useful to comment on the spatial distribution of lipid droplets and their proximity to these PEWM complexes. Are they nearby? Presumably since these complexes are proposed to be sites of lipid oxidation, lipid droplets would need to transfer fatty acids to these organelles during periods of lipolysis (or lipophagy)?

PEWM complexes do not necessarily appear to reside near LD. This is evident in Fig. 1C-D, which show a large portion of the hepatocyte lacking LDs but populated by PEWM complexes. In addition, Fig. 3C shows that, in the postprandial liver, half of the peroxisomal population is engaged to form PEWM complexes; in this metabolic state, however, the liver is nearly devoid of LD (Fig. 1A-B of Li et al., 2018); Fig. 2C of (Inagaki et al., 2007)). In general, we find that this is an interesting point that deserves to be raised and discussed in a commentary article on our study, or in a review, but that it is too speculative to be addressed here.

Comment 2: The conclusion that the formation of PEWM complexes is associated with β -oxidation activation either needs to be supported by functional data or needs to be toned down to be more

speculative. Currently, no functional data related to fatty acid oxidation or flux is included. The proteomic increase in factors associated with β -oxidation is interesting and suggestive but does not necessarily indicate a functional change or output.

We respectfully disagree.

In our study we showed that the number of PEWM complexes increase during fasting and in *Mttp*^{-/-} livers. It is universally accepted that during fasting liver fatty acid respiration is highest (Heimberg et al., 1962; Mayes and Felts, 1967). This is established by measuring the level of circulating ketone bodies; an increase in ketone bodies in the plasma indicates a switch from pyruvate to fatty acid respiration in the liver (Puchalska and Crawford, 2017). In our study, we have not measured plasma ketone bodies in fasted mice because it would have been futile to prove something so widely unanimously accepted. Instead, we focused on collecting molecular evidence of liver fatty acid respiration. We did this by performing sensitive and accurate quantitative proteomic analysis aimed at measuring and comparing the expression of key positive regulators of mitochondrial and peroxisomal β -oxidation (ACSS3, ACOT2, ACOT3, ACOT 4 and EHHADH) and of a negative regulator of pyruvate respiration, PDK4 (Grassian et al., 2011).

However, we take the comment of the Reviewer very seriously. Therefore, in this revised manuscript we have added Fig. 4D which shows upregulation of HMGCS2, the enzyme of the mitochondrial matrix that drives ketogenesis (d'Avignon et al., 2018; Puchalska and Crawford, 2017). More specifically, for this figure we analysed by estimation statistic (Bernard, 2019; Ho et al., 2019) our proteomic data on PDK4 and HMGCS2. This analysis shows their upregulation in *Mttp*^{-/-} livers, a condition that, like in fasting, is known to upregulate liver fatty acid respiration by increasing circulating ketone bodies (Fig. 6F of (Newberry et al., 2017)).

Based on the evidence in the literature and in our data, we did not to change the text of the manuscript. However, we will do so at the request of the Editor.

Comment 3: What is the evidence that these cells are hepatocytes and not some other type of liver cell (stellate etc.)?

In general, the anatomical structure of the liver is relatively simple. The hepatic tissue is composed of hepatocytes (80%), stellate cells, and Kupfer cells. Each of these cell types has a very distinct morphology and unique position within the hepatic acinus. However, we welcome the constructive comment of the Reviewer, which we addressed by adding the following paragraph in the Materials and Methods:

“For this analysis, hepatocytes were identified as cells: i) with a polarized cellular architecture, ii) polygonal in shape, iii) with their sides in contact either with sinusoids (sinusoidal face) or neighboring hepatocytes (lateral faces); iv) with microvilli abundantly present on the sinusoidal face and projecting sparsely into bile canaliculi, and v) containing glycogen granules as chrysanthemum-like clusters of electron-dense particles (Burdon and van Knippenberg, 1991; Hooser et al., 1990; Sorenson and Brelje, 2014)”

Minor comment 1: “wrapper” should be “wrappER” (line 160 page 8).

Done

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Second decision letter

MS ID#: JOCES/2021/259091

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ARTICLE TYPE: Research Article

I have read the reviews and your responses, and I am happy to tell you that your manuscript has been accepted for publication in the Journal of Cell Science, pending standard ethics checks. Thank you for submitting this interesting research to the Journal of Cell Science!