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Plasma Membrane - Endoplasmic Reticulum Contact Sites Regulate Phosphatidylcholine Synthesis

Shabnam Tavassoli, Jesse T. Chao, Barry P. Young, Ruud C. Cox, William A. Prinz, Anton I.P.M. de Kroon and Christopher J.R. Loewen

Corresponding author: Christopher J.R. Loewen, University of British Columbia

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(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 24 September 2012

Thank you very much for the submission of your research manuscript to our editorial office. We have now received the full set of reports from the referees that were asked to assess it. As the detailed reports are pasted below I will only repeat the main points here. As you will see, while the referees appreciate the potential interest of the study, they also feel that as it stands, the claims put forward are not yet fully substantiated by the data at hand.

For example, referee 1 suggests testing whether Osh2/3 can rescue Opi3 localization in scs2/ice2 mutant cells and in this regard referee 3 also sees the need for further clarification: s/he states that since Osh2/3 have been shown to depend on Scs2 for recruitment to membrane contact sites, how can they rescue the growth defects seen in scs2/ice2 mutant cells? This reviewer also feels that it should be tested whether Opi3 can rescue PC synthesis in scs2/ice2 mutant cells. Referee 1 is not yet fully convinced that Opi3 acts in trans and states that this should at least be discussed. S/he also makes a comment with regard to statistical analysis and in this regard I would like to point out that all experiments should be repeated at least three times independently (biological replicates). Finally, both referees 2 and 3 feel that the part on the role of Pah1 in establishing PM-ER contact site formation would require additional support.

From the analysis of these comments it becomes clear that significant revision is required before the manuscript may become suitable for publication in EMBO reports. However, given the potential

interest of your study, I would like to give you the opportunity to revise your manuscript, with the understanding that the main concerns of the referees must be addressed and their suggestions taken on board. Acceptance of the manuscript will depend on a positive outcome of a second round of review and I should also remind you that it is EMBO reports policy to allow a single round of revision only and that, therefore, acceptance or rejection of the manuscript will depend on the completeness of your responses included in the next, final version of the manuscript.

Revised manuscripts should be submitted within three months of a request for revision; they will otherwise be treated as new submissions. If you feel that this period is insufficient for a successful submission of your revised manuscript I can potentially extend this period slightly. Also, the length of the revised manuscript should not exceed roughly 28,000 characters (including spaces).

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We also welcome the submission of cover suggestions or motifs that might be used by our Graphics Illustrator in designing a cover.

I look forward to seeing a revised form of your manuscript when it is ready.

REFEREE REPORTS:

Referee #1:

The data presented in this manuscript provide compelling evidence that one role for contacts sites between the plasma membrane and endoplasmic reticulum is the regulation of phosphatidycholine synthesis. The figures are very nicely presented (if the micrographs are a little small) and the data are largely convincing. The paper stems from the finding that delta-Scs2-ice2 mutant cells show a choline-based growth defect. Addition of choline or overexpression of OPI3 rescues this defect. Intriguingly, localization of OPI3 to plasma membrane associated ER (pmaER) was absent in the mutant consistent with a requirement for pmaER localization in OPI3 function.

Further to this the authors identify a non-catalytic role for PAH1 in the process and support this with EM data suggesting that it is required for pmaER site formation and/or maintenance. This ultra-structural characterization provides strong support for the authors' model.

Osh2 and Osh3 have previously been show to localize to pmaER contact sites in an Scs2-dependent manner. Osh2 partially and Osh3 completely restored growth to delta-scs2-ice2 mutants consistent with the authors model. It would have been nice to see the localization of OPI1 in these cells as one would expect localization to pmaER to be restored. If possible this should be shown (perhaps using Opi3-GFP as in figure 1) to support the model proposed.

Finally the authors use an in vitro assay that supports the idea that Opi3 acts in trans. This is the weakest component of the manuscript. How can the authors rule out lipid transfer between the liposomes and microsomes during the 2 hour incubation. Could this explain the distribution of lipids without Opi3 acting in trans? While I agree that both published and new data support the notion of acting in trans, this possibility should be discussed. It appears that the assays have not be repeated; error bars from at least 3 independent experiments should be shown. The use of two colours to define four experimental conditions in Figures 4D/E also leads to some confusion; this could easily be rectified by use of four colours or different shades of gray.

Referee #2:

This manuscript describes the importance of plasma membrane-ER contact sites (PM-ER) in the methylation pathway of PC synthesis. The authors find a genetic interaction of the SCS2 and ICE2 double deleted strains with several genes controlling PC synthesis. SCS2 and ICE2 are ER proteins that localize proteins to the ER and also determine ER-PM contacts and the authors found that the overexpression of the OPI3 gene can alleviate the choline auxotrophy of the delta-scs2,delta-ice2 strains.. With a series of deletion and complementation experiments the authors showed that OPI3, a methyl transferase enzyme that functions on the pathway of PE to PC conversion, is found in PM-ER contacts where its localization depends on SCS2 and ICE2. They also showed that phosphatidic acid phosphohydrolize, PAH1 has a non-catalytic role in regulating the number of PM-ER contact sites and that the OSH3 protein is also able to correct the choline auxotrophy of the delta-scs2,delta-ice2 strains. Finally the authors demonstrate that OPI3 can methylate monomethylated PE to PC in trans. The authors conclude that PM-ER contact sites are important for the PE to PC pathway controlling OPI3 enzyme function.

This is a thorough study with interesting and convincing new data on the spatial organization of the PE methylation pathway. There are only a few issues that need clarification.

1. The authors showed the dependence of Opi3 PM-ER localization on Scs2/Ice2 in the double-deleted strains. They also showed a non-catalytic role of Pah1 in establishing more PM-ER contacts. What is missing is the effect of Pah1 (wt and dead) on Opi3 distribution. Does Pah1 overexpression also correct the Opi3 distribution defect?

2. Does OSH3 also have an effect on Opi3 distribution or its effect is only to help the enzyme to access its substrate as speculated in the Discussion. Does the presence of OSH3 affect the activity of Opi3 in the liposome assay shown in Fig. 4CD?

3. The authors noted from the literature that the PE-PME precursors are present in very low level in the PM. They explain this as an indication of the rapid conversion of these precursors to PC in the membrane. However, if that is so, one would expect that Opi3 deleted (or the Scs2/Ice2 double-deleted) strains would accumulate PME in the PM. Is there any indication for this in the authors' experiments or in the literature?

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This is a potentially interesting paper that presents data to suggest that Opi3, a phosphatidylethanolamine (PE) methyltransferase in the endoplasmic reticulum (ER), acts on PE or partially methylated PE in the plasma membrane (PM) to generate PC. A subtext to this conclusion is that Opi3 acts in trans, at ER-PM contact sites. The authors arrive at their conclusions by a very convoluted path, building uncritically on a variety of data.

The authors begin by linking a morphological defect in cortical ER (caused by simultaneously eliminating Scs2 and Ice2 function) to phosphatidylcholine biosynthesis and a growth defect evident in cells cultured in SD media. This can be rescued by providing choline (but not ethanolamine) in the medium, or by over-expressing Opi3 (Fig. 1C and 1D). In contrast with the rate of incorporation of [3H]ethanolamine into PC in wt cells, PC synthesis via the methylation route is slow in the scs2ice2 double mutant (Fig. 1G). It is also unnecessary to characterize the rate of PC synthesis in wt cells as 'rapid' (page 5, three lines from the bottom) - rapid, compared with what? The authors should show the chromatograms associated with Fig. 1G, and provide data to establish that the rate of PC synthesis can be improved by over-expressing Opi3 in the scs2ice2 double mutant.

What are the relative contributions of the Kennedy pathway and the methylation pathway to PC synthesis in wt cells grown on SD and YPD media? Perhaps the methylation pathway provides a particular PC molecular species?

Fig. 1-H, I is a bit odd. The scs2ice2 double mutant has scarce pmaER so it is no surprise that Opi3 is not localized to this almost 'non-existent' compartment in the mutant cells. What is the level of Opi3-GFP expression in Fig. 1-I? Is the growth defect of the double mutant grown on SD without choline rescued by Opi3-GFP under these conditions? If so, is the rescue occurring from Opi3-GFP located at the nuclear ER?

The Pah1 story (highlighted in the middle-section of the paper) is interesting but irrelevant to the bottom line. The authors spend a fair amount of time and space on this but form no mechanistic conclusions. To summarize simply: a catalytically dead Pah1 protein rescues the pmaER deficiency in scs2ice2 cells (Fig. 3), and simultaneously restores the ability of the cells to synthesize PC from PE (Fig. 2D). The authors should include data for Pah1-D398E in the plot shown in Fig. 2D. The coincidence between restoration of pmaER and PE->PC conversion is clear, but this adds more unknowns to the mix as the function of Ice2 is not known and the presumably structural role of Pah1 is not known.

The authors then move on to the role of Osh2 and Osh3 in forming/controlling ER-PM contacts through their interaction with Scs2 and presumably phosphoinositides. They could have gone straight to this section, without the Pah1 interlude. They show that over-expressing Osh2 or Osh3 rescues the growth phenotype of the scs2ice2 double mutant even in the absence of added choline. How does this work? Is Scs2 not an essential ingredient of the Osh2/3 bridging function? Related to this, do Osh2 or Osh3 proteins lacking PH domains or FFAT motifs behave the same way as wt Osh in this system? Does the expression of Osh2/3 also improve the ability of the cells to synthesize PC from [3H]ethanolamine (the in vivo Opi3 activity assay)? The authors build on data from the Emr lab concerning the activation of Sac1 by Osh proteins. Do the Osh proteins activate Opi3? Is their sterol-binding function necessary for these effects?

1st Revision - authors' response 08 February 2013

Changes to the manuscript:

Fig. 1. For the in vivo PE methylation assays, we have plotted PC synthesis as rates determined from the initial linear portion of the reactions, which are given in the supplementary figures. We have included new data on rescue of PC synthesis in scs2ice2 by Opi3. We have added new data on DTT sensitivity of scs2ice2.

Fig. S1. Supports Fig. 1. Now includes new data on PSD1-ICE2 genetic interaction.

Fig. 2. Now contains the microscopy data supporting a specific defect in pmaER in scs2ice2, suppression of choline auxotrophy by Osh2&3, and new data on rescue of PC synthesis by Osh3.

Fig. S2. Supports Fig. 1. Now includes new data on pmaER structure in scs2ice2 upon overexpression of Opi3 and Osh3.

Fig. 3. Now contains rescue by Pah1 data and ultrastructural assay.

Fig. S3. Supports Fig. 3. Contains PC synthesis plots. Contains new data on rescue of DTT sensitivity by Pah1 and rescue of Opi3-GFP localization to pmaER by Pah1.

Fig. S4. Supports Fig. 3. Now contains larger representative EM images corresponding to plots in Fig. 3.

Fig. 4. Contains new data using NP-40 detergent sensitivity assay that demonstrates PM instability in scs2ice2. Contains new data for in vitro trans methylation assay, replicated at least three times.

Fig. S5. Supports Fig. 4. Contains new data for in vitro trans methylation assay. Contains new data for lipid transport assays using scs2ice2 that suggest no defects in transport, supporting in trans methylation defects in the mutant.

Responses to Reviewers:

Referee #1:

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Osh2 and Osh3 have previously been show to localize to pmaER contact sites in an Scs2-dependent manner. Osh2 partially and Osh3 completely restored growth to deltascs2-ice2 mutants consistent with the authors model. It would have been nice to see the localization of OPI1 in these cells as one would expect localization to pmaER to be restored. If possible this should be shown (perhaps using Opi3-GFP as in figure 1) to support the model proposed.

We have now added data that demonstrates that Pah1 restores localization of Opi3 to pmaER Δscs2Δice2 mutant (Fig. S3), consistent with Pah1's rescue of pmaER structure in the mutant. We have also added data showing that overexpression of Osh3 does not restore pmaER in the mutant (Fig. S2), indicating Osh3 rescues Opi3 function, not by restoring contacts, but by activating the enzyme in some manner, perhaps through increasing access of Opi3 to lipid substrate, as has been proposed for Osh3 activation of Sac1 [3]. This data supports that contacts are required for Opi3 function and that Osh3 likely does not play a structural role at contacts. Overexpression of Opi3 in the mutant also does not restore contacts (Fig. S2), consistent with contacts being required for Opi3 function and not vice-versa, that defects in PC synthesis in the methylation pathway cause defects in contacts. It is important to note that our ultrastructural characterization of contacts as well as using Tcb3-GFP as a marker for contacts were done using yeast grown in the presence of choline, which rescues the PC synthesis defect, but clearly does not rescue contacts.

Finally the authors use an in vitro assay that supports the idea that Opi3 acts in trans. This is the weakest component of the manuscript. How can the authors rule out lipid transfer between the liposomes and microsomes during the 2 hour incubation. Could this explain the distribution of lipids without Opi3 acting in trans? While I agree that both published and new data support the notion of acting in trans, this possibility should be discussed. It appears that the assays have not be repeated; error bars from at least 3 independent experiments should be shown. The use of two colours to define four experimental conditions in Figures 4D/E also leads to some confusion; this could easily be rectified by use of four colours or different shades of gray.

These experiments have been reproduced with multiple replicates (Fig. 4). If lipid transfer accounted for the PC synthesized in the liposomal fraction, PME would need to spontaneously transfer from liposomes to microsomes, therein be converted to PC by Opi3, and transfer back to liposomes. Given the incredibly slow rates of spontaneous transfer for these lipids it is unlikely that lipid transfer within the short 2 hr time frame of the assay would account for the lipid synthesis observed in the assay (conversion of

~35% of the PME), and soluble lipid transport proteins would not be expected to be present in the reaction mix since they should be lost during microsome purification and washing.

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1. The authors showed the dependence of Opi3 PM-ER localization on Scs2/Ice2 in the double-deleted strains. They also showed a non-catalytic role of Pah1 in establishing more PM-ER contacts. What is missing is the effect of Pah1 (wt and dead) on Opi3 distribution. Does Pah1 overexpression also correct the Opi3 distribution defect?

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We have added data that shows that overexpression of Osh3 and Opi3 in the Δscs2Δice2 mutant does not rescue pmaER structure (Fig. S2) and measured methylation in the presence of Osh3 (Fig. 2).

3. The authors noted from the literature that the PE-PME precursors are present in very low level in the PM. They explain this as an indication of the rapid conversion of these precursors to PC in the membrane. However, if that is so, one would expect that Opi3 deleted (or the Scs2/Ice2 double-deleted) strains would accumulate PME in the PM. Is there any indication for this in the authors' experiments or in the literature?

We have now added a detergent sensitivity assay that suggests that PME accumulates in the plasma membrane of the Δscs2Δice2 mutant (Fig. 4). Although PME is know to accumulate in Δopi3 cells [4], it has not been directly measured in the PM. These experiments pose significant challenges because of the difficulty in isolating highly pure PM that is devoid of ER, thus making it near-impossible to conclude that the PME in such a prep originated in the PM. It is also likely that any highly pure PM fraction that is devoid of ER would not include regions of PM in physical contact with ER, in which the PME would likely reside. These issues we believe make a biochemical approach to investigating the location of PME in the mutants largely untenable. What is needed for the future are fluorescent molecular probes that can be used in living cells to study PE/PME/PDE by high resolution microscopy, similar to what exists for the study of phosphoinositides.

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We have now added data that demonstrates that Opi3 overexpression restores PC synthesis in the Δscs2Δice2 mutant (Fig. 1). With respect to the HPLC lipid analysis, we have not included chromatograms because, as is described in the methods, we run purified nonradiolabelled PE and PC standards (Avanti) along with the labelled yeast lipid extracts to identify the elution times for the labelled lipids in the samples, and we count these peaks by scintillation spectrometry. Therefore the HPLC chromatograms themselves are not informative of the composition of the yeast lipid samples.

What are the relative contributions of the Kennedy pathway and the methylation pathway to PC synthesis in wt cells grown on SD and YPD media? Perhaps the methylation pathway provides a particular PC molecular species?

In the presence of choline, the Kennedy pathway is sufficient to provide the cell's requirement of PC for growth and is the reason Δcho2 and Δopi3 cells are completely rescued on media containing choline (e.g. YPD or SD + 1 mM cho). In the absence of choline, the Kennedy pathway is important for PC remodeling, in which PC synthesized de novo by the methylation pathway is deacylated, liberating GroPC and then choline, which can then be combined with DAG in the Kennedy pathway to produce PC with new lipid acyl chains. It is unclear what the physiological importance of this is, but aggravating genetic interactions between Kennedy and methylation pathway genes in the absence of choline in the media (e.g. CKI1 and CHO2/OPI3, Fig. 1A), suggest an important function exists. However, we have no data to support that a unique PC species generated by Opi3 at contacts might be physiologically important, although it is an intriguing and plausible hypothesis.

Fig. 1-H, I is a bit odd. The scs2ice2 double mutant has scarce pmaER so it is no surprise that Opi3 is not localized to this almost 'non-existent' compartment in the mutant cells. What is the level of Opi3-GFP expression in Fig. 1-I? Is the growth defect of the double mutant grown on SD without choline rescued by Opi3-GFP under these conditions? If so, is the rescue occurring from Opi3-GFP located at the nuclear ER?

Quantification of Opi3-GFP in the ER by confocal microscopy in WT and the Δscs2Δice2 mutant was presented in the original version of the manuscript and can now be found in Fig. S2. There is no change in Opi3-GFP level in the mutant, suggesting Opi3 is not functional in the nuclear envelope. Therefore, we expect this to be true even when Opi3 is overexpressed from a plasmid. We have not looked for rescue by Opi3-GFP, although we show it is functional (Fig. S2) and therefore should likely rescue. Since we now have added data that Opi3 overexpression does not rescue pmaER in the Δscs2Δice2 mutant (Fig. S2), it is most likely the case that increasing the amount of Opi3

at existing PM-ER contacts rescues PC synthesis and cell growth in the mutant. However, we cannot rule out that under these nonphysiological circumstances, Opi3 functions in cis in the nuclear ER, except that for scs2ice2 cells without the Opi3 overexpression, this does not appear to be the case.

The Pah1 story (highlighted in the middle-section of the paper) is interesting but irrelevant to the bottom line. The authors spend a fair amount of time and space on this but form no mechanistic conclusions. To summarize simply: a catalytically dead Pah1 protein rescues the pmaER deficiency in scs2ice2 cells (Fig. 3), and simultaneously restores the ability of the cells to synthesize PC from PE (Fig. 2D). The authors should include data for Pah1-D398E in the plot shown in Fig. 2D. The coincidence between restoration of pmaER and PE->PC conversion is clear, but this adds more unknowns to the mix as the function of Ice2 is not known and the presumably structural role of Pah1 is not known.

We feel that rescue by Pah1 provides a critical piece of genetic data that strengthens the argument that PM-ER contacts regulate Opi3 and PC synthesis. This is because Pah1 rescues the structure of contacts, which we provide detailed ultrastructural analysis to support. We have provided additional data which shows that Opi3-GFP also returns to pmaER with Pah1 overexpression (Fig. S3), consistent with Opi3 acting at PM-ER contacts. With respect to the mechanism of rescue by Pah1, we provide new data that it does not rescue the Δcho2 choline auxotrophy (Fig. S3), indicating that Pah1 rescues by a mechanism separate from Scs2 and Osh3, consistent with Pah1, but not Scs2 or Osh3, rescuing contact structure. We show that Pah1 also restores PM stability in a new detergent sensitivity assay (Fig. 4), consistent with rescue of PM-ER contacts and reestablishment of PM lipid homeostasis. Pah1 also rescues the DTT sensitivity of the scs2ice2 mutant independent of UPR activation (Fig. S3) further supporting that Pah1 fixes the underlying defect that is the source of the ER stress in the mutant.

With respect to Ice2 function, we have added additional data that ICE2 interacts genetically with PSD1 (Fig. S1), showing that it clearly functions in the methylation pathway. It is not included in the manuscript, but Pah1 overexpression in the Δice2Δpsd1 mutant does not rescue its ethanolamine auxotrophy, demonstrating that PE is required for Pah1 rescue. This data provides additional support that the mechanism of rescue by Pah1 involves increased methylation of PE by Opi3. Pah1 associates with the ER and has know roles in regulating ER structure [5] suggesting that Pah1 could directly control PMER contacts.

The authors then move on to the role of Osh2 and Osh3 in forming/controlling ER-PM contacts through their interaction with Scs2 and presumably phosphoinositides. They could have gone straight to this section, without the Pah1 interlude. They show that over-expressing Osh2 or Osh3 rescues the growth phenotype of the scs2ice2 double mutant even in the absence of added choline. How does this work? Is Scs2 not an essential ingredient of the Osh2/3 bridging function? Related to this, do Osh2 or Osh3 proteins lacking PH domains or FFAT motifs behave the same way as wt Osh in this system? Does the expression of Osh2/3 also improve the ability of the cells to synthesize PC from [3H]ethanolamine (the in vivo Opi3 activity assay)? The authors build on data from the Emr lab concerning the activation of Sac1 by Osh proteins. Do the Osh proteins activate Opi3? Is their sterol-binding function necessary for these effects?

We have now added data that demonstrates that Osh3 overexpression restores PC synthesis in the Δscs2Δice2 mutant (Fig. 3). We rationalize that since Osh3 overexpression activates Opi3 in the Δscs2Δice2 mutant as well as the Δcho2 mutant, Osh3 likely modifies Opi3 function directly. This is further supported by our new data showing that Osh3 does not rescue pmaER in the Δscs2Δice2 mutant and therefore likely does not play a structural role (Fig. S2). Perhaps, the function of Scs2 is to enable Osh2/3 to more easily access Opi3 at contacts under physiological Osh2/3 and Opi3 expression levels. In the absence of Scs2, overexpression of Osh2/3 (non-physiological conditions) bypasses the requirement of Scs2 perhaps because at high Osh2/3 expression, their targeting to the PM through their PH domains is sufficient for

localization to existing contacts, hence activating Opi3. Consistent with this, we have found that some localization of Osh2/3 to the plasma membrane remains in Δscs2 cells [6]. This same reasoning also can be used to explain why overexpression of Opi3 rescues the PC synthesis defect but not pmaER structure in the Δscs2Δice2 mutant. Under normal physiological conditions, Opi3 activity at contacts is limiting, perhaps by Osh2/3 or by its affinity for PME in the plasma membrane. By increasing the amount of total Opi3 in the ER, more will be present at contacts (even though they are reduced in number) to facilitate PC synthesis. In any case, these genetic analyses establish that Opi3 function is downstream of a role for Scs2 and Osh2/3 at contacts. It will be interesting to test roles for the FFAT motif and PH domain in Osh3 function at contacts in the future as well as to determine if there is a role for sterols, although sterol binding has yet to be demonstrated for Osh2/3.

References:

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Correspondence - Editor 27 February 2013

Many thanks for submitting the revised version of your manuscript to EMBO reports. We have now received the assessment of the referees on the revision and I am happy to tell you that all reviewers now support publication of your study in our journal.

Before we proceed with the official acceptance may I kindly ask you for one more piece of information: Please indicate for each experiment how many independent times it was repeated and which statistical tests have been used throughout the study. This information does not seem to be present for all experiments at the moment.

The easiest way is if you modify the text accordingly, for example by including a section to the Materials and Methods section and/or by adding it to each individual figure legend. Once you have modified the text, simply send it as an email attachment and we will replace the old version.

Please do not hesitate to contact me if you have any further questions.

With best wishes and congratulations on a nice piece of work.

REFEREE REPORTS:

Referee #1:
The revisions to this manuscript fully address any concerns I had. I am happy to recommend publication.

Referee #2:
This is a revised version of the manuscript by Tavassoli et. al. The authors have addressed my comments and provided additional information on the effect of Pah1 expression on Opi3 distribution. The revised manuscript presents important new data of high significance.

Referee #3:
The authors have satisfactorily addressed my comments and those of the other reviewers. New data are provided and also corresponding text changes.

2nd Editorial Decision 01 March 2013

Thank you very much for sending us the LTP and the updated SI. I am very pleased to accept your manuscript for publication in the next available issue of EMBO reports (probably May, even though the study will of course be AOP before that). I thought I'd let you know that we will have a short News&Views piece written by David Stephens to accompany your paper. I really liked the story and thought it would be great to promote it a little more..

As part of the EMBO publication's Transparent Editorial Process, EMBO reports publishes online a Review Process File to accompany accepted manuscripts. As you are aware, this File will be published in conjunction with your paper and will include the referee reports, your point-by-point response and all pertinent correspondence relating to the manuscript.

If you do NOT want this File to be published, please inform the editorial office within 2 days, if you have not done so already, otherwise the File will be published by default [contact: emboreports@embo.org]. If you do opt out, the Review Process File link will point to the following statement: "No Review Process File is available with this article, as the authors have chosen not to make the review process public in this case."

Finally, we provide a short summary of published papers on our website to emphasize the major findings in the paper and their implications/applications for the non-specialist reader. To help us prepare this short, non-specialist text, we would be grateful if you could provide a simple 1-2 sentence summary of your article in reply to this email.

Thank you again for your contribution to EMBO reports and congratulations on a successful publication. Please consider us again in the future for your most exciting work.

