EMBO reports

Manuscript EMBO-2016-43311

Mitochondrial-nuclear co-evolution leads to hybrid incompatibility through pentatricopeptide repeat proteins

Han-Ying Jhuang, Hsin-Yi Lee, and Jun-Yi Leu

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Submission Date:	07 September 2016
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Editor: Martina Rembold

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 28 September 2016

Thank you for the submission of your research manuscript to our journal. We have now received the full set of referee reports that is copied below.

As you will see, all three referees acknowledge the potential interest of the findings and have only some minor comments that can be addressed with textual changes. In addition, referee 2 suggests to test 15S rRNA and Cox protein levels for some of the suppressor mutations.

Given these very positive and constructive comments, we would like to invite you to revise your manuscript with the understanding that the referee concerns (as detailed above and in their reports) must be fully addressed and their suggestions taken on board. Please address all referee concerns in a complete point-by-point response. Acceptance of the manuscript will depend on a positive outcome of a second round of review. It is EMBO reports policy to allow a single round of revision only and acceptance or rejection of the manuscript will therefore depend on the completeness of your responses included in the next, final version of the manuscript.

REFEREE REPORTS

Referee #1:

The manuscript by Jhuang et al. on Mitochondrial-nuclear co-evolution and hybrid incompatibility is an extension of previous work from the Leu group where a couple of examples of asymmetric incompatibility were found. Here they describe a thorough analysis of a PPR motif containing gene involved in mitochondrial RNA processing where there is symmetric incompatibility between two closely related yeasts. This in itself is an advancement in our understanding of how such

incompatibility evolves. As 2 PPR proteins were now found to be involved in incompatibilities between different yeast species they looked at the entire set of PPR genes in the Saccharomyces sense stricto yeasts and tested them in pairwise comparisons between S. cerevisiae and 4 other species. 9 out of 14 of these exhibited incompatibility when the gene was provided by the other species but the mitochondria was provided by S. cerevisiae, indicating that this is a prevalent mode of nuclear-mitochondrial incompatibility. The interactors of these proteins are not known but they are mitochondrial.

Although beyond the scope of this paper it would be interesting to know how many of these are symmetric vs asymmetric, whether there are incompatibilities between other pairs of species and where in the larger tree os S. sense stricto yeasts the various incompatibilities arose. One could hazard a guess that all of the PPR genes will be involved in incompatibility between some yeast species based not the findings so far.

Referee #2:

The Jhuang, Lee & Leu manuscript reports a mito-nuclear incompatibility between S. cerevisiae and S. bayanus. The authors further show that the incompatibility is bidirectional, which is unusual. The authors map the incompatibility down to CCM1, a PPR domain containing protein encoded by the nuclear genome, and 15S rRNA in the mitochondrial genome. Using experimental evolution, authors were able to map at least a couple of residues in CCM1 that might be directly involved in causing the incompatibility. Finally, the authors provide evolutionary and experimental evidence to suggest that PPR proteins might be routinely involved in creating mito-nuclear incompatibilities.

Overall, this is an exceptionally strong manuscript. It is clearly written and the experiments are elegant, comprehensive and rigorous. The insight this work provides, that PPR genes are likely a common cause of incompatibilities outside of plants, is novel and worthy of publication. The authors go above and beyond in terms of performing experiments to present a compelling case. For example, experimental evolution to identify suppressors is an elegant means of gaining insights into mechanism of PPR domain evolution and its role in causing incompatibility. In another example, the authors make a good effort to investigate the role of all PPR genes in all sensu scrito yeast species.

I have only one concern that the authors should address before accepting the manuscript for publication:
1) The authors identify 16 distinct suppressor mutations in the Sb-CCM1 gene and one in the 15S rRNA gene in the mtDNA. While the authors show that these rescue growth on glycerol, it would be very interesting and informative to know how they are able to rescue. The authors show earlier that the incompatibility results in decreased 15S rRNA levels and decrease in protein levels for Cox1, Cox2, and Cox3. Are the 15S rRNA levels and Cox protein levels rescued by the nuclear and the mitochondrial suppressor mutations? If they are, then this further provides evidence supporting their proposed mechanism of incompatibility. Alternatively, if the levels are not rescued, then it suggests that other mechanisms can be employed for rescue. Either way, the results would be informative. The authors don't have to check the 15S rRNA levels and the Cox protein levels for all their suppressors. However, they should check them in the one mtDNA suppressor, one nuclear suppressor that changes Sb allele to Sc allele (i.e. D400N), and one nuclear suppressor that is a de novo mutation (e.g. E375Q would be a good one given that it arose multiple times). This is a very doable and I would think easy experiment since they have all the strains already made. They also have all the reagents needed and they already know that they work.

Other minor comments:

In the introduction, page 3, lines 5-6, the authors say that PPR proteins constitute the largest protein family in eukaryotes. However, this is only true for plants. The authors should reword this sentence to clarify this.

Referee #3:

Jun-Yi Leu and colleagues map the genes involved in mitochondrial-nuclear DNA incompatibilities between two species of yeast, S. cerevisiae and S. bayanus. The approach they used is very clever. They substituted individually S. cerevisiae chromosomes for S. bayanus chromosomes. They found that S. cerevisiae strains with S. bayanus chromosome 7 had respiratory defects, consistent with abnormal mitochondrial function. They used a library of S. cerevisiae to complement this defect and ended up with candidate genes that include CCM1. They confirmed that the S. bayanus CCM1 allele is incompatible with the S. cerevisiae mt genome. The gene codes for a mitochondrial protein involved in the pre-mRNA intron removal of two mtDNA-encoded genes and the processing of rRNAs. In addition, the incompatibilities appear to be symmetric, i.e. the mtDNA of S. bayanus is also incompatible with the CCM1 allele from S. cerevisiae, which is quite interesting. The authors show that Ccm1 seems to be located in the mitochondrial fraction even in the incompatible combinations. In addition, they show that 15S rRNA seem to be specifically affected and their results suggest that the defects are caused by an abnormal interaction between Sb Cmm1 and the Sc 15S RNA. The authors then used experimental evolution to find mutations that may rescue the genetic incompatibility and found suppressor mutations in CCM1 and in mtDNA as well. These were confirmed and sequenced. Some of the mutants had changed the bayanus residue to the cerevisiae residue, strengthening the causal relationships between these changes and the incompatibility. All mutations were in a motif called PPR (pentatricopeptide repeat) that are involved in RNA interaction. As a final set of experiments, they show that other PPR containing proteins could also be involved in incompatibilities.

The experiments are well performed and strongly support the points made. The implications of the work are quite interesting because they clearly show that some types of genes could be more prone to lead to nuclear-mitochondrial incompatibilities. The issues addressed are universal in the life-sciences and are thus of interest to a journal such as EMBO reports. This is a beautiful and well done study.

Minor comments:

Line 9 page 1: some developments have been made along these lines recently that might be worth citing, for instance by Leducq et al. 2016.

Line 24 page 1: Is it really the co-evolution that lead to this transfer?

Line 9 page 3: Not sure mutational robustness has anything to do here. Maybe just mention rapid evolution?

Line 10, page 5, line 10: Strong rescue might be optimistic, at least from the image we have in the manuscript.

Line 11, page 18: reference for the plasmid collection?

Figure 2, panel A: protein bands for the Sc and Sb mito CCM1 are quite different. Anything to mention about this? Are they known to vary in size?

1st Revision - authors' response 09 October 2016

Response to Editor (referee's comments in plain text, responses in italics):

Regarding data quantification, can you please specify also the test used to calculate p-values in the respective figure legends? This information is currently incomplete and must be provided in the figure legends.

As suggested, we have added information about statistic methods used in the figures (see figure legends of Figure 2C, 6A, 6B, S1).

Response to Referee #1 (referee's comments in plain text, responses in italics):

The manuscript by Jhuang et al. on Mitochondrial-nuclear co-evolution and hybrid incompatibility is an extension of previous work from the Leu group where a couple of examples of asymmetric incompatibility were found. Here they describe a thorough analysis of a PPR motif containing gene involved in mitochondrial RNA processing where there is symmetric incompatibility between two closely related yeasts. This in itself is an advancement in our understanding of how such incompatibility evolves. As 2 PPR proteins were now found to be involved in incompatibilities between different yeast species they looked at the entire set of PPR genes in the Saccharomyces sense stricto yeasts and tested them in pairwise comparisons between S. cerevisiae and 4 other species. 9 out of 14 of these exhibited incompatibility when the gene was provided by the other species but the mitochondria was provided by S. cerevisiae, indicating that this is a prevalent mode of nuclear-mitochondrial incompatibility. The interactors of these proteins are not known but they are mitochondrial.

Although beyond the scope of this paper it would be interesting to know how many of these are symmetric vs asymmetric, whether there are incompatibilities between other pairs of species and where in the larger tree os S. sense stricto yeasts the various incompatibilities arose. One could hazard a guess that all of the PPR genes will be involved in incompatibility between some yeast species based not the findings so far.

Indeed, it will be interesting to know how many of the PPR incompatibilities are symmetric since reported cases are rare even in other organisms. It is possible that more cases of symmetric incompatibility will be found in this gene family since PPR genes are fast evolving. For the PPR genes that we did not observe incompatibility, it is also possible that the incompatibility will show up if we test different species pairs. In the future, we will test both hypotheses by genetically manipulating other yeast species. We thank the reviewer for providing these valuable suggestions.

Response to Referee #2 (referee's comments in plain text, responses in italics):

The Jhuang, Lee & Leu manuscript reports a mito-nuclear incompatibility between S. cerevisiae and S. bayanus. The authors further show that the incompatibility is bidirectional, which is unusual. The authors map the incompatibility down to CCM1, a PPR domain containing protein encoded by the nuclear genome, and 15S rRNA in the mitochondrial genome. Using experimental evolution, authors were able to map at least a couple of residues in CCM1 that might be directly involved in causing the incompatibility. Finally, the authors provide evolutionary and experimental evidence to suggest that PPR proteins might be routinely involved in creating mito-nuclear incompatibilities.

Overall, this is an exceptionally strong manuscript. It is clearly written and the experiments are elegant, comprehensive and rigorous. The insight this work provides, that PPR genes are likely a common cause of incompatibilities outside of plants, is novel and worthy of publication. The authors go above and beyond in terms of performing experiments to present a compelling case. For example, experimental evolution to identify suppressors is an elegant means of gaining insights into mechanism of PPR domain evolution and its role in causing incompatibility. In another example, the authors make a good effort to investigate the role of all PPR genes in all sensu scrito yeast species.

I have only one concern that the authors should address before accepting the manuscript for publication:

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suppressor that changes Sb allele to Sc allele (i.e. D400N), and one nuclear suppressor that is a de novo mutation (e.g. E375Q would be a good one given that it arose multiple times). This is a very doable and I would think easy experiment since they have all the strains already made. They also have all the reagents needed and they already know that they work.

As the reviewer suggested, we have examined the levels of 15S rRNA and Cox proteins in two nuclear suppressor strains (D400N and E375Q) and one mitochondrial suppressor strain. Both 15S rRNA and Cox protein levels were improved in the suppressor strains, suggesting that the incompatibility was rescued by the proposed mechanism. The results are shown in new Figure 5.

Other minor comments:

In the introduction, page 3, lines 5-6, the authors say that PPR proteins constitute the largest protein family in eukaryotes. However, this is only true for plants. The authors should reword this sentence to clarify this.

It is very true that while the PPR protein family is one of the largest protein families in eukaryotes, plants are the major contributors. We have modified the sentence to clarify this.

Response to Referee #3 (referee's comments in plain text, responses in italics):

Jun-Yi Leu and colleagues map the genes involved in mitochondrial-nuclear DNA incompatibilities between two species of yeast, S. cerevisiae and S. bayanus. The approach they used is very clever. They substituted individually S. cerevisiae chromosomes for S. bayanus chromosomes. They found that S. cerevisiae strains with S. bayanus chromosome 7 had respiratory defects, consistent with abnormal mitochondrial function. They used a library of S. cerevisiae to complement this defect and ended up with candidate genes that include CCM1. They confirmed that the S. bayanus CCM1 allele is incompatible with the S. cerevisiae mt genome. The gene codes for a mitochondrial protein involved in the pre-mRNA intron removal of two mtDNA-encoded genes and the processing of rRNAs. In addition, the incompatibilities appear to be symmetric, i.e. the mtDNA of S. bayanus is also incompatible with the CCM1 allele from S. cerevisiae, which is quite interesting. The authors show that Ccm1 seems to be located in the mitochondrial fraction even in the incompatible combinations. In addition, they show that 15S rRNA seem to be specifically affected and their results suggest that the defects are caused by an abnormal interaction between Sb Cmm1 and the Sc 15S RNA. The authors then used experimental evolution to find mutations that may rescue the genetic incompatibility and found suppressor mutations in CCM1 and in mtDNA as well. These were confirmed and sequenced. Some of the mutants had changed the bayanus residue to the cerevisiae residue, strengthening the causal relationships between these changes and the incompatibility. All mutations were in a motif called PPR (pentatricopeptide repeat) that are involved in RNA interaction. As a final set of experiments, they show that other PPR containing proteins could also be involved in incompatibilities. The experiments are well performed and strongly support the points made. The implications of the work are quite interesting because they clearly show that some types of genes could be more prone to lead to nuclear-mitochondrial incompatibilities. The issues addressed are universal in the life-sciences and are thus of interest to a journal such as EMBO reports. This is a beautiful and well done study.

Minor comments:

Line 9 page 1: some developments have been made along these lines recently that might be worth citing, for instance by Leducq et al. 2016.

As the reviewer suggested, we have included the Leducq study and several others in the revised manuscript.

Line 24 page 1: Is it really the co-evolution that lead to this transfer?

Selective pressures for better maintenance or enhanced fixation of beneficial mutations in mitochondrial genes are the previously proposed causes for the transfer. We have rewritten the sentence and also added new references to clarify this point.

Line 9 page 3: Not sure mutational robustness has anything to do here. Maybe just mention rapid evolution?

The sentence has been changed as the reviewer suggested.

Line 10, page 5, line 10: Strong rescue might be optimistic, at least from the image we have in the manuscript.

We have removed the word "strong" from the sentence.

Line 11, page 18: reference for the plasmid collection?

The information about the plasmid collection has been added in the Materials and Methods section (page 18, line 10).

Figure 2, panel A: protein bands for the Sc and Sb mito CCM1 are quite different. Anything to mention about this? Are they known to vary in size?

Myc-tagged Sc- and Sb-Ccm1 proteins are predicted to be 121.9 kD and 122.7 kD, respectively, which should be hard to tell by Western blot analyses. However, we always observe a slight difference in size between these two proteins. In addition, they appear as double bands, suggesting that the protein may be post-translationally modified. However, we do not have a clear idea what kind of modification causes this. We have discussed this point in the figure legends of Figure 2A.

2nd Editorial Decision 21 October 2016

I am very pleased to accept your manuscript for publication in the next available issue of EMBO reports. Thank you for your contribution to our journal.

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PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: Jun-Yi Leu
Journal Submitted to: EMBO Reports
Manuscript Number: EMBOR-2016-43311

Reporting Checklist For Life Sciences Articles (Rev. July 2015)

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. These guidelines are consistent with the Principles and Guidelines for Reporting Preclinical Research issued by the NIH in 2014. Please follow the journal's authorship guidelines in preparing your manuscript.

A- Figures

1. Data

The data shown in figures should satisfy the following conditions:

- the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- figure panels include only data points, measurements or observations that can be compared to each other in a scientifically meaningful way.
- graphs include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
- if n< 5, the individual data points from each experiment should be plotted and any statistical test employed should be justified
- Source Data should be included to report the data underlying graphs. Please follow the guidelines set out in the author ship guidelines on Data Presentation.

2. Captions

Each figure caption should contain the following information, for each panel where they are relevant:

- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.

- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple $\chi 2$ tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

Any descriptions too long for the figure legend should be included in the methods section and/or with the source data.

Please ensure that the answers to the following questions are reported in the manuscript itself. We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

In the pink boxes below, provide the page number(s) of the manuscript draft or figure legend(s) where the information can be located. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable).

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B- Statistics and general methods

Please fill out these boxes ↓ (Do not worry if you cannot see all your text once you press return)

1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	NA
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2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	N.A.
3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe.	N.A.
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5. For every figure, are statistical tests justified as appropriate?	Yes
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C- Reagents

6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right).	Anti-G6PDH antibody was purchased from Sigma (St. Louis, MO). Anti-c-Myc antibody (sc-40) was from Santa Cruz Biotechnology (Dallas, TX). Anti-TAP antibody (CAB1001) was from Thermo Fisher Scientific (Waltham, MA). Anti-Cox1 (MS418), anti-Cox2 (MS419) and anti-Cox3 (MS406) were purchased from MitoScience, Abcam (Cambridge, UK)
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	N.A.

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D- Animal Models

8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals.	N.A.
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	N.A.
10. We recommend consulting the ARRIVE guidelines (see link list at top right) (PLoS Biol. 8(6), e1000412, 2010) to ensure that other relevant aspects of animal studies are adequately reported. See author guidelines, under 'Reporting Guidelines'. See also: NIH (see link list at top right) and MRC (see link list at top right) recommendations. Please confirm compliance.	N.A.

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	N.A.
12. Include a statement confirming that informed consent was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.	N.A.
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	N.A.

14. Report any restrictions on the availability (and/or on the use) of human data or samples.	N.A.
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	N.A.
16. For phase II and III randomized controlled trials, please refer to the CONSORT flow diagram (see link list at top right) and submit the CONSORT checklist (see link list at top right) with your submission. See author guidelines, under 'Reporting Guidelines'. Please confirm you have submitted this list.	N.A.
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F- Data Accessibility

18. Provide accession codes for deposited data. See author guidelines, under 'Data Deposition'. Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	N.A.
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22. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biomodels (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information.	N.A.

G- Dual use research of concern

23. Could your study fall under dual use research restrictions? Please check biosecurity documents (see link list at top right) and list of select agents and toxins (APHIS/CDC) (see link list at top right). According to our biosecurity guidelines, provide a statement only if it could.	N.A.