The mitospecific domain of Mrp7 (bL27) supports mitochondrial translation during fermentation and is required for effective adaptation to respiration

Jessica Anderson, Jodie Box, and Rosemary Stuart

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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.)

RE: Manuscript #E21-07-0370
TITLE: The mitospecific domain of Mrp7 (bL27) supports mitochondrial translation during fermentation and is required for effective adaptation to respiration

Dear Rosemary,

Two expert reviewers have evaluated your manuscript, and I am happy to report that both of them are enthusiastic.

The reviewers have some constructive suggestions. Please consider these comments carefully, use your judgment to determine the most effective way to enhance the story, and provide a cover letter outlining your responses to the points that were raised. I will then send the manuscript to one of the reviewers for another quick look.

I look forward to seeing the revised manuscript.

Sincerely,

Benjamin Glick
Monitoring Editor
Molecular Biology of the Cell

Dear Dr. Stuart,

The review of your manuscript, referenced above, is now complete. The Monitoring Editor has decided that your manuscript is not acceptable for publication at this time, but may be deemed acceptable after specific revisions are made, as described in the Monitoring Editor's decision letter above and the reviewer comments below.

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When submitting your revision include a rebuttal letter that details, point-by-point, how the Monitoring Editor's and reviewers' comments have been addressed. (The file type for this letter must be "rebuttal letter"; do not include your response to the Monitoring Editor and reviewers in a "cover letter.") Please bear in mind that your rebuttal letter will be published with your paper if it is accepted, unless you haveopted out of publishing the review history.

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Please contact us with any questions at mboc@ascb.org.

Thank you for submitting your manuscript to Molecular Biology of the Cell. We look forward to receiving your revised paper.

Sincerely,

Eric Baker
Journal Production Manager
MBoC Editorial Office
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Reviewer #1 (Remarks to the Author):

Despite their bacterial ancestry, mitochondrial ribosomes have evolved to adapt to the requirements of organellar protein synthesis and have acquired new proteins or mitochondrion-specific extensions of those proteins that are conserved in prokaryotes. One case, bL27, is studied in this paper by Anderson and colleagues. The mito-specific extension of bL27, and the protein Mam33 are required for organellar protein synthesis in fermentative conditions. This study has uncovered an unexpected mechanism by which mitochondrial protein synthesis functions at a high rate under fermentation in a manner uncoupled from OXPHOS complex assembly. The authors propose that in this way, mitochondrial protein synthesis may provide a signal for the transition from fermentable to respiratory conditions.

Major points:
1- To substantiate the claim that newly synthesized mitochondrial proteins are degraded during growth in fermentable carbon sources, the authors need to perform pulse-chase experiments.
2- The authors show that Mrp7 and Mam33 proteins interact with each other, and both serve to support the adaptation of cells from glucose fermentation to respiration conditions. They should test whether overexpression of MAM33 in the mrp7(1-187) and mrp7(1-146) mutants restores protein synthesis in fermentative and/or respiratory conditions.
3- All mitoribosome proteins, except Var1, assembly factors and translation factors are encoded in the nuclear genome. Therefore, glucose repression should affect their abundance and import into mitochondria. The authors should explain how the mitochondrial protein synthesis rate is maintained with less ribosomes. Also, differences in translation rate in glucose and ethanol could be seen if the authors would perform a time-course labeling of newly-synthesized products (from 2.5min to 15min).

Reviewer #2 (Remarks to the Author):

In the manuscript by Stuart and colleagues, they investigate the regulation of mitochondrial translation of mitochondrial ribosomes under conditions that favor glycolysis vs. oxidative phosphorylation (OXPHOS). The mitochondrial ribosomal protein Mrp7, which has a mitochondrial specific domain, is initially analyzed. Experiments are carefully done, and include an assessment of whether mitochondrial DNA is being lost. Because mtDNA is lost, RNR1 is expressed to maintain the mtDNA genome. The C-terminal mitoribosome specific domain of Mrp7 is unexpectedly important for mitochondrial translation in glucose media, but not required for translation in OXPHOS conditions. It is also interesting that the level of mitochondrial translation is so robust in glucose media, which are conditions in which OXPHOS is typically inactive. Additional analysis of Mrp7 shows that interactions with Mam33 are also important for maintaining mitochondrial translation under fermentation conditions.
This is an exciting manuscript in which focused experiments are done to characterize key proteins involved in mitochondrial translation in fermentation vs. OXPHOS conditions. The fact that mitochondrial translation is maintained in fermentation contradicts previous ideas that have been proposed in the literature about mito translation only being active during OXPHOS. As a result, this warrants publication, even though the specific mechanism has not been determined. It is interesting that porin from the outer membrane is much lower and it is assumed to be caused by a decreased transcription. Analyzing porin RNA abundance as done in Fig 1 might be interesting to confirm if this is the mechanism. Alternatively regulation of the protein by turnover might be considered (see below)
Points to address
1. In Fig 2A, it is interesting that the porin levels, but not Tim44, are very low for the 1-187 and 1-146 constructs in the presence of glucose. The authors indicate that porin expression is repressed, but is it possible that there is also increased turnover of the mitochondrial outer membrane proteins? Perhaps the mitochondrial network could be compared in the mutants and the WT to confirm that the mitochondrial network is not perturbed.
2. I do not know if this can be done easily (is an antibody available), but analyzing the levels of the Mrp7 protein and truncations in isolated mitochondria would be useful in understanding the function of Mrp7 and potential problems with assembly defects for the ribosome subunits. However, if an antibody is not available, this reviewer understands that these experiments cannot be done easily.

Minor points
1. In describing the initial experiments in Fig. 1 in the results section, brief details about the plasmid for Mrp7 expression would be helpful (i.e., expression from own promoter, centromeric) as the general reader may not understand how the strains are established.
2. In Fig. S1, the symbol Ø is somewhat confusing for the delta Mrp7 strain. Perhaps, delta Mrp7 can be included or the empty vector name can be included?

Department of Biological Sciences

KLINGLER
College of Arts & Sciences
MARQUETTE UNIVERSITY

October 27, 2021

RE: Manuscript #E21-07-0370

Dear Ben,

Thank you so much for your email of 08/27/21 detailing the reviewers' comments to our manuscript entitled "*The mitospecific domain of Mrp7 (bL27) supports mitochondrial translation during fermentation and is required for effective adaptation to respiration*" and authored by Jessica Anderson, Jodie Box and myself. We thank you for providing us with the opportunity to submit a revised version of the manuscript to *Molecular Biology of the Cell (MBoC)*.

We thank the reviewers for their careful review and detailed feedback. We have revised the manuscript in accordance with their comments and we are of the opinion the revised manuscript is stronger because of their constructive and helpful suggestions. A detailed response to the reviewers' comments is given here below.

Response to Reviewer #1

1. In the manuscript we showed that robust mitochondrial translation was observed under fermentation growth conditions (e.g. glucose growth) despite these being metabolic conditions which do not support a high level of OXPHOS complex assembly and function. We had presented both *in vivo* labeling (new mitoprotein synthesis) and steady state protein analysis of OXPHOS components (Western blot) to substantiate this finding. In addition, and as requested by the reviewer, we have now performed pulse chase experiments (Figure S4 of the revised manuscript) to demonstrate that mitochondrially-encoded proteins synthesized during glucose growth conditions are turned over at a faster rate than their glycerol/ethanol (OXPHOS assembly promoting conditions) synthesized counterparts. We have also added a comment to this finding in the Results section of the revised manuscript (page 10, lines 15-17).
2. The reviewer asked if over-expression of Mam33 could improve mitochondrial translation and growth of the *mrp7* mutants. In response to the reviewer's question, we have now overexpressed Mam33 (under control of GAL10 promoter) in both the wild type and *mrp7*(1-146) mutant. We found that an excess of Mam33 does not alleviate the perturbed mitotranslation observed in the *mrp7*(1-146) mutant under fermentation (galactose) conditions, nor does it restore growth under respiratory conditions. Indeed, our observations would suggest that an excess of Mam33 may be detrimental to the *mrp7* mutant phenotypes in these respects. These new findings are discussed in the revised manuscript (page 17, lines 20-23) and presented as Figure S6 of the revised manuscript.
3. The reviewer commented on the elevated level of mitoprotein synthesis observed during glucose repression conditions, i.e. conditions when the expression and thus import of many nuclearly-encoded mitochondrial proteins are glucose-suppressed, as these conditions may have been expected to also contribute to a reduction in mitoribosomes and translation factor levels. Quantitative mitochondrial proteomics (e.g. Morgenstern et al., 2017) has shown that the steady state levels of nuclearly-encoded mitochondrial ribosomal components and TIM23 import machinery subunits are however not significantly reduced under glucose-repression growth conditions. Our comparative Western blot analysis of glucose and glycerol/ethanol grown cells in the original manuscript had supported this finding (Figure 3).

However, we did observe that the levels of mitoribosomes (as indicated by Mrp20/uL23 levels) were slightly higher under respiratory (glycerol/ethanol) growth conditions (relative to glucose), yet overall incorporation of [^{35}S]methionine into mitotranslation products was lower (relative to the fermentation grown cells). As noted also by the reviewer, collectively these findings do beg the question as to how the observed elevated glucose radiolabeling is maintained by less mitoribosomes. As requested by the reviewer, we have now added mitochondrial translation kinetic experiments requested to the revised manuscript. Our findings support that *in vivo* radiolabeling of mitochondrial translation products is faster during fermentation (especially for Var1, Cox1, Cox2, Cox3, Atp8 and Atp9), relative to respiration-based labeling conditions, suggesting that the glucose/fermentation and glycerol/ethanol/respiratory mitoribosomes may be differentially regulated. These data are presented (Figure S3 in the revised manuscript) and discussed in the revised manuscript, both in the Results section (page 10, lines 13-15) and in the Discussion (page 21). Taking all these findings together, we suggest in the Discussion that mitochondrial ribosomes under glucose fermentation conditions may be less tightly regulated than under respiration growth conditions (where rate and level of translation may need to be tightly coordinated with import of nuclear encoded OXPHOS partner proteins).

Response to Reviewer #2

Points to address

1. We agree with the reviewer that it is interesting that the levels of porin (and citrate synthase), two proteins whose expression are known to be glucose-repressed, are further reduced in the *mrp7* mutants when grown under fermentation conditions. Our initial analysis (Figure 5A) in the original manuscript suggests that glucose derepression of porin expression when adapting from high glucose to limiting glucose concentrations may be problematic in the *mrp7*(1-146) mutant. Our analysis of Tim44 and mitoribosomal proteins (encoded by nuclear genes known not to be glucose repressed) appear normal in the *mrp7* mutant, thus suggesting there is not a global impact on mitochondrial network/protein import process. We have added a comment in the revised manuscript highlighting the significance of this result in this context (Results section page 16, lines 11-14). We are in the process of further studying the expression of porin (repression and derepression) in the *mrp7* mutants, which we hope to report on in the future, but consider this analysis beyond the scope of the current study presently.
2. We agree with the reviewer that it would been interesting to determine the steady state levels of the truncated *mrp7* derivatives. The currently available antibody against Mrp7 originates from the lab of Dr. T Mason and is a monoclonal one (epitope site was previously undetermined). Our analysis indicates that the epitope of this antibody maps to the C-terminal mitospecific region of Mrp7 and specifically between residues 187 and 261. Consequently, the monoclonal antibody recognizes both full-length Mrp7 and the *mrp7*(1-325) and *mrp7*(1-216) mutant derivatives (all appear similar in steady state levels) but failed to detect the *mrp7*(1-187) and *mrp7*(1-146) mutant proteins. Efforts in the lab to generate a new Mrp7 antibody specifically against the N-terminal (bL27 homology domain) unfortunately were not successful. While we cannot directly demonstrate the physical presence of the *mrp7*(1-187) and *mrp7*(1-146) mutant derivatives, the observed normal steady state levels of mitoribosomal proteins and assembly state of the mitochondrial ribosome (e.g. Figure 4) and the observed mitotranslational activities of the *mrp7*(1-187) and *mrp7*(1-146) mutants under glycerol/ethanol growth conditions, would support the presence of truncated *mrp7* derivatives, as the presence of Mrp7 is required for ribosome assembly and activity. We have a now added a commented on this issue in the Results section of the revised manuscript (page 8, lines 15-23).

Minor points:

3. We had detailed in the Materials and Methods section of the manuscript that we used a centromeric plasmid (pRS316 and pRS413) system and where expression of the Mrp7/*mrp7* proteins was achieved using MRP7's own 5'-promoter and 3'-terminator regions. As suggested by the reviewer we have added further clarification in this section that the chosen genetic system was designed to mimic endogenous expression levels of Mrp7 (page 24) and have also highlighted the use of the

MRP7's promoter region to drive the expression of the *mrp7* derivatives in the revised Results section (page 5)

4. We have revised Fig. S1A as requested by the reviewer to remove the symbol Ø for the delta Mrp7 strain and included information of the plasmid (pRS413) into the figure and that the empty plasmid was used in the uppermost sample.

We thank you and the reviewers once again for the valuable feedback, constructive comments and the opportunity to submit a revised version of this manuscript.

With every best wish,

Rosemary A. Stuart, Ph.D.
Professor - Dept. Biological Sciences
Phone: 414-288-1472
e-mail rosemary.stuart@marquette.edu

RE: Manuscript #E21-07-0370R
TITLE: "The mitospecific domain of Mrp7 (bL27) supports mitochondrial translation during fermentation and is required for effective adaptation to respiration"

Dear Rosemary,

I asked Reviewer #1 to take another look, and I'm happy to report that the revisions were deemed to be satisfactory. I am marking the manuscript as accepted for publication in its current form.

Thanks for sending this nice work to MBoC.

Sincerely,
Benjamin Glick
Monitoring Editor
Molecular Biology of the Cell

Dear Dr. Stuart:

Congratulations on the acceptance of your manuscript.

A PDF of your manuscript will be published on MBoC in Press, an early release version of the journal, within 10 days. The date your manuscript appears at www.molbiolcell.org/toc/mboc/0/0 is the official publication date. Your manuscript will also be scheduled for publication in the next available issue of MBoC.

Within approximately four weeks you will receive a PDF page proof of your article.

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We are pleased that you chose to publish your work in MBoC.

Sincerely,

Eric Baker
Journal Production Manager
MBoC Editorial Office
mbc@ascb.org

Reviewer #1 (Remarks to the Author):

The authors have performed an excellent revision of the manuscript and responded to all my previous criticism. I only doubt whether the new Fig S3 should be part of the main figures, given the relevance of the data presented, but I will leave it to the discretion of the authors and editor.