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Retrograde Signaling Mediates an Adaptive Survival Response to Endoplasmic Reticulum Stress in *Saccharomyces cerevisiae*

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Original submission

First decision letter

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MS TITLE: Retrograde Signaling Mediates an Adaptive Survival Response to Endoplasmic Reticulum Stress

AUTHORS: Imaddedin Hijazi, Jeffrey Knupp, and Amy Chang
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 raise a number of substantial criticisms that prevent me from accepting the paper at this stage. They suggest, however, that a revised version might prove acceptable, if you can address their concerns. If you think that you can deal satisfactorily with the criticisms on revision, I would be pleased to see a revised manuscript. We would then return it to the reviewers.

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

The manuscript by Hijazi et al. demonstrates potential signaling pathways that modulate mitochondrial oxidative phosphorylation activity in response to ER stress. Using budding yeast

model system, the authors found that, without glucose shortages, ER stress can upregulate specific sets of mitochondrial proteins, thereby enhancing mitochondrial respiratory function. This selective change in mitochondrial activity is responsible for the adaptive survival program in ER-stressed cells, independently of the unfolded protein response (UPR) program. The authors' finding supports that, to cope with ER stress TORC1 inhibition and RTG activation are the key signaling event that is necessary for the cells potentiating mitochondrial survival response.
With diverse genetic and biochemical tools, the authors elucidate the potential crosstalk between AMPK and TORC1-RTG pathways for mitochondrial metabolic remodeling and cellular stress adaptation. The manuscript is clearly written, and to this reviewer, the work is solid and should fulfills the journal's standard after addressing the following concerns.

Comments for the author

Comments:
In Fig 1A, please include PgK1 blot data for ire1 delta and rtg1 delta cells as loading controls.
In Fig 3D, the labeling for each treatment is missing.
In Fig 5C, please include the loading controls (e.g., Pgk1).
In Fig 5D, "TORC1" should inhibit "Snf1 activation" since TORC1 inhibition leads to Snf1 activation according to the authors' model.
On page 15, line 9, "Fig 4A" should be Fig 3A.

Reviewer 2

Advance summary and potential significance to field

The manuscript by Hijazi and colleagues defines a signal transduction pathway activated during endoplasmic reticulum stress that results in mitochondrial remodeling and increased respiration. The authors demonstrate a surprising and novel link between the retrograde response (RTG) and ER dysfunction. And, they demonstrate that RTG activation is required to survive ER stress. In general, the manuscript is well written and likely of general interest.

Comments for the author

My primary concern of the manuscript is that it remains unclear why increased respiration via the retrograde response is required during ER stress. One could imagine that increased ATP is required to promote protein folding or degradation of poorly folded ER proteins consistent with CPY* activating the response. ERAD, or folding/trafficking of ER proteins could be examined during ER stress comparing cells with and without the retrograde response. At the very least, does inhibition of respiration or ATP synthesis sensitize to ER stress (antimycin oligomycin)?

Are mtDNA-encoded respiratory chain components increased during tunicamycin treatment (in addition to Cox2)?

The authors suggest that the increase in Cox2 protein is due to an increase in Rtg1 and Pet111-dependent translation from mitochondrial ribosomes. Is Cox2 mRNA also increased during tunicamycin exposure?

The authors demonstrate that Snf1/AMPK is not required to down-regulate TORC1 and elicit the retrograde response. How is TORC1 activity reduced during ER stress?

First revision

Author response to reviewers' comments

Dear Dr. Lippincott-Schwartz,

Thanks very much to you and the reviewers for your comments on our manuscript “Retrograde Signaling Mediates an Adaptive Survival Response to Endoplasmic Reticulum Stress”. Based on your suggestions we have added new data and made revisions to the text; changes are marked by a border at the revised text.

Point-by-point responses to the reviewers are as follows:
Reviewer 1: As requested, we have added Pgk1 loading controls to the experiments from which they were missing, including Fig. 1A and 5C. Labeling of Fig. 3D is now properly visible. In Fig. 1A, Atp1 serves as a loading control for the blot of rtg1Δ cells.
In Fig. 5D, the correction to our model has been made.

Reviewer 2: The reviewer asks why increased respiration is required during ER stress. In our previous paper in 2018 Cell Death & Differentiation, we proposed that cells die when mitochondrial reactive oxygen species accumulate in response to ER stress. We proposed that ER stress-induced ROS production is reduced as electron flow is increased through the electron transport chain. Based on this hypothesis, we have now performed a new experiment using dihydroethidium to measure ROS accumulation in wild-type cells with and without treatment with tunicamycin or antimycin or both. As shown in Fig. 1E, we find that wild-type cells are fairly resistant to ROS production in response to low dose tunicamycin (0.5 μg/ml) or antimycin (4 μM) treatment. Strikingly, however, tunicamycin and antimycin together have a synthetic effect on ROS production, supporting the idea that inhibition of respiration sensitizes cells to ER stress. We now include in the Discussion the possibility that respiration is increased by ER stress to meet increased ATP demand. However, we do not favor this hypothesis as we have previously found that ER stress is ameliorated by CCCP, a protonophore that dissipates the mitochondrial membrane potential (Knupp et al., 2018); this previous finding does not support the idea that respiration is increased to make more ATP for chaperones and protein folding.

The reviewer asks whether mtDNA-encoded respiratory components other than Cox2 are increased during tunicamycin treatment. We have not yet been able to confirm that the other 6 mtDNA-encoded ETC proteins are increased by ER stress because we do not have antibodies that work well enough for Western blot. Although mitochondrial proteomics analysis has been initiated, we do not have at present a comprehensive list of components contributing to increased oxygen consumption after ER stress. At present, however, we do know that mitochondrial response to ER stress is dependent on mitochondrial translation as Cox2 increase is inhibited by pentamidine (Suppl. Fig. 1).

The reviewer asks whether COX2 mRNA is increased during tunicamycin exposure in order to effect increased Cox2 protein levels. Based on the literature, key control points in mitochondrial gene expression occur post-transcriptionally via mRNA stabilization for instance (Rogowska et al., 2006). On the other hand, we cannot at present rule out translational regulation as a mechanism by which ER stress leads to Cox2 increase. Previously, we found via a genetic screen that overexpression of a mitochondrial methyltransferase Mrm1 leads to increased Cox2 translation and increased oxygen consumption, and Mrm1 overexpression protects against ER stress (Knupp et al., 2018).

The reviewer asks how does ER stress signal to inactivate TORC1. This is an excellent question that we are currently pursuing. It has been suggested that membrane contact sites may play a role in regulating TORC1 signaling (Murley et al., 2017). We are currently examining whether ER-mitochondrial, ER-vacuolar or mitochondrial-vacuolar contact sites are involved in conveying information about ER stress to TORC1 which resides at the vacuolar/endosomal membrane. We think this question is important but will require a substantial effort beyond the scope of the present manuscript.

We believe the manuscript has been significantly improved by our revisions. We hope the manuscript is now acceptable for publication in the Journal of Cell Science. We very much appreciate your help and that of the reviewers to improve the manuscript.

Sincerely,

Amy Chang, PhD

Knupp, J., Arvan, P. and Chang, A. (2018). Increased mitochondrial respiration promotes survival from endoplasmic reticulum stress. Cell Death and Differentiation 26, 487-501.
Murley, A., Yamada, J., Niles, B. J., Toulmay, A., Prinz, W. A., Powers, T. and Nunnari, J. (2017). Sterol transporters at membrane contact sites regulate TORC1 and TORC2 signaling. J. Cell Biol. 216, 2679-2689.
Rogowska, A. T., Puchta, O., Czarneccka, A. M., Kaniak, A., Stepien, P. P. and Golik, P. (2006). Balance between transcription and RNA degradation is vital for Saccharomyces cerevisiae mitochondria: reduced transcription rescues the phenotype of deficient RNA degradation. Mol. Biol. Cell 17, 1184-1193.

Second decision letter

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ARTICLE TYPE: Research Article

I am happy to tell you that your manuscript has been accepted for publication in Journal of Cell Science, pending standard ethics checks.