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The RtcB RNA ligase is an essential component of the metazoan unfolded protein response

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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.)

Editor: Esther Schnapp

1st Editorial Decision 17 September 2014

Thank you for the submission of your research manuscript to EMBO reports. We have now received 2 referee reports on it and given that they are in agreement that the study can essentially be published as is, I am making a decision now in order to safe some time.

I would like to ask you to address and incorporate all referee comments in a revised manuscript, which should be submitted until the end of this month, in order to meet the deadline for our December issue, as we would like to publish your paper as fast as possible and certainly this year, given the recent Mol Cell paper reporting similar findings.

I look forward to seeing a revised version of your manuscript by the end of this month. Please let me know if you have questions or comments regarding the revision.

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Referee #1:

In this extremely interesting and experimentally rigorous paper, Kosmaczewski and colleagues clearly demonstrate a role for C. elegans RtcB in tRNA ligation and the regulation of the unfolded protein response by ligating xbp-1 mRNA following splicing by IRE-1. In many ways it is unfortunate that the authors were scooped by the Molecular Cell paper, as this study is considerably stronger. The data is of exceptional quality and I think the paper should be published largely as is.

A couple of suggestions:
-Metazoan XBP-1's role in the UPR was essentially identified simultaneously by Shen et al (Cell) and Calfon et al (Nature) and should be referenced as such.

-The functional ortholog of XBP-1 in yeast should be referred to as Hac1 to limit confusion and be consistent with the literature.

Referee #2:

This is a very elegant piece of work demonstrating that RtcB ligase in addition to its established role in tRNA splicing also functions during unusual splicing of XBP1 mRNA during UPR.
The current work demonstrates the RtcB function at the organismal level, in C. elegans, what nicely complements recent data from Wang's lab describing similar findings for mammalian cells grown in culture. Interestingly, the authors also present preliminary data that RtcB might also have function(s) additional to that in tRNA and XBP1 mRNA splicing, either by preventing accumulation of tRNA fragments or being involved in ligation of other substrates. I have only few remarks, mainly related to discussion and references to the previous work biochemically characterizing RtcB-like ligase in mammals. Without that one is left with impression that nothing was known about metazoan RNA ligases until 2011, which is definitely not the case.
1. Introduction, p. 3, l. 3 top. When referring to Laski et al the authors should also quote papers by Filipowicz and Shatkin, Cell 1983, and Filipowicz et al. NAR 1983 (nb these papers appeared even earlier than that of Laski et al.). The three papers together clearly demonstrated that the mammalian RNA ligase differs from the plant /yeast enzymes by utilizing specific RNA termini and ligating RNA by an orthodox phosphosphodiester without the 2'- phosphate involvement; this information should be mentioned in Introduction. The three papers did not just "suggest" that mammals contain RNA ligase activity but demonstrated it and provided its initial biochemical characterization. Hence, also the statement in l. 9 top on this page "until recently no RNA ligases were known in metazoans....." should be modified. Rather: "Until recently the gene for the ligase has not been cloned...."
Also next paragraph should rather replace recent "discovery" with "recent cloning and molecular characterization .....".
2. P. 5, lines 2-4 top. Although authors discuss here only the metazoan-related issues they should also refer to much earlier work of Abelson's group in yeast who demonstrated unique character of tRNA splicing ~30 years earlier.
3. P. 10 Discussion, l. 4 bottom. RNase P produces 5'-p and 3'-OH termini. Robertson et al. were wrong in their initial assessments. Hence, remove reference to RNAseP.
4. Titles of subchapters should be formatted.
5. 5. The Mol. Cell RtcB/UPR paper from Wang's lab should be referred to in Discussion.

1st Revision - authors' response 25 September 2014

We very much appreciate the reviewers' interest and detailed suggestions. We have made all the recommended changes, and the manuscript now does a much better job of accurately accounting for previous discoveries.

1. Metazoan XBP-1's role in the UPR was essentially identified simultaneously by Shen et al (Cell) and Calfon et al (Nature) and should be referenced as such.

We added these references (e.g., p. 3: "Second, the function of the XBP mRNA--encoding a transcription factor that mediates the unfolded protein response-- requires ligation after cleavage by the IRE1 endonuclease [5,6].")

2. The functional ortholog of XBP-1 in yeast should be referred to as Hac1 to limit confusion and be consistent with the literature.

We now say (p. 6) “However, the RNA ligase TRL1 that ligates Hac1, the functional ortholog of XBP1, during the yeast UPR is not found in metazoans, and the identity of the ligase or ligases that mediate the metazoan UPR is not known [20,21].”

3. Introduction, p. 3, l. 3 top. When referring to Laski et al the authors should also quote papers by Filipowicz and Shatkin, Cell 1983, and Filipowicz et al. NAR 1983 (nb these papers appeared even earlier than that of Laski et al.). The three papers together clearly demonstrated that the mammalian RNA ligase differs from the plant /yeast enzymes by utilizing specific RNA termini and ligating RNA by an orthodox phosphosphodiester without the 2'- phosphate involvement; this information should be mentioned in Introduction. The three papers did not just "suggest" that mammals contain RNA ligase activity but demonstrated it and provided its initial biochemical characterization. Hence, also the statement in l. 9 top on this page "until recently no RNA ligases were known in metazoans....." should be modified. Rather: "Until recently the gene for the ligase has not been cloned...."
Also next paragraph should rather replace recent "discovery" with "recent cloning and molecular characterization .....".

We made these changes. We now say:

(p. 3) “Metazoan cell extracts contain RNA ligase activity, demonstrating that RNA ligation does occur in vivo in metazoans [1–3].”

(p. 3) “Until recently, however, the molecular identity of the ligase was not known in metazoans, as the RNA ligases identified in other phyla (such as LigT in bacteria, *At*RNL in *Arabidopsis thaliana*, and TRL1 in yeast) are absent from known metazoan genomes [7–9].”

(p. 3) “The initial biochemical characterization showed the metazoan RNA ligase differs from the yeast and plant enzymes by utilizing specific RNA termini and by ligating RNA by an orthodox phosphosphodiester without the 2'- phosphate involvement [1–3].”

(p. 3) “The recent cloning and molecular characterization of RtcB/HPC117, an RNA ligase that is highly conserved from bacteria to metazoans, raises the possibility that this RNA ligase mediates multiple aspects of RNA ligation in metazoans.”

4. P. 5, lines 2-4 top. Although authors discuss here only the metazoan-related issues they should also refer to much earlier work of Abelson's group in yeast who demonstrated unique character of tRNA splicing ~30 years earlier.

We added this reference (p. 5): “Ligation of intron-containing tRNAs is defective in RtcB mutants. In all branches of life, bacteria, archea, and eukaryotes, some precursor tRNA transcripts contain introns [12–14].”

5. P. 10 Discussion, l. 4 bottom. RNase P produces 5'-p and 3'-OH termini. Robertson et al. were wrong in their initial assessments. Hence, remove reference to RNAseP.

We removed this statement and associated reference (p. 10): “Multiple nucleases can produce RNA fragments with 5'-OH and 2',3'-cyclic phosphate ends, including tRNA splicing endonucleases and IRE1 [15,28].”

6. Titles of subchapters should be formatted.

The reviewer is correct, the first sentence of the paragraphs in the results section could be used and formatted as subchapter headings. We have left this to the discretion of EMBO Reports.

7. The Mol. Cell RtcB/UPR paper from Wang's lab should be referred to in Discussion.

We now say: (p. 10): “These findings agree with and complement the recent work identifying RtcB as a component of the mammalian UPR [24].”

2nd Editorial Decision 26 September 2014

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.

