EMBO reports

TEFM regulates both transcription elongation and RNA processing in mitochondria

Shan Jiang, Camilla Koolmeister, Jelena Misic, Stefan Siira, Inge Kühl, Eduardo Silva Ramos, Maria Miranda, Min Jiang, Viktor Posse, Oleksandr Lytovchenko, Ilian Atanassov, Florian A. Schober, Rolf Wibom, Kjell Hultenby, Dusanka Milenkovic, Claes M. Gustafsson, Aleksandra Filipovska, Nils-Göran Larsson.

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Transaction Report: **This manuscript was transferred to *EMBO reports* following peer review and revision at *The EMBO Journal***

1st Editorial Decision 19th March 2019

Thank you for the transfer of your manuscript to EMBO reports. We would like to invite you to address the remaining referee concerns below in a revised manuscript for publication here. Please also submit a point by point response to the comments below. It will be important to tone down all overstatements and to adapt the title.

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REFERE REPORTS

Referee 1:

The authors have responded robustly to the review and provided additional evidence in support of their claims that TEFM is somehow involved in RNA processing in addition to its known role in transcription elongation. The new BioID data in particular point to an involvement of TFEM in RNA processing or, at least, RNA granule localization. However, the data presented still do not provide any direct evidence that transcription elongation per se is coupled or linked to RNA processing via TFEM. This is clearly the authors interpretation and may even be correct, but to conclude this requires evidence of multi-protein complexes involving POLRMT, TEFM and the processing factors that are travelling together in space and time. The other possibility is that TFEM has a function (direct or indirect) in RNA processing that is completely independent of POLRMT (i.e. uncoupled or unlinked from transcription elongation). For this reason, the title is misleading and should be changed to "TFEM is linked..." as opposed to "Transcription elongation is linked..." and the authors should discuss this alternative possibility/limitation. Finally, although the authors discount a potential role for MRPL12 in binding POLRMT based completely on their own and others' negative data, it does seems reasonable to discuss this as a possible mechanism that stabilizes POLRMT in the absence of TFEM, given the extensive positive results published (e.g. Nouws et al. JBC 2016, Mitochondrial Ribosomal Protein L12 Is Required for POLRMT Stability and Exists as Two Forms Generated by Alternative Proteolysis during Import, and other papers cited in the original critique).

Referee 2:

The major difference from the previous submission is that the authors have performed some solid experiments with proximity labelling using a TEFM-BIOID fusion protein to address our question of whether TEFM really does associate in any way with parts of the RNA processing machinery. Indeed, they find evidence for interaction with numerous processing proteins. I'm afraid I still have concerns about the interpretation of some of their data. This is an excellent group and the data is of interest. Clearly, TEFM is an important mitochondrial protein that facilitates transcription elongation, as has been shown beautifully in vitro by Posse and colleagues. Evidence suggests that TEFM forms part of the transcription machinery but is the data really convincing that it is somehow involved in RNA processing as well ? I do not believe this data is sufficient clear to allow the conclusion that transcription elongation is linked to RNA processing, as stated in the title, although it is quite likely. The authors claim that TEFM somehow orchestrates transcription elongation and RNA processing. I find it difficult to conceptualise this and feel it is an overextrapolation. For example, if one was to perform the proximity labelling by fusing BIOID to RNA polymerase itself, I would expect the expt would pick up numerous members of the RNA processing enzymes that are found in the RNA granule but what would be the conclusion ?

I am also confused by the authors response to one of my original points:
From northern blot data, there does not seem to be much of a problem with processing 12S rRNA, as based on levels of three species: mt tRNAF, 12S rRNA and mt tRNAV.

Response:
The transcription of the 12S rRNA is not affected as it is promoter proximal and as such the processing of this transcript is not severely impaired.

Perhaps I am not understanding their point ? However, if TEFM is somehow involved in orchestrating RNA processing, why does it matter whether the transcript is promoter proximal ?
Overall, I think there is a lot of very sound and publishable data here, but I don't like the overextrapolation. I have no problem with the data being published and then allowing readers to make up their own mind but the claims should be toned down.

1st Revision - authors' response 21st March 2019

Point by Point Response:

Referee 1:

The authors have responded robustly to the review and provided additional evidence in support of their claims that TEFM is somehow involved in RNA processing in addition to its known role in transcription elongation. The new BioID data in particular point to an involvement of TFEM in RNA processing or, at least, RNA granule localization. However, the data presented still do not provide any direct evidence that transcription elongation per se is coupled or linked to RNA processing via TFEM. This is clearly the authors interpretation and may even be correct, but to conclude this requires evidence of multi-protein complexes involving POLRMT, TEFM and the processing factors that are travelling together in space and time. The other possibility is that TFEM has a function (direct or indirect) in RNA processing that is completely independent of POLRMT (i.e. uncoupled or unlinked from transcription elongation).
For this reason, the title is misleading and should be changed to "TFEM is linked..." as opposed to "Transcription elongation is linked..." and the authors should discuss this alternative possibility/limitation.
Response:
We agree with the referee as our manuscript provides no detailed molecular mechanism whereby TEFM is impacting RNA processing. However, our data clearly show that loss of TEFM impairs RNA processing. Also, the BioID data suggest a role for TEFM in RNA processing. As we have no defined molecular link explaining the coupling between transcription elongation and RNA

processing, we have down tuned our conclusions in the text and also changed the title of the manuscript to: "Impaired transcription elongation leads to RNA processing defects in mammalian mitochondria".

Finally, although the authors discount a potential role for MRPL12 in binding POLRMT based completely on their own and others' negative data, it does seems reasonable to discuss this as a possible mechanism that stabilizes POLRMT in the absence of TFEM, given the extensive positive results published (e.g. Nouws et al. JBC 2016, Mitochondrial Ribosomal Protein L12 Is Required for POLRMT Stability and Exists as Two Forms Generated by Alternative Proteolysis during Import, and other papers cited in the original critique).
Response:
We thank the review for this suggestion and have added a paragraph in the discussion section about possible interactions between MRPL12 and POLRMT.

Referee 2:

The major difference from the previous submission is that the authors have performed some solid experiments with proximity labelling using a TEFM-BIOID fusion protein to address our question of whether TEFM really does associate in any way with parts of the RNA processing machinery. Indeed, they find evidence for interaction with numerous processing proteins. I'm afraid I still have concerns about the interpretation of some of their data. This is an excellent group and the data is of interest. Clearly, TEFM is an important mitochondrial protein that facilitates transcription elongation, as has been shown beautifully in vitro by Posse and colleagues. Evidence suggests that TEFM forms part of the transcription machinery but is the data really convincing that it is somehow involved in RNA processing as well ? I do not believe this data is sufficient clear to allow the conclusion that transcription elongation is linked to RNA processing, as stated in the title, although it is quite likely. The authors claim that TEFM somehow orchestrates transcription elongation and RNA processing. I find it difficult to conceptualise this and feel it is an overextrapolation. For example, if one was to perform the proximity labelling by fusing BIOID to RNA polymerase itself, I would expect the expt would pick up numerous members of the RNA processing enzymes that are found in the RNA granule but what would be the conclusion ?
Response:
The data in this manuscript indicate that RNA processing enzymes associate with TEFM and that loss of TEFM causes low level accumulation of RNA precursors. These findings suggest that elongation is closely associated with RNA processing, which is the next step in RNA biogenesis. We agree with this reviewer that there is no direct molecular link and we discuss this in the revised manuscript as suggested by both reviewers. It is also worth to mention that the RNA processing defect is remarkable in the TEFM KO mice because we have only found RNA processing defects in MRPP3 KO mice [1], lacking the nuclease subunit of the mitochondrial RNase P complex that is required for 5' tRNA cleavage. We have previously analyzed 5 additional mouse models where DNA- or RNA-binding proteins have been knocked out [2] and have not identified any RNA processing impairments, suggesting that this is unique to the TEFM KO mice that would be of interest to report for the scientific community.

I am also confused by the authors response to one of my original points:
From northern blot data, there does not seem to be much of a problem with processing 12S rRNA, as based on levels of three species: mt tRNAF, 12S rRNA and mt tRNAV.
Response:
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Perhaps I am not understanding their point ? However, if TEFM is somehow involved in orchestrating RNA processing, why does it matter whether the transcript is promoter proximal ?
Overall, I think there is a lot of very sound and publishable data here, but I don't like the overextrapolation. I have no problem with the data being published and then allowing readers to make up their own mind but the claims should be toned down.
Response:
We apologize for not explaining this better, the processing of the 12S rRNA is also affected, however this defect may not as apparent as the others because there is increased transcription from the proximal HSP promoter resulting in much greater abundance of the 12S rRNA. We do not claim

that TEFM orchestrates RNA processing and we have re-phrased sections of the manuscript and changed the title as suggested by the reviewers.

1. Rackham O, Busch JD, Matic S, Siira SJ, Kuznetsova I, Atanassov I, Ermer JA, Shearwood A-MJ, Richman TR, Stewart JB, et al. (2016) Hierarchical RNA Processing Is Required for Mitochondrial Ribosome Assembly. *Cell Rep* **16**: 1874–1890.
2. Kuehl I, Miranda M, Atanassov I, Kuznetsova I, Hinze Y, Mourier A, Filipovska A, Larsson N-G (2017) Transcriptomic and proteomic landscape of mitochondrial dysfunction reveals secondary coenzyme Q deficiency in mammals. *Elife* **6**:.

2nd Editorial Decision 28th March 2019

Thank you for the submission of your revised manuscript. In principle, it looks good now, but a few more changes will be required.

Accepted 2nd April 2019

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: Nils-Göran Larsson
Journal Submitted to: EMBO Reposts
Manuscript Number: EMBOR-2019-48101-T

Reporting Checklist For Life Sciences Articles (Rev. June 2017)

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.

In the pink boxes below, please ensure that the answers to the following questions are reported in the manuscript itself. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable). We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

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?	At least n=5 was chosen as a standard in the field.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	The sample size has been indicated individually in figure legends.
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	No exclusion has been made.
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.	The grouping of animals were selected randomly according to their genotypes.
For animal studies, include a statement about randomization even if no randomization was used.	The grouping of animals were selected randomly according to their genotypes.
4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe.	The investigator didn't see the animals when choosing the animals for experiments.
4.b. For animal studies, include a statement about blinding even if no blinding was done	The investigator didn't see the animals when choosing the animals for experiments.
5. For every figure, are statistical tests justified as appropriate?	YES
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	We assume the normal distribution.
Is there an estimate of variation within each group of data?	We used Welch's correction assuming unequal variance
Is the variance similar between the groups that are being statistically compared?	We used Welch's correction assuming unequal variance

C- Reagents

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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).	Included in the manuscript
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	NA

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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.	Included in the manuscript
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	Included in the manuscript
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.	Included in the manuscript

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	NA
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.	NA
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	NA
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	NA
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	NA
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.	NA
17. For tumor marker prognostic studies, we recommend that you follow the REMARK reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines.	NA

F- Data Accessibility

18: Provide a "Data Availability" section at the end of the Materials & Methods, listing the accession codes for data generated in this study and deposited in a public database (e.g. RNA-Seq data: Gene Expression Omnibus GSE39462, Proteomics data: PRIDE PXD000208 etc.) Please refer to our author guidelines for '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	Included in the manuscript
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20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or EGA (see link list at top right).	NA
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G- Dual use research of concern

22. 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.	NA