Nuclear encoded mitochondrial ribosomal proteins are required to initiate gastrulation

Agnes Cheong, Danielle Archambault, Rinat Degani, Elizabeth Iverson, Kimberly D. Tremblay and Jesse Mager
DOI: 10.1242/dev.188714

Editor: Liz Robertson

Review timeline
Original submission: 27 January 2020
Editorial decision: 6 March 2020
First revision received: 26 March 2020
Accepted: 30 March 2020

Original submission

First decision letter

MS ID#: DEVELOP/2020/188714

MS TITLE: Nuclear encoded mitochondrial ribosomal proteins are required to initiate gastrulation

AUTHORS: Agnes Cheong, Danielle Archambault, Rinat Degani, Elizabeth Iverson, Kimberly D Tremblay, and Jesse Mager

I have now received all the referees reports on the above manuscript, and have reached a decision. The referees' comments are appended below, or you can access them online: please go to BenchPress and click on the 'Manuscripts with Decisions' queue in the Author Area.

The overall evaluation is positive and we would like to publish a revised manuscript in Development, provided that the referees' comments can be satisfactorily addressed. Please attend to all of the reviewers' comments in your revised manuscript and detail them in your point-by-point response. If you do not agree with any of their criticisms or suggestions explain clearly why this is so.

Reviewer 1

Advance summary and potential significance to field

The authors present a characterisation of the phenotype of homozygous mutations in 21 mitochondrial ribosomal genes (Mrp mutant embryos). These mutants display a developmental delay, as at E7.5 they resemble E6.0 embryos and fail to undergo gastrulation. The evidence presented supports the explanation that this phenotype is due a G2/M arrest, without a significant contribution of apoptosis. Characterization of how these mutations affect mitochondria function revealed altered mitochondria shape and cristae density dimished ATP production, but no change in mitochondrial DNA copy number. Together, these results indicate that mitochondrial activity is essential for proper early post-implantation mouse development.
The study is nicely presented and provides an important characterisation of embryonic development in the absence of the complete family of Mrp proteins. It provides a timely demonstration that mitochondrial activity is required in the lead-up to gastrulation, a finding that

contrasts with many conclusions drawn about mitochondria function using pluripotent stem cell models. Therefore it is an important paper for the field.

Comments for the author

There are some minor concerns that would benefit from being addressed. Specifically:
1. The numbers of embryos analysed for each mutation per marker needs to be indicated. For the immunohistochemistry stainings where n>1 quantification should be provided (as done in Fig. 7E), specially for markers that that are essential for the conclusions of the paper (e.g T, c-Casp3). The manuscript would benefit from n= 3 or more for at least one mutation for those markers that are essential for the conclusions of the paper (e.g T, c-Casp3)
2. When comparing immunohistochemistry stainings images, the authors should make sure the same region is selected across all genotypes and controls.
3. Lines 84-87: This part of introduction would benefit from being rephrased – the changes in maternal mitochondria only begin to take place post-implantation and not immediately after fertilization, as suggested.
4. Figure 4 (line 206). Why do the authors use of Trp53 as a marker of apoptosis? Only some of Trp53 roles are related to apoptosis. What do the authors define as active trp53? Maybe they should specify that p-p53 at Ser15 is being analysed. The data with cleaved caspase 3 is clear, therefore the differences .
5. Figure 6 (line 257): Why is mtDNA/nDNA quantification data normalised to E6.5 wt embryo? Did the authors analyse the mtDNA/nDNA content in whole embryos or only in epiblast cells? Could this account for the variability observed?
6. Can the authors speculate why ATP production is reduced in the mutant embryos (e.g. low translation of ETC proteins, hence low OXPHOS)?
7. The discussion would benefit from some streamlining.

Reviewer 2

Advance summary and potential significance to field

The main advance made by this paper is the surprising revelation that each of the ~70 mitochondrial ribosomal proteins appears to be essential for initiation of gastrulation.
In this manuscript 21 knockout mouse lines for various MRPs are used for generating embryos by heterozygous intercrosses. Each of them generates null blastocysts that implant in the uterus, form normal egg cylinders, but then fail to advance beyond this point. When the littermates have reached E8.5, the mutants resorb. More detailed analysis to investigate the cause of the phenotype is performed on 5 mutants. The authors find that the cause of the mutant phenotype is reduced energy production (ATP) and perturbation of cell cycle. Interestingly, there is no increase in apoptosis in any mutants, but Histone H3 pSer10 is increased, suggesting arrest of the cell cycle at G2. There is also no evidence of oxidative stress in mutant embryos. Mitochondrial morphology is, however, dramatically affected, but the genome is not. Expression of each MRP is ubiquitous, apart from mrps22. Translation in mitochondria depends on mtCO2 protein, and this is absent in mutant embryos. Reduction of ROS leads to cell cycle arrest via prevention of Cdc25c activation. This causes arrest prior to the G2/M checkpoint. Cell cycle arrest at G2/M most probably contributes to the retarded phenotype of the MRP mutant embryos; mitochondrial number is not affected in mutant embryos. the results highlight the importance of OXPHOS during gastrulation.
The introduction is written in a very engaging and informative way and the summary diagram showing the proposed positions of activity of the various proteins studied is helpful. This is the first study to address the roles of mitochondria during mammalian development in such detail and the results are suitably intriguing to be of wide interest to the developmental biology community.

Comments for the author

I would request the addition of scale bars on the confocal images when littermates at E7.5 are being compared with mutant embryos arresting at ~E6. Also, although wild type and het embryos are generally pooled in one group, it would be worth noting whether there might be a dosage

phenotype for any of the mutations studied. A table quantifying embryo phenotypes/sizes by genotype would be useful.

First revision

Author response to reviewers' comments

Reviewer 1 Advance summary and potential significance to field
The authors present a characterisation of the phenotype of homozygous mutations in 21 mitochondrial ribosomal genes (Mrp mutant embryos). These mutants display a developmental delay, as at E7.5 they resemble E6.0 embryos and fail to undergo gastrulation. The evidence presented supports the explanation that this phenotype is due a G2/M arrest, without a significant contribution of apoptosis. Characterization of how these mutations affect mitochondria function revealed altered mitochondria shape and cristae density, dimished ATP production, but no change in mitochondrial DNA copy number. Together, these results indicate that mitochondrial activity is essential for proper early post-implantation mouse development. The study is nicely presented and provides an important characterisation of embryonic development in the absence of the complete family of Mrp proteins. It provides a timely demonstration that mitochondrial activity is required in the lead-up to gastrulation, a finding that contrasts with many conclusions drawn about mitochondria function using pluripotent stem cell models. Therefore it is an important paper for the field.

Reviewer 1 Comments for the author
There are some minor concerns that would benefit from being addressed. Specifically:

1.The numbers of embryos analysed for each mutation per marker needs to be indicated. For the immunohistochemistry stainings where n>1 quantification should be provided (as done in Fig. 7E), specially for markers that that are essential for the conclusions of the paper (e.g T, c-Casp3). The manuscript would benefit from n= 3 or more for at least one mutation for those markers that are essential for the conclusions of the paper (e.g T, c-Casp3)

Yes, all results presented were repeated on a minimum 3 mutants and 3 controls from each knockout line except for one panel of one figure - Mrpl22 knockout line shown in figure 7E. We have now stated this clearly in the methods. We have quantified the number of Caspase-3 positive cells comparing mutants and controls. Quantification shows that Mrp mutants display on average 3.5 active-Caspase-3 positive cells per 100 cells counted while littermates show 1.7 active-Caspase-3 positive cells per 100 cells counted. Although the difference in active capase-3 cells between the Mrp mutants and littermates is statistically significant (p = 0.02), we believe that this very mild increase in caspase-3 mediated apoptosis is not a major contributing factor of mutant phenotype between E5.5-E7.5. We have added these details to the results section (Lines 258-262) and further in the discussion that we think phenotype is likely resulting from the lack of ATP production and disruption in cell cycle that prevent the Mrp mutants from initiating gastrulation, rather than cell death. We cannot quantify T as there literally are no T positive cells in mutants. We have verified this by RT-PCR but did not include these results since the protein (or absence) is a better functional readout for gastrulation.

2.When comparing immunohistochemistry stainings images, the authors should make sure the same region is selected across all genotypes and controls.

We have shown the embryo regions most representative between mutants and controls. Some slightly different views are presented in order to provide both stage-matched and littermate controls.

3.Lines 84-87: This part of introduction would benefit from being rephrased – the changes in maternal mitochondria only begin to take place post-implantation and not immediately after fertilization, as suggested.

We have rephrased the statement for clarification. (line 85-87) "Following fertilization, any paternal mtDNA arriving from the sperm is eliminated via mitophagy. In post-implantation embryos, the maternal mitochondria..."

4.Figure 4 (line 206). Why do the authors use of Trp53 as a marker of apoptosis? Only some of Trp53 roles are related to apoptosis. What do the authors define as active trp53? Maybe they should specify that p-p53 at Ser15 is being analysed.

We have specified throughout that we are using anti phospho-ser15 antibody that only recognizes active Trp53.

5.Figure 6 (line 257): Why is mtDNA/nDNA quantification data normalised to E6.5 wt embryo? Did the authors analyse the mtDNA/nDNA content in whole embryos or only in epiblast cells? Could this account for the variability observed?

The quantification is normalized to E6.5 stage matched controls to ensure comparison with developmentally similar embryos. The same difference is observed when compared to E7.5 littermates. Whole embryos were used in these assays.

6.Can the authors speculate why ATP production is reduced in the mutant embryos (e.g. low translation of ETC proteins, hence low OXPHOS)?

We have added to the discussion, (line 345-350) "The OXPHOS system requires the translation of essential ETC components within mitochondria. Our data show that the lack of functional Mrp proteins leads to the disruption of the mitochondrial translation machinery and therefore a lack of ETC related proteins as reflected by the absence of mtCO2 protein in mutant cells (fig 6C-G). The absence of functional ETC components in turn is most likely responsible for the drastic reduction of ATP in mutant embryos."

7.The discussion would benefit from some streamlining.

We have edited the discussion for clarity and efficient use of language.

Reviewer 2 Advance summary and potential significance to field
The main advance made by this paper is the surprising revelation that each of the ~70 mitochondrial ribosomal proteins appears to be essential for initiation of gastrulation. In this manuscript 21 knockout mouse lines for various MRPs are used for generating embryos by heterozygous intercrosses. Each of them generates null blastocysts that implant in the uterus, form normal egg cylinders, but then fail to advance beyond this point. When the littermates have reached E8.5, the mutants resorb. More detailed analysis to investigate the cause of the phenotype is performed on 5 mutants. The authors find that the cause of the mutant phenotype is reduced energy production (ATP) and perturbation of cell cycle. Interestingly, there is no increase in apoptosis in any mutants, but Histone H3 pSer10 is increased, suggesting arrest of the cell cycle at G2. There is also no evidence of oxidative stress in mutant embryos. Mitochondrial morphology is, however, dramatically affected, but the genome is not. Expression of each MRP is ubiquitous, apart from mrps22. Translation in mitochondria depends on mtCO2 protein, and this is absent in mutant embryos. Reduction of ROS leads to cell cycle arrest via prevention of Cdc25c activation. This causes arrest prior to the G2/M checkpoint. Cell cycle arrest at G2/M most probably contributes to the retarded phenotype of the MRP mutant embryos; mitochondrial number is not affected in mutant embryos. the results highlight the importance of OXPHOS during gastrulation.
The introduction is written in a very engaging and informative way and the summary diagram showing the proposed positions of activity of the various proteins studied is helpful. This is the first study to address the roles of mitochondria during mammalian development in such detail and the results are suitably intriguing to be of wide interest to the developmental biology community.

Reviewer 2 Comments for the author
1. I would request the addition of scale bars on the confocal images when littermates at E7.5 are being compared with mutant embryos arresting at ~E6.

Scale bars are present in all figures in the revised submission.

2. Also, although wild type and het embryos are generally pooled in one group, it would be worth noting whether there might be a dosage phenotype for any of the mutations studied. A table quantifying embryo phenotypes/sizes by genotype would be useful.

We did examine all assays with respect to hets and wt embryos but there are no significant differences in any assays presented so we therefore presented simply mut vs. controls. We have added this information in the methods section.

Second decision letter

MS ID#: DEVELOP/2020/188714

MS TITLE: Nuclear encoded mitochondrial ribosomal proteins are required to initiate gastrulation

AUTHORS: Agnes Cheong, Danielle Archambault, Rinat Degani, Elizabeth Iverson, Kimberly D Tremblay, and Jesse Mager
ARTICLE TYPE: Research Article

I am happy to tell you that your manuscript has been accepted for publication in Development, pending our standard ethics checks.