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Developmental programming of mitochondrial biology: a conceptual framework and review

Lauren E. Gyllenhammer, Sonja Entringer, Claudia Buss and Pathik D. Wadhwa

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1st revised submission:	25 March 2020
2nd revised submission:	31 March 2020
Final acceptance:	31 March 2020

Note: Reports are unedited and appear as submitted by the referee. The review history appears in chronological order.

Review History

RSPB-2019-2713.R0 (Original submission)

Review form: Reviewer 1

Recommendation
Accept with minor revision (please list in comments)

Scientific importance: Is the manuscript an original and important contribution to its field?
Excellent

General interest: Is the paper of sufficient general interest?
Excellent

Quality of the paper: Is the overall quality of the paper suitable?
Good

Is the length of the paper justified?
Yes

Should the paper be seen by a specialist statistical reviewer?
No

Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.
No

It is a condition of publication that authors make their supporting data, code and materials available - either as supplementary material or hosted in an external repository. Please rate, if applicable, the supporting data on the following criteria.

Is it accessible?
N/A

Is it clear?
N/A

Is it adequate?
N/A

Do you have any ethical concerns with this paper?
No

Comments to the Author
File attached. (See Appendix A)

Review form: Reviewer 2 (Dr. Amin Cheikhi)

Recommendation
Accept with minor revision (please list in comments)

Scientific importance: Is the manuscript an original and important contribution to its field?
Excellent

General interest: Is the paper of sufficient general interest?
Excellent

Quality of the paper: Is the overall quality of the paper suitable?
Good

Is the length of the paper justified?
Yes

Should the paper be seen by a specialist statistical reviewer?
No

Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.
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Is it accessible?
Yes

Is it clear?
Yes

Is it adequate?
Yes

Do you have any ethical concerns with this paper?
No

Comments to the Author
The notion that the likelihood of pathology in later life may can be traced back to some preconceptional and gestational conditions is concordant with an extensive body of work on the implications of such conditions for offspring mitochondria, and their potential role in directing a wide array of pathogenic processes as cells and organisms develop and age.
Although models and frameworks for mitochondrial biology systems exist, few are explicitly designed to guide a comprehensive multi-scale research. This review, however, provide a unifying conceptual framework that may reveal new aspects of developmental programming that were not previously appreciated.
While this work is important and should be published, some minor additions/clarifications could improve the review:
- Long-standing challenges and limitations of the most widely used methods for detecting and measuring reactive oxygen species and the redox state of cells are known. Table S1 would be more informative if it includes an additional column for the methods/probes utilized to quantify the ROS. Potential methodological shortcomings and their bearing on the author's interpretation of the referenced studies need to be clarified in the text. For instance, low micromolar concentrations of the superoxide probe MitoSOX uncouple mitochondria and inhibit complex IV activity.

- The notion of "Temporal stability of gestational condition-related variability in offspring mitochondrial biology" begs more clarification. While "the persistence (stability) of features of the initial setting of mitochondria function" is certainly encompassed in such temporal stability, it is not entirely clear how such notion can be reconciled for instance with fundamentally dynamics aspects of extra-mitochondrial biology in health and disease (e.g. the long-range physical cell-to-cell transfer of mitochondria between different cell types and organs via cellular nanotunnels or extracellular vesicles). Such dynamics can not only "amplify" mitochondrial dysfunction as suggested by the authors, but can potentially correct and/or diminish it over time.

- A more rigorous evaluation of mitochondrial heterogeneity at the cell population level and its underlying causes and consequences, as well as the development of methods to dissect and control it, will be critical to understand the molecular mechanisms of developmental programming of mitochondrial biology. This crucial aspect could be addressed in subsection 4c and/or section 5 notably with regard to the impact of molecular mechanisms of asymmetric division in PGCs and oocytes on mitochondrial segregation and their cellular transgenerational inheritance, the lack of such asymmetric division in mesenchymal stem cells (MSCs) and the emerging evidence suggesting a biparental inheritance of mitochondrial DNA in humans.

- The age-related features exhibited by the mtDNA mutator model are also displayed but other accelerated aging models where no mtDNA mutations are involved. Subsection 5a needs to consider known limitations of the mtDNA mutator mice and their significance for the proposed framework.

Decision letter (RSPB-2019-2713.R0)

02-Mar-2020

Dear Dr Gyllenhammer,

I apologise for the time it has taken to reach a decision on your manuscript, but it has now been peer reviewed . The reviewers' comments (not including confidential comments to the Editor) are included at the end of this email for your reference. As you will see, the reviewers have raised some concerns with your manuscript and we would like to invite you to revise your manuscript to address them.

We do not allow multiple rounds of revision so we urge you to make every effort to fully address all of the comments at this stage. If deemed necessary, your manuscript will be sent back to one or more of the original reviewers for assessment. If the original reviewers are not available we may invite new reviewers. Please note that we cannot guarantee eventual acceptance of your manuscript at this stage.

To submit your revision please log into http://mc.manuscriptcentral.com/prsb and enter your Author Centre, where you will find your manuscript title listed under "Manuscripts with Decisions." Under "Actions", click on "Create a Revision". Your manuscript number has been appended to denote a revision.

When submitting your revision please upload a file under "Response to Referees" in the "File Upload" section. This should document, point by point, how you have responded to the reviewers' and Editors' comments, and the adjustments you have made to the manuscript. We require a copy of the manuscript with revisions made since the previous version marked as 'tracked changes' to be included in the 'response to referees' document.

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All supplementary materials accompanying an accepted article will be treated as in their final form. They will be published alongside the paper on the journal website and posted on the online figshare repository. Files on figshare will be made available approximately one week before the accompanying article so that the supplementary material can be attributed a unique DOI. Please try to submit all supplementary material as a single file.

Online supplementary material will also carry the title and description provided during submission, so please ensure these are accurate and informative. Note that the Royal Society will not edit or typeset supplementary material and it will be hosted as provided. Please ensure that the supplementary material includes the paper details (authors, title, journal name, article DOI). Your article DOI will be 10.1098/rspb.[paper ID in form xxxx.xxxx e.g. 10.1098/rspb.2016.0049].

Please submit a copy of your revised paper within three weeks. If we do not hear from you within this time your manuscript will be rejected. If you are unable to meet this deadline please let us know as soon as possible, as we may be able to grant a short extension.

Thank you for submitting your manuscript to Proceedings B; we look forward to receiving your revision. If you have any questions at all, please do not hesitate to get in touch.

Best wishes,
Innes Cuthill

Prof. Innes Cuthill
Reviews Editor, Proceedings B
mailto: proceedingsb@royalsociety.org

Reviewer(s)' Comments to Author:
Referee: 1

Comments to the Author(s)
File attached

Referee: 2

Comments to the Author(s)
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Author's Response to Decision Letter for (RSPB-2019-2713.R0)

See Appendix B.

Decision letter (RSPB-2019-2713.R1)

27-Mar-2020

Dear Dr GYLLENHAMMER

I am pleased to inform you that your manuscript RSPB-2019-2713.R1 entitled "Developmental Programming of Mitochondrial Biology: A Conceptual Framework and Review" has been accepted for publication in Proceedings B.

No further revisions are required as I am happy with your responses to the referees' comments. Therefore, I invite you to upload the final version of your manuscript. Because the schedule for publication is very tight, it is a condition of publication that you submit the final version of your manuscript within 7 days. If you do not think you will be able to meet this date please let us know.

To upload your manuscript, log into https://mc.manuscriptcentral.com/prsb and enter your Author Centre, where you will find your manuscript title listed under "Manuscripts with Decisions." Under "Actions," click on "Create a Revision." Your manuscript number has been appended to denote a revision. You will be unable to make your revisions on the originally submitted version of the manuscript. Instead, revise your manuscript and upload a new version through your Author Centre.

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3) Electronic supplementary material: this should be contained in a separate file and where possible, all ESM should be combined into a single file. All supplementary materials accompanying an accepted article will be treated as in their final form. They will be published alongside the paper on the journal website and posted on the online figshare repository. Files on figshare will be made available approximately one week before the accompanying article so that the supplementary material can be attributed a unique DOI.

Online supplementary material will also carry the title and description provided during submission, so please ensure these are accurate and informative. Note that the Royal Society will not edit or typeset supplementary material and it will be hosted as provided. Please ensure that the supplementary material includes the paper details (authors, title, journal name, article DOI). Your article DOI will be 10.1098/rspb.[paper ID in form xxxx.xxxx e.g. 10.1098/rspb.2016.0049].

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• Phylogenetic data: TreeBASE accession number S9123
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Once again, thank you for submitting your manuscript to Proceedings B and I look forward to receiving your revision. If you have any questions at all, please do not hesitate to get in touch.

Best wishes,
Innes Cuthill

Reviews Editor, Proceedings B
mailto: proceedingsb@royalsociety.org

Decision letter (RSPB-2019-2713.R2)

31-Mar-2020

Dear Dr Gyllenhammer

I am pleased to inform you that your manuscript entitled "Developmental Programming of Mitochondrial Biology: A Conceptual Framework and Review" has been accepted for publication in Proceedings B.

You can expect to receive a proof of your article from our Production office in due course, please check your spam filter if you do not receive it. PLEASE NOTE: you will be given the exact page length of your paper which may be different from the estimation from Editorial and you may be asked to reduce your paper if it goes over the 10 page limit.

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Thank you for your fine contribution. On behalf of the Editors of the Proceedings B, we look forward to your continued contributions to the Journal.

Sincerely,
Proceedings B
mailto: proceedingsb@royalsociety.org

Appendix A

The focus of this review centers around mitochondrial biology as a common cellular pathway in the intergenerational transmission of developmentally programmed phenotypes. The review is timely, focuses on a highly relevant topic and very comprehensive.

The premise leading to the focus on mitochondrial function in terms of developmental programming is that various intrauterine insults leads to a wide range of phenotypes suggestive of potential mediation via common cellular mechanisms. A schematic linking various insults with several parallel phenotypic outcomes leading up to the postulate on mictochondrial focus would be helpful,

As opposed to the multitude of developmental programming reviews focusing on nuclear DNA epigenetic modifications, this review takes a novel direction, addresses the yin-yan relationship that exists between nuclear DNA epigenetic modifications and mitochondrial function with nuclear DNA epigenetic modifications affecting mitochondrial function and vice versa. Inclusion of a schematic highlighting this relationship between nuclear DNA epigenetics mechanisms and mitochondrial function would help nail this concept better.

The focus of this review clearly deviates from several other reviews that focus on mitochondrial bioenergetics and quality in that it narrows it down to the developmental plasticity of the mitochondrial system. Authors rightfully point out the need for undertaking several concurrent measures of mitochondrial function that involve static as well as dynamic measures to understand this fully.

In the section on mitochondrial mechanisms on developmental programming authors should dvelve deeper into functional aspects of mitochondria – OxPhos and its role particularly in generation of oxidative stress and contributing to programming.

Similarly, in the epigenetics section, the fact that ROS generation plays a major role in inducing programmed phenotype also was not capitalized upon to the fullest.

An aspect that was completely ignored is sex differences and the influence of sex on the mitochondrial function and consequences.

Another aspect that requires attention is the role of maternal hormonal milieu

Appendix B

22-March-2020

Dear Prof. Cuthill,

Thank you for your consideration and review of our manuscript "Developmental Programming of Mitochondrial Biology: A Conceptual Framework and Review." We have incorporated the reviewers' feedback in this revision. Please find below a response to each of the reviewers' comments. The specific sections of the reviewers' comments that require an author response are highlighted in purple, and our detailed responses for each of these are provided here. Space was limited, and in order to respond to the suggestions and recommendations of the referees' we needed to remove some text to fit within the article length limits. We have changed figure 1 to a supplemental figure in order to accommodate the new text. The figure caption included essential concepts and the text was moved to the introductory section of the paper. All changes to the text body or figure legends are track changed in the revised manuscript. Additionally, line numbers have been added, and a reference to the exact line additions are included in this response.

Referee: 1

Comments to the Author(s)

The focus of this review centers around mitochondrial biology as a common cellular pathway in the intergenerational transmission of developmentally programmed phenotypes. The review is timely, focuses on a highly relevant topic and very comprehensive.

- The premise leading to the focus on mitochondrial function in terms of developmental programming is that various intrauterine insults leads to a wide range of phenotypes suggestive of potential mediation via common cellular mechanisms.

1) A schematic linking various insults with several parallel phenotypic outcomes leading up to the postulate on mitochondrial focus would be helpful,

AUTHOR RESPONSE- This concept has been reviewed fairly extensively by others, and in response to this comment a list of reviews were added to the text "(reviewed in[1-3]) (line 96). Given the space limitation we have chosen to focus the figures and the body of the text on the novel data and figures related to mitochondrial biology.

- As opposed to the multitude of developmental programming reviews focusing on nuclear DNA epigenetic modifications, this review takes a novel direction, addresses the yin-yan relationship that exists between nuclear DNA epigenetic modifications and mitochondrial function with nuclear DNA epigenetic modifications affecting mitochondrial function and vice versa.

2) Inclusion of a schematic highlighting this relationship between nuclear DNA epigenetics mechanisms and mitochondrial function would help nail this concept better.

AUTHOR RESPONSE- We agree that this is an essential concept in this review, which is now better emphasized in this revision. Accordingly, we have updated Figure 1 (previously figure 2) and the figure legend (lines 31-36) to better illustrate the bi-directional relationship between mitochondrial function and DNA epigenetic modification. In addition, we have added further information in section 5b (lines 390-398).

- The focus of this review clearly deviates from several other reviews that focus on mitochondrial bioenergetics and quality in that it narrows it down to the developmental plasticity of the mitochondrial system. Authors rightfully point out the need for undertaking several concurrent measures of mitochondrial function that involve static as well as dynamic measures to understand this fully.

  AUTHOR RESPONSE- We appreciate this comment. We agree this issue is important for the development and design of future studies in this area.

- In the section on mitochondrial mechanisms on developmental programming authors should

3) delve deeper into functional aspects of mitochondria – OxPhos and its role particularly in generation of oxidative stress and contributing to programming. Similarly, in the epigenetics section, the fact that

ROS generation plays a major role in inducing programmed phenotype also was not capitalized upon to the fullest.

AUTHOR RESPONSE- We agree. We have better emphasized the importance of oxidative stress and the major role that ROS plays in the epigenome in the text (lines 144, 146-147, 395-397) and in the Figure 1 (previously figure 2) legend (line 31-32).

4) An aspect that was completely ignored is sex differences and the influence of sex on the mitochondrial function and consequences.

AUTHOR RESPONSE- We agree that the issue of sex differences warrants consideration. The animal and human studies we have reviewed unfortunately have not consistently or adequately address sex differences. Clearly, this is an important concept that needs to be addressed in future studies. This point has been added in the section on "key knowledge gaps" and directions for future studies (lines 430-431, 433).

5) Another aspect that requires attention is the role of maternal hormonal milieu

AUTHOR RESPONSE- We note that our conceptual model (previously figure 1, now supplemental figure S1) emphasizes the role that the maternal hormonal milieu plays- "(b) transmission occurs primarily via the effects of various maternal states and conditions on stress-related maternal-placental-fetal (MPF) oxidative, immune/inflammatory, endocrine and metabolic pathways that participate in the process of developmental programming of health and disease risk." In response to this comment, the figure caption was moved to the introductory section (lines 62-72) to better emphasize this concept, and we have added additional text in the body of the manuscript (section 4 -lines 175) to further highlight the proposed role of the maternal hormonal milieu (i.e. the MPF signals).

Referee: 2

Comments to the Author(s)

The notion that the likelihood of pathology in later life may can be traced back to some preconceptional and gestational conditions is concordant with an extensive body of work on the implications of such conditions for offspring mitochondria, and their potential role in directing a wide array of pathogenic processes as cells and organisms develop and age.

Although models and frameworks for mitochondrial biology systems exist, few are explicitly designed to guide a comprehensive multi-scale research. This review, however, provide a unifying conceptual framework that may reveal new aspects of developmental programming that were not previously appreciated.

While this work is important and should be published, some minor additions/clarifications could improve the review:

1) Long-standing challenges and limitations of the most widely used methods for detecting and measuring reactive oxygen species and the redox state of cells are known. Table S1 would be more informative if it includes an additional column for the methods/probes utilized to quantify the ROS. Potential methodological shortcomings and their bearing on the author's interpretation of the referenced studies need to be clarified in the text. For instance, low micromolar concentrations of the superoxide probe MitoSOX uncouple mitochondria and inhibit complex IV activity.

AUTHOR RESPONSE- We agree that this is an important point, and we have added specific ROS method information in the table. Not every study measured ROS, so rather than making a new column, we have now included the details in the existing column "mitochondrial biology outcomes" for the relevant studies. The limitations in direct ROS measurement are noted, and an additional relevant review has been added to the Section 4a (lines 215-221).

2) The notion of "Temporal stability of gestational condition-related variability in offspring mitochondrial biology" begs more clarification. While "the persistence (stability) of features of the initial setting of mitochondria function" is certainly encompassed in such temporal stability, it is not entirely clear how such notion can be reconciled for instance with fundamentally dynamics aspects of extra-mitochondrial biology in health and disease (e.g. the long-range physical cell-to-cell transfer

of mitochondria between different cell types and organs via cellular nanotunnels or extracellular vesicles). Such dynamics can not only "amplify" mitochondrial dysfunction as suggested by the authors, but can potentially correct and/or diminish it over time.

AUTHOR RESPONSE- We agree with the need for a more nuanced discussion of the concept of temporal stability of mitochondrial dysfunction. We have added additional text in section 4b that discusses programmed susceptibility versus determinism, and how this is consistent with the inherently dynamic nature of mitochondria (lines 299-306). We also have changed the title of 4b to "Evidence for temporal stability of gestational condition-related variability in offspring mitochondrial biology."

3) A more rigorous evaluation of mitochondrial heterogeneity at the cell population level and its underlying causes and consequences, as well as the development of methods to dissect and control it, will be critical to understand the molecular mechanisms of developmental programming of mitochondrial biology. This crucial aspect could be addressed in subsection 4c and/or section 5 notably with regard to the impact of molecular mechanisms of asymmetric division in PGCs and oocytes on mitochondrial segregation and their cellular transgenerational inheritance, the lack of such asymmetric division in mesenchymal stem cells (MSCs) and the emerging evidence suggesting a biparental inheritance of mitochondrial DNA in humans.

AUTHOR RESPONSE- Mitochondrial subpopulations are important to consider in this review for both conceptual and methodological reasons. We have emphasized the need to quantify and measure mitochondrial subpopulations at the end of section 3 (lines 162-163), and we have given more information as suggested in section 4c. In addition, we have directed readers to a review with more detailed information regarding these concepts (lines 329-332).

4) The age-related features exhibited by the mtDNA mutator model are also displayed but other accelerated aging models where no mtDNA mutations are involved. Subsection 5a needs to consider known limitations of the mtDNA mutator mice and their significance for the proposed framework.

AUTHOR RESPONSE- Our model proposes that developmental programming may additionally (not instead) exert effects through mitochondrial biology, and we don't preclude the possibility of other broad cellular or tissue specific effects. To emphasize this point in our framework, we added "(not instead)" to line 101 of the section 2. Furthermore, we use cautious language when introducing section 5 and state that the proposed stable changes to mitochondrial biology "may be mediated, in part, by 5a-5c." We don't expect that 5a-5c explain all of mitochondrial mediated changes in prenatal programming, let alone explain all of human aging.

The mtDNA mutator mouse does not perfectly recapitulate human aging, but it was presented in the text not as a perfect representation of human aging, but instead for the observation that the induced mutation load occurred early in embryonic/fetal life. The POLG mutation is not gestationally specific (and induced mutations can/should accumulate throughout the lifespan), however we found it interesting that something about the early process of development/rapid growth concentrated the mutation load in that window. This point is not necessary for the text (we are only reviewing gestational environmental exposures), and so, in order to avoid any possible confusion, we have removed this sentence from section 5a.