Chen Lesnik

Sections B1 and B2

Project 15194

Summary Comments Edit 1

**Summary**

**1. This summary is critical for the first round, so I focused on it**. It reads well as written. Congrats! However, there are many comments in the spirit of finding any potential improvements for you to consider for success.

**2. Character count.** The character count is now 1885 vs 2001 as written. This provides space to address the comments below if you accept them.

**3. Please read the summary carefully to be sure I have not altered your intent.** I have compacted the writing and reorganized it where indicated. These are suggestions. The words and intent of the proposal should be yours.

**4. Have you listed all of the outputs**? Line 26. A major suggestion is to be explicit about the output of the proposal. So far, I understand that specific proteins and pathways regulating toxicity during reproductive aging will be identified. For example, if there are other key outputs like markers for reproductive aging or any scientific community benefits, I suggest stating them clearly around line 26.

**5. Translatability of C. elegans.** I suggested stating that C. elegans is translatable to humans. A: though the mt genome is much smaller, the conserved elements of oxidative phosphorylation are present. It is implicit as written but not explicit. It seems important for your goal of human health.

**6. Figure 1.** Please note the comments about Fig 1.

**Extended synopsis**

**1. Please read carefully so I have not changed any intent**. This should reflect your writing and concepts.

**2. Addressing ERC criteria. I** have added comments and suggestions to address some of the questions ERC indicated would be review criteria. As indicated, I tried to add points about timeliness and the model's uniqueness and your lab. I suggest stating more strongly why your model is the best for the research and why you and your lab are the clear choice. I suggest a strong match between your knowledge and skills and the proposal content. You want the reviewers to conclude that this model and your lab are the only ones for this research. I suggest a subheading on or about line 82 stating explicitly why your lab is uniquely qualified for this research. Presumably, it is because of your expertise with worms and background as a reproductive aging pioneer/leader or similar. You don't want to be boastful because you are writing to your colleagues, but you should be informative. Otherwise, the reviewers will have to find it in the text somewhere and may not bother because they are busy.

**3. Self-evident descriptions of the objectives.** Objectives (about line 91). I suggest thinking about how to make the descriptions, especially Objective 1, more self-evident. The methods are stated at levels such as cellular, biochemical, and whole organism. As a nonexpert, it is not obvious to me how these methods will provide the objective of identifying specific mitochondrial proteins and pathways related to reproductive aging. While this is explained later, I suggest it should also be clear in the descriptions below each numbered Objective.

**4. Font**. To save space, consider using Times font instead of Arial.

**5. What are your expected numbers of proteins and their identities**? Objective 1, section 1.2 and 13. As a friendly reviewer, it is unclear how you will decide which proteins to pursue other than the score cutoff. What seems missing is a sense of how many differential proteins you expect and what those proteins might be. There are around 70 genes in the mt genome of C elegans, and fewer than 12 are involved in oxidative phosphorylation/ROS production so the candidates will be limited in number. Even still, reviewers should understand that you have thought through the expectations.

One suggestion is to indicate how many differential proteins you expect from males and hermaphrodites to give reviewers a sense of scale. Another is trying to convey what proteins or regulators you expect based on previous research and the Mt sequence. This will provide a sense of what direction you will take regarding proteins.

**6. Have you fully argued your case?** Perhaps this is a naïve question from a non-worm expert, but it may occur to generalist reviewers. Given the small number of mt genes likely involved in altering ROS, why not just overexpress/knockdown mt gene in oxidative phosphorylation and assay for enhanced reproductive aging? Although biased, this seems easier than choosing candidates by proteomics. You might then check sex-specific aspects. Of course, the advantage of your approach is it is unbiased.

**7. It seems that an unbiased approach is a major selling point.** As an extension of Comment 6, if other studies have not used proteomics as an unbiased forward screen, can you state that your approach is beyond the current state of the art? The positive is that it can uncover mt proteins that might not be suspected of being regulators of ROS.

***8. What chemicals are being tested?*** The types of chemicals, examples of them, and their selection criteria are not discussed. This seems like important information for evaluating the proposal, especially for nonexpert reviewers or reviewers who have done drug screening.

**9. Equivocation.** This is a small point to be aware of. Words like perhaps, maybe, could, might, etc. express doubt unless they are necessary to state the truth. I indicated instances in the text. One example in this proposal is "Will help" at about line 270 (Impacts of Objective 2) as opposed to "will." The proposal mostly avoids this common issue, which is excellent!

**10. Hypotheses rather than questions.** As proposals are generally hypothesis-driven, I suggest seeking opportunities to express questions in this form. I provide an example about line 290.

**11. Risk assessment.** As a friendly reviewer, the most significant risk to the project seems to be that no proteins are involved in ROS regulation and aging. This statement highlights the issue clearly. I suggest arguing more vigorously that it is likely or even highly probable that there is a mechanism regulating ROS during reproductive aging. Because ROS does change during sexual aging, it seems logical there is a regulatory mechanism that is detectable by protein or transcription changes. As written, I suggest the statement communicates funding for a short period (rather than five years) to do exploratory work to validate the hypothesis that there are natural mechanisms during aging. I am sure you have strong reasons to pursue this proposal. I suggest expressing them. I hope this makes sense!

**12. Objective 3.1 and transcriptomics.** As a friendly reviewer, 3.3. reads as a bit untethered to the rest of the objectives. I suggest explaining how transcriptomics will provide a chemical mechanism. Will you look at nuclear transcripts? Knowledge of chemical structure, protein targets, and related pathways may provide more helpful information because transcriptomics mainly reveals downstream responses and does not always correlate with protein levels. It seems that the combination of chemical identification and MOA, the transgenerational effects identified, and perhaps transcriptomics will lead to mechanistic insights. I suggest giving this some thought. 3.3 is the last objective in the proposal and should be read as well integrated. Maybe you can propose to combine all your information from Objectives 2 and 3 to understand mechanisms. Based on my experience with chemical screening on whole organisms, this is how you will come to understand mechanisms in any event. I hope this helps.

**13. Summary Statement.** The statement reads as an abstract. I suggest using the statement to describe the scientific impacts of the research forcefully and as a route to address human health. You can also use it as an opportunity to restate why your lab will be successful if you chose not to do this earlier in the proposal. You have the expertise to do this beyond cutting research and are in the best position to overcome any obstacles. This or similar seems important because it is the last thing reviewers will read and their final impression.

**14. A possible question to address in the text.** Perhaps naïve, but is it possible that regulators of mtROS are cytosolic in sperm or ovules?

**15. Misc.** Do you have any collaborators to identify for the proteomics, transcriptomics, or other approaches?

**Section B2**

**1. Hypotheses.** I suggest that after you have posed questions initially, they be stated as hypotheses. Proposals are usually hypothesis-driven. The questions lead you to make hypotheses that you address via your Objectives.

**2. I did not edit this section as heavily because it does not appear very complete**. I made comments and some edits that may be helpful as you progress

**3. Credit for results and ideas line 85.** Can you be more specific than we did things in the lab? You and a graduate student? Did you supervise a student in this discovery? Being specific where advantageous could demonstrate leadership and differentiate what you did in the lab. Your advisor will also get credit for work done in her lab. You are trying to highlight what you did to be judged as independent. Short of having pubs from your own lab, I realize this is tricky because you don’t want to overclaim or offend anyone. If you are worried about what you can claim to differentiate yourself, you might discuss with your past advisor to think through what you can honestly claim was your idea vs the lab.

**4. Position of Objectives.** At line 196, you have Objectives. I suggest placing the section at line 201 prior to the Objectives. Thus, you are describing the advantages of your system followed by how you will apply the system (Objectives).

**5. Organization.** I did not comment much on organization because B2 seem incomplete.

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**Summary Comments (Edit 2).**

**Summary**

**1. Please note the limits for each section of the proposal.** The Summary is now within the 2000-character limit. As written, it was more than 2600 characters. Please read carefully to be sure I have not altered your intent.

**Extended synopsis**

**1. To save additional space.** I removed all spaces between paragraphs and sections, which makes the proposal look more crowded.

**2. Clearer organization.** I also suggested changes to make the organization more apparent to reviewers.

 a. I italicized highlighted sentences to distinguish them from the bold titles.

 b. I used multiple font sizes. 14 pt for the main title and 12 pt for subtitles.

c. I changed colons to periods in the titles. Perhaps it's a personal preference, but it looks a bit less cluttered to me.

It seems easier to see the organization now, but please see what you think.

**2. Saving space.** Again, I streamlined the text throughout to save space. Please read carefully to be sure I have not altered your intent. Please note that I shortened many sentences.

**3. Prioritizing your proteins.** At line 241,it is great that you added a sense of the number of candidates for reviewers! I think this portion of the objective is very important.

a. I presume that even 50 to 100 proteins would be a lot for functional analysis.

Thus, I suggest stating clearly how you will reduce this number further at about line 247. I am not a worm expert, but 50 to 100 proteins for functional analysis (RNAi, transcription analysis, oocyte quality, etc. seems like a large number, especially for a relatively small research group. Regardless, I suggest providing a rationale for choosing your top candidates. The concept is to define a multistep-step selection process to rationally decide the highest-priority proteins for functional characterization.

b. Define the criteria for the highest-priority candidates. I have moved the sentence at line 248 sentences to line 244. In this example, I indicate a second-tier selection beyond 50-100 proteins based on the protein type, as written. What seems missing is a clear idea of how you will select the highest-priority candidates. Some of this language is at line 267. The highest-priority characteristics are, of course, for you to decide. It could be proteins known to be involved in mtROS, transcription factors, proteins not known to related to mtROS, or simply those that display the greatest differential expression. Regardless, I suggest indicating the characteristics of the highest-priority candidates you will pursue through functional characterization. How would you rank your top 10 candidates for deeper analysis? By stating additional criteria, you will reduce the 50 to 100 proteins, perhaps greatly.

c. State how many proteins you anticipate functionally analyzing based on the second-tier analysis.

d. I suggest it is critical to state the maximum number of proteins you can reasonably expect to analyze functionally regardless of how many candidates you have. Does your labor force and equipment allow you to evaluate 25 proteins using your various methods? 10? This is crucial for reviewers because it will set the boundaries of the proposal to a reasonable and feasible number, indicating that you understand the limitations and are realistic about what you can achieve. As a friendly reviewer, I would suggest that you avoid any hint of an open-ended analysis.

Again, I modified the sentences to highlight how to communicate to reviewers what you expect to achieve reasonably, indicating maturity and forethought. The text and ideas are for you to express.

**4. Abbreviation.** To save space, I suggest using the abbreviation TRT for transgenerational reproductive toxicity.

**5. Nuclear or cytosolic factors**. At line 372, it is acknowledged that there could be regulation due to nuclear or cytosolic factors. While true, I gently suggest this undercuts the rationale for your proposal. If you are not sure, why investigate mitochondria? I may be incorrect, as I am not a worm expert. But as a friendly reviewer, moving the project to look for cytosolic or nuclear factors seems like a major shift beyond the scope of the proposal. If shifting to cytosolic or nuclear factors is not difficult, then I suggest making this clear. Perhaps it is better to use your best arguments that regulators are in the mitochondria. Are there other known mitochondrial regulators for other pathways, for example? Logically, if the synthetic machinery for ROS exists in the mitochondria, then it seems likely there are endogenous regulators to control synthesis, even if there is influence from cytosolic or nuclear sources. ROS production is from Ox Phos, so at minimum, that pathway must coordinate its component stoichiometry. Some mitochondrial regulators may control mtROS synthesis locally, whereas extramitochondrial regulation may coordinate overall cellular activity. I hope this makes sense and is helpful.

**6. The Extended Synopsis is now within the 5-page limit.** I simplified many sentences to get to this point. So please read carefully to be sure I have maintained your intent. Please keep in mind that the Summary is limited to 2000 characters and separate from the Synopsis, which has a five-page limit. So we should be In good shape.

I hope this second edit is helpful. Overall, I think it is much improved, and the organization is more easily seen now!

**Section B2**

**Section a. State of the art and objectives**

**1. Consistency with section B1.** Please note that font sizes, underlines, italics, etc. were edited to be consistent with the second edit of section B1

**2. Less streamlining.** I was less aggressive in streamlining the writing because you are under the 14-page limit for B2. However, I simplified text where simpler sentences seem to aid clarity.

**3. Contributions.** At line 15, I commented on being clear about what you discovered vs. what was done in the lab more generally. This is tricky, I realize. But it is useful to provide reviewers with a clear idea of what you did via your own intellectual and laboratory work. This can be balanced with proper attributions to your advisors who supported you. Rather than saying “we” for your achievements in the lab, I can offer a few suggestions. In this example, if you discovered the link between bcat-1 and mt function, you might consider stating that you discovered this link with the support of your advisor. Or you found this link while a postdoc for your advisor. No matter what you write, it will be clear to the reviewers that the work was done in an advisor’s lab. Advisors will always get credit. They set the conditions that permit your discoveries. For early career scientists, independent publications are lacking, so intellectual contributions must be pointed out acceptably. I am not from the Israeli system, so I cannot comment on the societal context. Presumably, your advisors will write supporting letters indicating your contributions. However, sections B1 and B2 are what the reviewers will see initially. I don’t know if letters are included in the B2 review. Perhaps you can check this. If not, your contributions and independence will be determined by section B1 first, then B2. Another suggestion is to state that you found this link as a postdoc in your advisers lab, resulting in a first author publication, if this is the case. First author will signal that you did most of the work intellectually and physically. Finally, if you have good communication, you may wish to contact your advisors and ask them if they would be accepting of you stating your specific contributions in the proposal. I presume they will write you letters anyway, so perhaps discussing it directly is the best way to avoid any hurt feelings. Most advisors understand the situation. As an editor, my job is to point out potential weaknesses in the submission. The final wording is for you, of course. But I hope this helps.

**4. State your innovations explicitly.** For the section starting at line 229, I suggest stating explicitly what the proposal offers that is new and exciting. As an example, I added headings to each point you are making. The final titles are for you to decide, but this example presents the concept. Reviewers will not have to derive the innovations from the text because they are stated clearly. I suggest adding additional innovations or benefits of the proposal here as headings so reviewers will see clearly why they should fund it. You might also consider a section title. For example, “Benefits of our approach to reproductive aging and environmental toxicity” reads more generic and encompasses all the points you will make below. Make sense?

**Section b. Methodology**

**1. The sentence at line 256 is an impact**. I moved it to line 245 under its own heading and modified the existing sentence at line 256.

**2. Preliminary data section.**

a.One of your hypotheses is there are age and sex-specific changes in mt function. This preliminary data is essential to convince reviewers you are on the right track because the objectives depend upon the hypothesis being true. As a friendly reviewer, I may think this proposal is too preliminary. That is, you should first prove convincingly that there are real differences before funding. I suggest trying to avoid this potential issue by arguing vigorously that your data is convincing to the extent possible.

b. To this end, at line 303, I suggest explaining the data, especially panel B. Are there any trends? Are there any sex-specific differences in the data? This seems important because the underlying assumption of the proposal is that sex-specific differences will be found. So, I suggest arguing here as vigorously as possible that the results point to a high probability of differences if true.

c. I suggest indicating the robustness of the analysis if possible. Can you say anything about the experiment? How many times was it repeated independently? How many worms per measurement within a repetition? Any statistics would be helpful, even if preliminary.

d. If you are in the act of repeating the experiment, then I would indicate this. Remember that you may modify the proposal until the due date so new data can be added if available.

**3. Nuclear and cytosolic factors.** Please see the comment 5 about section B1.

**4. Communicating that you are well-prepared** For Objective 2.2, line 489, and noted in Comment 3 for Section B1, I suggest adding a sentence here explaining how many top hits you expect and how many you can characterize. For example, “We estimate from previous studies (ref) that we will obtain 5-10 top hits”. As a further example, “If we obtain more, we will characterize up to 20 toxicants, prioritizing those with the greatest sex-specific potency”. The concept again is to communicate that you have thought through the limits of your resources and have a clear rubric for ordering your top candidate toxicants in anticipation of having more hits that can be characterized initially. As an aside: Based on my experience with high throughput compound screening in whole organisms, it may be difficult to handle more than a small number of compounds simultaneously because the characterization work expands exponentially with each new compound. So, it is important to have a selection process for top compounds, especially if you expand the screening past 133 compounds or need to prove that a compound interacts physically with the protein target. Whereas this is not proposed, it seems that you will want this knowledge for a drug because you will need to understand the nature of the interaction.

**5. More details about the screen.** I suggest providing a bit more detail about the screening. How many compounds are you planning to screen? At line 457, it is stated that initially, 133 known toxicants will be screened. Are you planning to screen more? What will be the source of the additional compounds? Under what conditions would you screen additional compounds? If you do not obtain any hits? If you obtain fewer than ten hits, for example? If your screen is high throughput, then how many more compounds can you screen, and in what timeframe? As written, 133 compounds x 3 concentrations x 3 times? = ~3000 wells. Adding DMSO and other controls would result in fewer than 35 96-well plates for the entire screen. Are you planning to repeat the screen for confirmation or re-test just the positives? I suggest you provide enough information that reviewers will understand that you have thought through the details and effort required.

**6. Ending statement.** At line 614, I suggest adding a short paragraph outlining the project's significance to scientific knowledge and society through improved human health.The concept is to balance the risks stated previously with the gain. This is your final chance to sell the project. I would also consider stating in an acceptable manner that the project will permit the lab to be a leader in this burgeoning field of improved sexual aging. The expertise and knowledge will be shared through collaboration and publication and will help develop the field further. Something in the vein. This communicates that the funding is not just for your lab but for your field and broader community.

**7. Important overall comment.** Objective 1 will identify sex-specific proteins involved in mtROS. Objectives 2 and 3 will screen for toxicants that affect mitochondrial function, possibly across generations. What does not quite come across as written is how you will integrate both sets of results. They seem stand-alone as written.

a. For example, are you hoping that some sex-specific proteins will also be targets from the toxicant screening? I may have missed this, but that indicates it is not obvious. I suggest a statement, perhaps at line 225 (Benefits). At line 585, I also suggest a separate section explaining how the results will be integrated (Integration of Objectives). Since you have space, you might also consider a figure explaining how the results from the objectives will integrate into targets and potential agents to assist with reproductive aging. This will make it obvious how you meld the three Objectives toward the overall output of the project.

b. If you agree with this assessment, I suggest that you also insert a sentence indicating the integration in the Extended Synopsis. There is little or no room for this, so it must be short.