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Mitochondrial plasticity in trypanosomatids as a stress adaptation mechanism

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Reviewer 2: Alena Panicucci Zíková
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Manuscript reviewed 2022-06-28: *Only major points included.*

Reviewer 2

The review paper by Menna-Barreto ate al. on Mitochondrial plasticity in trypanosomatids as a stress adaptation mechanism is an interesting summary of the major achievements of the group that contribute to the general knowledge of mitochondrial metabolism and ETC of trypanosomatid parasites. The review briefly describes mitochondrial plasticity between *T. cruzi* stages and Leishmania species. It also focuses on mitochondrial metabolism and ROS resistance in Strigomonas culicis, a monoxenous trypanosomatid that harbors a bacterial endosymbiont. In general, the review is easy to read and summarizes the main scientific findings, but the target audience seems to be scientists from this field, as many terms are not properly explained (the different stages of the life cycle, trypanothione metabolism, specific redox players). It is a bit sad that mitochondrial plasticity is described only for two life cycle stages of *T. cruzi* (epimastigotes and bloodstream trypomastigotes ?) and only for one Leishmania life cycle stage (promastigotes ?). The review could provide a deeper insight into the remodeling of mitochondrial metabolism of all major life cycle stages of these trypanosomatid species or at least refer to other reviews addressing this exciting part of the parasite's biology. Another "problem" is that *T. brucei* is completely omitted, with the exception of the mention of alternative oxidase, an enzyme that has been studied most extensively in this parasite. Considering that the title includes "Trypanosomatids," I think it should be acknowledged that *T. brucei* is not covered and the reader can find most of the information in several recent reviews (Smith et al., 2017, Metabolic reprogramming during the *Trypanosoma brucei* life cycle; Zikova et al., 2022, Mitochondrial adaptations throughout the *Trypanosoma brucei* life cycle, Michels et al., 2021 Carbohydrate metabolism in trypanosomatids: New insights revealing novel complexity, diversity and species-unique features).

Authors

Thanks for the comments. In the invitation, the suggested length of the manuscript was small; then, we opted to review our contribution to the field in last decades, well focused on the scientists of our field. Before answer these queries, we contacted the editors (Dr. Vito de Pinto and MSc. Lisa Tindle-Solomon) to understand the journal position in relation to the length. The answer was "We realize that since the time of the authors' submission the strategy went from a print collection to a strictly online volume,

removing the limit on length. Our suggestion would be to continue with the short review to publish now and then create a living communication whereby the authors take the additional 2-3 months to create a full review which addresses the literature gaps mentioned by the reviewers (especially reviewer #2), which would then be submitted as a second edition (version) of the manuscript." For sure, we agree with the reviewer, and we intend to add, in the second manuscript version, topics related to trypanosomatids' antioxidant system (trypanothione metabolism and others antioxidant players), carbohydrate and fatty acid metabolisms, and pyruvate metabolism. We will focus in metabolic adaptations described to *T. cruzi*, *Leishmania* spp., monoxenous trypanosomatids (*Strigomonas culicis* and others) and, as highlighted by the reviewer, *T. brucei*; thus, we believe that the readers will have a good overview about bioenergetic and mitochondrial metabolism of trypanosomatids. At the moment, we corrected the points specified by the reviewer in the questions below, adding some important information about mitochondrial metabolism of *T. cruzi*, *Leishmania* spp. and *T. brucei* in the topic "*background*".

Reviewer 2

In addition, although ETS is described in the manuscript, the role of alternative dehydrogenase is not mentioned, although it plays a very important role in all parasitic trypanosomatids and may be a source of ROS. In addition, the last comprehensive review of NADH dehydrogenase activities was published in 2014, and there might be room for an update (e.g. Duarte at al, 2021, Leishmania type II dehydrogenase is essential for parasite viability irrespective of the presence of an active complex I, Duarte and Tomas, 2014, The mitochondrial complex I of trypanosomatids--an overview of current knowledge, PNAS PMC8545495

Authors

Thanks for the comments. An update about NADH dehydrogenase activities, as well as, alternative dehydrogenase importance to trypanosomatids' ETS was included.

Reviewer 2

Page 2: Curiously, NADH:ubiquinone oxidoreductase (complex I) in these parasites presents partial functionality, being the protozoa respiration exclusively dependent of succinate:ubiquinone oxidoreductase (complex II) and, consequently, by the succinate availability. I do not believe that these parasites respire exclusively on succinate. The metabolism of all trypanosomatid species is much more complex and depends on the availability of nutrients (proline, glucose glutamine etc.) and the stage of the life cycle (mammalian/insect forms). Considering that succinate is the end product of at least some trypanosomatid parasites, I think this view is too simplistic.

Authors

Thanks for the comments. We agree with the reviewer and remove the simplification about succinate-dependent respiration, adding a brief description about the importance of succinate to trypanosomatids' ETS, especially in *T. cruzi*. Although the complexity of trypanosomatid metabolism has been discussed in the specific topics of each species, especially in relation to nutrient availability; we believe that the second

manuscript version will provide a more complete overview about energy substrates (especially carbohydrates) used by each parasite form and its availability in the host.

Reviewer 2

Page 2: Previous studies showed a succinate-dependent respiration in *Trypanosoma cruzi*, *Trypanosoma brucei* and *Leishmania* spp., very similar to that present in vertebrates... What is meant by "similar"?

Authors

Thanks for the comments. We clarify the sentence.

Reviewer 2

Moreover, it is sometimes difficult to know for which life cycle stage of the particular parasite the described phenotypes are explained. It would be helpful if this was always indicated.

Authors

Thanks for the comment. The parasite stages were included in the whole manuscript in order to clarify the information.

Reviewer 2

Page 3: Ubiquinol: cytochrome *c* oxidoreductase (complex III) and ubiquinone Q cycle are the main ROS producers in these parasites (Murphy 2009; Wang &Hekini 2016). The III complex is one of the major ROS producers in general in aerobic eukaryotes, but to my knowledge, I cannot recall any published evidence that the III complex and ubiquinone Q cycle are the major ROS producers in these parasites. This is supported by the fact that the attached references are well-written general reviews of ROS and ubiquinone, but do not mention parasites specifically. These parasites contain other enzymes in their mitochondrion that can potentially contribute to the ROS pool (complex I, II, III, glycerol-3-phosphate dehydrogenase, alternative dehydrogenase, fumarate reductase). To the best of my knowledge, which molecular entity is responsible for the majority of the mitochondrial ROS has not been determined (for example see: Mantilla et all., Higher expression of proline dehydrogenase altered mitochondrial function and increased *Trypanosoma cruzi* differentiation in vitro and in the insect vector, Alencar MB, Ramos EV, Silber AM, Zikova A, Oliveira Marcus F (2022) The extraordinary energy metabolism of the bloodstream Trypanosoma brucei forms: a critical review and a hypothesis. Bioblast 2022: BEC Inaugural Conference.)

Authors

Thanks for the comment. We agree with the reviewer and, until the moment, no scientific work has compared the mitochondrial complexes of trypanosomatids regarding the production of reactive species. However, all evidences points to Q-cycle presence in these parasites; thus, we corrected the information about complex III (adding other electron leak points in the trypanosomatids' ETS), but we raised the question of high reactive species production by Q-cycle and emphasize the lack of information about the theme.

Reviewer 2

Page 4: In metacyclogenesis, low nutrient availability and an acid environment are pivotal features to the transformation of epimastigotes into metacyclic trypomastigotes. Please specify the environment in which metacyclogenesis occurs?

Authors

It is well-known that epimastigotes must be submitted to starvation and acid stresses to differentiate to metacyclic forms. The axenic medium to perform metacyclogenesis in vitro named TAU (triatomine artificial urine), was described by Contreras et al in late 1980s and consists in a kind of buffer adding 3 aminoacids and glucose in pH 5.0.