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The extraordinary energy metabolism of the bloodstream Trypanosoma brucei forms: a critical review and hypothesis

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Reviewer 2: Pavel Dolezal

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Manuscript reviewed 2022-08-07: *Only major points included.*

Reviewer 2

There has been a number of similar studies that highlight the uniqueness of the mitochondrial metabolic switch in kinetoplastids so the added value comes according to the authors in the following: glycerol-3-P shunt and TAO act as antioxidant system that also prevents programmed cell death processes related to the dissipation of mitochondrial membrane potential. I don't think that there is enough experimental support for such claims as the cited observations of the defective phenotypes could just purely reflect the experimentally manipulated systems with logical consequences (see below). Yet, there is always space for new hypothesis but these should be carefully scrutinized. I guess the manuscript can be later accepted for publication after some of the parts of the manuscript are modified. Although, I think the idea behind this opinion paper is not well supported.

Authors

We thank the reviewer for the valuable comments and insights. We agree that the experimental support for our main claims are scarce but considering our contribution in a hypothesis paper, we believe that we have room for discussing some open issues on bloodstream forms of T. brucei that other researchers in the field should develop further. We hope to provide the required basis for our hypothesis along this rebuttal letter and in the revised manuscript.

Reviewer 2

The paragraph about number of papers on trypanosoma mitochondrion is somewhat misleading. Why would 26 % of papers on BSF mitochondria mean limited understanding? Please rephrase.

Authors

We have removed that sentence in the revised manuscript.

Reviewer 2

Why do you compare ROS production in *T. cruzi* epimastigotes to bloodstream trypomastigotes? I assume that *T. brucei* trypomastigotes are meant as *T. cruzi* is intracellular for most of the time in the mammalian host.

Authors

In this sentence we aimed to discuss the changes in mitochondrial function along *T. cruzi* parasite forms, the epimastigotes and bloodstream trypomastigotes. The reason to discuss only *T. cruzi* lies on the fact that there is no available information regarding the changes in mitochondrial ROS production along *T. brucei* life forms, a key topic in our point of view. Therefore, in order to meet the reviewer comment, we have changed this sentence to emphasize that we are discussing the mitochondrial ROS production only in *T. cruzi* parasite forms, as following:

"The assessment of ROS production in *T. cruzi* mitochondria revealed that in epimastigote forms the oxidant production is low compared to bloodstream trypomastigotes."

Reviewer 2

Also when you say low ROS production, what does it mean?

Authors

The work which is referenced has measured mitochondrial hydrogen peroxide production in digitonin permeabilized parasites. Considering that hydrogen peroxide is a reactive oxygen species (ROS), we then kept this statement as it was.

Reviewer 2

There are no higher and lower eukaryotes, it is old concept.

Authors

We thank the reviewer for this comment and we have changed all statements of "higher eukaryotes" to " animals".

Reviewer 2

The manuscript says: "GPSh represents a critical hub that interconnects glucose and lipid metabolism,….." The link to lipid metabolism is not explained in this context.

Authors

Given that glycolysis produces G3P, which can be utilized to support phospholipid and triacylglycerol synthesis, glucose and lipid metabolism are linked through G3P and ultimately by GPSh (Mráček et al 2013).

Reviewer 2

"GPSh is mostly regulated at mG3PDG either through its content or by….." what is meant by "content"? please rephrases (amount?)

Authors

We thank the reviewer for pointing out this misspell. We have changed this sentence to the following one:

"GPSh is mostly regulated at mG3PDH level mostly either through its content or by allosteric regulation by specific signals including free fatty acids and Ca^{2+}"

Reviewer 2

"In most mammalian cells,…" what does it mean? Just say in mammalian cells or mammals/animals/metazoans/opisthikonts….

Authors

We have corrected this.

Reviewer 2

"sequences is slightly smaller…" the size of the protein can be smaller but sequences are shorter.

Authors

Corrected.

Reviewer 2

I do not think it is necessary to mention the number of amino acid residues, neither there is any reason to mention prediction by quite outdated Mitoprot II.

Authors

We have removed the mitoprot II statements in this paragraph but we maintained the protein size and the number of aminoacids to emphasize the differences in size between the BSF and mammalian mG3PDH.

Reviewer 2

Also, consider citing Tryptag database with all localization data.

Authors

We thank the reviewer for this comment but, considering that mitochondrial localization of both mG3PDH was already demonstrated (Skodova et al., 2013), we think the inclusion of the Tryptag localization of both isoforms was not necessary.

Reviewer 2

Why should Ca^{2+} promote the production of superoxide in mammalian mG3PDH, I guess it is there to promote the enzyme activity and the superoxide may come in as a side product. Please change that it is clear what is the role of calcium in the reaction.

Authors

The reviewer is right and we have changed the sentence to the following one to make the effect of calcium on mG3PDH clearer:

"In this regard, it is long known that Ca^{2+} binds to the EF-hand domain of mammalian mG3PDH which increases its affinity for G3P and also boosts mitochondrial superoxide production (Wernette et al 1981; Orr et al 2012)."

Reviewer 2

I do not think that there needs to be information about heat generation by plants and the attraction of pollinators as it has no link to the subject of the manuscript.

Authors

We think it is important to dissociate the notion of energy output to generate work and the biological significance. In this regard, several examples of biological significance of heat production were stated in the manuscript. Although the attraction of insect pollinators to thermogenic plants could be interpreted as energetically inefficient, it has a key adaptive role as these plants would not compete with others for pollinators and then increase reproduction efficiency during cold weather. The same applies for the thermogenic insects which maintain their activities even under low temperature. Importantly, in this particular case, the substrate used to sustain heat production in bumblebees is glycerol phosphate, the same substrate used by BSF mitochondria. Therefore, the collection of evidence to support the idea that BSF would generate heat as a side product of a reduced ETS would naturally make sense from the thermodynamic and biological point of view. Thus, it is important to consider that the absence of work as a consequence of any biological process (heat production) does not necessarily mean that there is no biological function. For these reasons, we kept these statements in the revised manuscript.

Reviewer 2

Again no need to describe protein presequences, instead refer to Tryptag.

Authors

Corrected. We have changed this sentence to the following one:

"Indeed, the protein products of Tb927.10.9760 and Tb927.10.7090 genes have their mitochondrial localization determined (Hamilton et al 2014)."

Reviewer 2

In this regard, the reversal of F_1F_0 ATP synthase activity seems to regulate T. brucei TAO activity by preventing matrix ATP accumulation.." I do not think it is correct, F_1F_0 ATP synthase simply used ATP to maintain the membrane potential and if TAO activity is affected secondarily, it is possible but has no direct link to F_1F_0 ATP synthase.

Authors

We have changed these sentences to better explain the relationship between ATP synthase and TAO, as following:

"In this regard, the inhibition of F_1F_o ATP synthase reversal activity by oligomycin reduces T. brucei TAO activity. Although direct evidence to explain this effect is missing, it is conceivable that reversal of F_1F_o ATP synthase activity would prevent the accumulation of matrix ATP and maintain TAO activity (Luévano-Martínez et al 2020;

Hierro-Yap et al 2021). Indeed, oligomycin treatment of BSF raises intramitochondrial ATP levels, strengthening the possibility of a regulatory axis between F_1F_o ATP synthase and TAO (Williams, et al., 2008)."

Reviewer 2

"..indicating that TAO activity has a preventive antioxidant role" I do not know the original references but it makes sense that if you block the final electron transfer, you generate superoxide and other ROS. But how does it relate to natural antioxidant role?

Authors

The reviewer is right in assuming that blockage of electron transfer along the inner mitochondrial membrane usually results in increased superoxide production. This is the case for all known protein complexes that mediate electron transfer in mitochondria. However, the extent by which these complexes generate superoxide is quite variable with complexes III and I as the major sites in mammals (PMID: 20064600). It is important to notice that preventive antioxidant mechanisms are those that avoid electron leakage and superoxide production. We stated TAO as a preventive antioxidant mechanism bearing in mind that mitochondrial oxidant production is very low in BSF, suggesting that the electron transfer through TAO does not involve electron leakage to generate superoxide. Supporting evidence has demonstrated a reduction in cellular oxidant levels when mice constitutively overexpress AOX (PMID: 23300486). Therefore, from the redox point of view, we think TAO activity can be considered as a preventive antioxidant mechanism.

Reviewer 2

Title of the chapter should not contain "programmed cell death" Why? Because it is not "programmed signal" to cell death, it is a metabolic adaptation to different carbon source.

Authors

We removed the word "programmed" from the title and throughout the revised manuscript to meet the issues raised by both reviewers about BSF cell death mechanisms. We understand the reviewer´s point of view, however we do not agree that it is "a metabolic adaptation to a different carbon source". The reason is that evidence has shown that under the same metabolic condition, pharmacological or genetic inhibition of ATP synthase promotes BSF cell death (Panicucci et al., 2017).

Reviewer 2

".. to the best of our knowledge, has no parallel in nature" it may be true but its somewhat a cherry picking because the entire metabolic switch of tryps is unparalleled, so must be also its elements.

Authors

We are discussing the uniquenesses of BSF mitochondria when compared to other eukaryotes. When one does that, the remarkable features of BSF mitochondria are stunning and defies the paradigm of electron transfer as a mechanism to provide energy to sustain ATP synthesis through chemiosmosis. In an attempt to dissect and highlight these features, we focused on the two principal components of this system: the ETS

mediated by GPSh and TAO and the ATP synthase. The induced "cherry-picking" we have made in this chapter, by choosing ATP synthase, is just another piece in the puzzle to understand how an eukaryotic cell can survive if the energy released by the ETS is not just coupled to generate protonmotive force to allow ATP synthesis, but rather the opposite. Therefore, we agree that our "cherry picking" works to explain piece by piece the complexity of mitochondria in BSF.

Reviewer 2

Page 13 mentions the lack of conserved anti-ROS response but later brings the presence of unique trypanothione systems, this information should be better mixed together so everything is clear from the beginning, also there is a repetitive information on the lack of particular reductases)

Authors

The understanding of BSF redox metabolism has several key points to be addressed and the contribution of specific antioxidant enzymes and their subcellular localization is just one of the missing points. We have stated on page 14 second paragraph that trypanosomatids have unique scavenging antioxidant mechanisms that regulate parasite redox balance. This is a major feature from the redox point of view considering that these organisms do not have classical scavenging antioxidant systems such as catalase and glutathione reductase for example. In this regard, another component to be considered is the subcellular compartmentalization of some of these scavenging antioxidants. This was further discussed in the next paragraph of page 14, when we describe the key role of cytosolic tryparedoxin to BSF tolerance to oxidant insults and parasite survival. We have rephrased some sentences at the end of the second paragraph to clarify the apparent dispensable role of mitochondrial antioxidant enzymes in BSF as following:

"This strongly suggests that mitochondrial scavenging antioxidant mechanisms do not play a key protective role for BSF survival. Indeed, evidence demonstrates that mitochondrial peroxidases seem to play a minor role for BSF growth and viability (Wilkinson et al 2003; Diechtierow, Krauth-Siegel 2011; Bogacz et al 2020). Conceivably, lower mitochondrial ROS production in BSF relative to PCF might explain the apparent dispensable role of mitochondrial peroxidases for BSF but the experimental evidence is still needed to fully address this aspect."

Reviewer 2

I just do not understand the idea of GPSh-TAO to be something else than two enzymatic systems if you regenerate NAD+ you must logically have an electron sink (oxygen reduced by TAO). Obviously, without TAO the system would collapse quickly, so where is the superoxide related function. You cannot separate the actual function of TAO that is sending the electrons to oxygen, from the suggested anti-superoxide response. The same is with the heat. Heat is the energy that cannot be used anymore. For tryps it is loss, but they do not care because they swim in glucose. The conclusion, in my opinion, exaggerates the role of these two enzymatic systems. Generally, the main hypothesis should be toned down or better supported.

Authors

We agree with the reviewer that we cannot separate the electron sink from the preventive antioxidant roles and heat production mediated by TAO. However, our inability to tie out these processes does not necessarily mean that they do not exist or are not biologically relevant. Considering in other eukaryotes inhibition of mitochondrial electron transfer promotes superoxide production, inhibition of TAO would inherently result in the increase of superoxide levels. Regarding the heat, we understand that our proposal that BSF produces heat as a natural by-product of an energetically inefficient ETS is highly speculative and should be experimentally supported. However, this point remains to be determined in the literature. In the context of an hypothesis paper, which is our case here, we think it is valuable to state and propose provocative ideas to be experimentally tested. More specifically, comparative calorimetry studies to determine the heat production in BSF relative to PCF would be critical to address this point. In this sense, one can speculate that the rate of heat produced by oxygen consumed would be much higher in BSF than in PCF. Finally, the proposed "role" of heat in BSF to increase mitochondrial enzyme activities could also be included as an unexpected side-effect as result of the energetically inefficiency of the BSF mitochondria.