

Bordeaux, France, May 18th, 2021

Review of the Master thesis of Michaela Husová, “Elucidating the source of bloodstream *Trypanosoma brucei* mitochondrial ATP”

Trypanosoma brucei is a flagellated unicellular protozoa and the causative agent of African Trypanosomiasis in humans, or Nagana in cattle. This parasite has a complex life cycle, alternating between the vector insect and the mammalian host. The metabolism of the parasite is quite different depending of the stage. In the insect vector, proline is the carbon source present most abundantly. In contrast, in the mammalian host glucose is the main carbon source for the bloodstream forms (BSF) of *T. brucei*. Many studies had focused on the glycolytic pathway of the parasite, but little is known about the mitochondrial energetic metabolism in BSF.

The aim of Ms. Husová master thesis is to study the energetic mitochondrial metabolism in *T. brucei* BSF, focusing on the contribution of ACC exchanger and the mitochondrial substrate phosphorylation on the membrane potential in the organelle. For asset these aims, Michaela used some mutants previously obtained in the research group, but also generated new mutants to complement the study. Michaela studied the role of ACC and SCoAS in the generation of mitochondrial potential using biochemical techniques, and determined the role of both during the infection. *in vivo*.

In general, the thesis is very well structured and it is written in a clear and concise way.

The introduction is appropriate, it includes the necessary information to understand the relevance of the project and provides the basis for understanding the discussion and development of the thesis in general. The references in this chapter are appropriate and used correctly. The figures are illustrative and help to visualize the concepts explained in the introduction and are also useful for consultation during the discussion.

The methodology is well designed, although sometimes seemed to me to be narrated as anecdotal and not as descriptive as it should be. References are also missing throughout this section.

The results are described concisely, especially since in this chapter Ms. Husová described the results of the RNAi and DKO mutants experiments without being reiterative or confusing. The figures are neat and well done, although occasionally they are not cited throughout the text. Furthermore, it is clear that each of the experiments were carefully thought out and focused to reach the aims of the project.

Finally, the discussion is very well conducted, it includes the results and discuss them accurately, in addition, Michaela proposes explanations for the results obtained citing the necessary bibliographic references. However, from my point of view, a small conclusion is needed.

In general, I found the thesis very interesting and relevant to our research area. The results obtained are outstanding and help us to understand in more detail the metabolism of *T. brucei*.

Sincerely,

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Questions

How is the activity of ATPase regulated in *T. brucei* to make it work in the sense of synthesis or hydrolysis of ATP?

- In Materials and Methods chapter, when the cells are being adapted to glycerol, why do they need a better aeration

- What is the mechanism of inhibition of CATR on ACC?, regarding this compound, it is dissolved in DMSO for the IC50 determination, did you check the effect of DMSO in the parasites?

- Did you try to adapt the SCoAS DKO to CMM + glycerol?

- What would be the prospects of your project?