Review of diploma thesis „Elucidating the source of bloostream *Trypanosoma brucei* mitochondrial ATP" of Bc. Michaela Husová

The diploma thesis by Bc. Michaela Husová represents the results of original research focused on the energy metabolism of the parasite *Trypanosoma brucei* that causes sleeping sickness in humans. The project was elaborated under supervision of RNDr. Alena Panicucci Zíková, Ph.D. at the Laboratory of Functional Biology of Protists, Biology Centre, CAS. The main goal of the project was to decipher the source of ATP that is necessary for generating mitochondrial membrane potential in bloodstream form of T. *brucei* and which is inevitable for its survival.

Theoretical part of the thesis briefly but sufficiently summarizes the *T. brucei* life cycle and more importantly its metabolism in both procyclic and bloodstream forms. The insufficiency of this part is the absence of references to figures.

To achieve the main goal, genetic models prepared by different techniques were employed, comprising knockout or RNAi models of mitochondrial transporter of glycolytic ATP or mitochondrial enzyme that produces ATP directly in mitochondria. Since the number of various cell lines is quite large, it would be helpful to include the table clarifying the vector and the selection reagent used in each particular genetic model. Also, the description of the method of double knockout cell lines construction is included in different places in the Results. The range of other techniques used is more than sufficient, they are described clearly, occasionally with minor inaccuracies (e.g. the antibiotic concentration is missing).

The Results part include large number of original experiments clearly focused on the aims of the project. The analyses in vitro are complemented by in vivo

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measurements that combine traditional methods with high-throughput omic approaches. I appreciate that when the result is not definite, it is critically discussed by the author. Overall, this part is above standard, with minor drawbacks, such as the sentence that does not make sense in first paragraph on the page 27 describing depolarization of inner mitochondrial membrane by ATP addition. Possible interpretation of the results as well as future focus of the project are discussed in the last part. In my opinion, some parts of the discussion should be included in the Results, namely the changes in nucleoside diphosphate kinase 4 or adenylate cyclase, since they seem to originate from the same dataset as the data shown in figures 29 and 30. If not, the source of the data should be mentioned (e.g. unpublished observation of lab member).

Despite minor criticisms, I believe that Bc. Michaela Husová is well acquainted with the topic and the methods and acquired novel insight into the biology of *T.brucei*. Therefore, the submitted diploma thesis fulfills all requirements to qualify Bc. Michaela Husová for MSc. title.

I have following questions:

1. Since the shape of mitochondrion may differ in *T. brucei* (e.g. between procyclic and bloodstream form), did you observe any structural changes of this organelle in media with different substrates, e.g. glycerol compared to glucose?
2. Could you specify metabolic processes in *T. brucei* that require maintenance of the mitochondrial membrane potential?
3. Does the bloodstream form have active mitochondrial translation and if yes, how sensitive it is to tetracycline antibiotic i.e. is it save to use Tet expression system without interfering with mitochondrial translation?
4. You suggested that complex I may be involved in the active proton transport and thus contribute to the mitochondrial membrane potential. Do you plan to measure activity of isolated complex (spectrophotometrically) or the respiration using complex I-dependent substrates?

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