Review of the Master Thesis written by Lucie Novotná

Summary:

The presented master thesis concerns functional characterization of a mitochondrial protein that has been suggested to be a component of putative MRB1 complex. Composition of this complex is very unclear since this complex associates with mitochondrial RNAs and different research groups purified different complexes with overlapping composition. Thus functional analysis of its subunits is a good way how to figure out the function and structure of this intriguing complex. The author has focused on a protein called Tb3010, which expression was silenced by means of RNAi. Author shows that this protein is essential for the survival of the PF and BF *T. brucei* cells and it is involved in the stability and/or editing of mitochondrial mRNA. Interestingly, this protein seems not to be involved in the stability of gRNAs. Furthermore, author has used immunofluorescence assay to elucidate the localization of two additional subunits of MRB1 complex.

In my opionion, the master thesis is very well written and I did not find any major mistakes in the text. I am also satisfied with the length of all presented sections. Further, I very appreciate that author did not make any strong conclusions about the function of the studied protein since the primary function of this protein is still unknown. I understand how difficult is to study unknown complex and I appreciate the author attempt to add one more piece into this very complicated mosaic. I will have only a few questions.

1. The author speculates that the lack of a signal for TB3010 transcript in BF Northern analysis is due to higher portion of rRNA loaded on the gel. Does this mean that BF cells have more rRNA than PF cells? Why it would be?
2. Another explanation might be that the abundance of Tb3010 mRNA is lower in BF than in PF cells. What experiment can be suggested to confirm or disconfirm this hypothesis?
3. Does the author plan to create an antibodies against Tb3010 or to tag Tb3010 in order to elucidate if this protein co-sediments with GAP1 and GAP2 proteins? Furthermore, experiments including RNase treatment may be very helpful to elucidate if Tb3010's association with GAPs is RNase sensitive. If I remember well, some experiments concerning the RNase treatment followed by MRB1 complex purification were performed by different groups. Did any of the results from these experimenst involve Tb3010?
4. The obtained qPCR data reminds data obtained when the components of editosome were knocked-out, thus Tb3010 may play a direct role in RNA editing or in stability of

the edited and/or partially edited mRNAs. Can author suggest some experiments that would distinguish between these two possibilities?

5. Based on GAP1 antibody western, the MRB1 complex has not been disrupted after Tb3010 RNAi-silencing. Similar observation was detected for two other subunits (Tb11.02.5390 – core subunit; Tb927.6.1680) (Acestor et al., 2009) using antibody against the second subunit of this complex GAP2. I just wonder if the GAP2/GAP1 antibodies are the best to track MRB1 complex. It seems that in glycerol gradient they recognize only the GAP1/GAP2 heterodimer. Since the antibody against Tb11.02.5380 is available (and potentially more antibodies against MRB1 subunits are also available) I would suggest to repeat the western blot analysis of the presented glycerol gradients using these antibodies. Were these western blots performed?

6. Acestor at al. showed that silencing of the MRB1 subunits leads to cytological anomalies (e.g. zoids or 1K2N cells). Did author notice similar abnormalities when Tb3010 was silenced?

In summary, Lucie Novotná showed that she is capable of performing skill demanding molecular biology techniques and she is able to write a scientifical text. This master thesis fulfils all the criteria given by the Faculty of Natural Sciences and I recommend it for the defense.

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Review - Lucie Novotna diploma thesis

Lucie Novotna in her thesis analyzed a function of the protein MRB3010 that is part of the mitochondrial binding complex 1 involved in RNA editing in *Trypanosoma brucei*. Lucie Novotna effectively knock-downed MRB3010 and showed that the protein is essential for both forms (bloodstream and procyclic) of *T. brucei*. In addition, she showed that the protein is important for editing of several mitochondrial genes. However, she was not able to uncover molecular details of MRB3010 function as its knock-down did not affect either gRNA or MRB1 stability.

The thesis is very well written and despite a few mistakes (e.g. referring to Fig 1.5. instead of Fig. 1.6. at p. 5) it was a pleasure to read it. Lucie Novotna showed that she is able to adopt different cell and molecular biology methods to tackle a basic biological question and I fully recommend this thesis to be accepted.

Questions:

1. Does MRB1 mediated editing work *in vitro*? If yes, can you isolate MRB1 from wild-type and knocked-down cells and test its function?

2. Fig. 3.2. - Did you test the growth of wild-type cells in the presence of tetracycline as a control?

3. Despite the lack of solid data, could you speculate on molecular function of MRB3010?

4. Just out of curiosity, why do you abbreviate procyclic as PS when there is no "S" in the word "procyclic"?

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