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I have evaluated the thesis of Zhenqui Huang favorably. His studies at your university incorporate collection and evaluation of primary literature, development of new methodology, a complete hypothesis-driven characterization of a trypanosome mitochondrial protein, and acquisition of preliminary descriptive data that could be the starting point for another student's research.

His Summary and Introduction (Chapters 1 and 2) are of a proper depth and scope for the rest of the work. There are a few items I wish him to address in this section. Utmost is his treatment of addition of non-encoded tails to mitochondrial mRNAs. While his language is similar to what I have seen in older reviews, some of the initial theories from the landmark papers he uses were overly simplistic. Examples in the primary literature make it abundantly clear that even short tails consist of uridine as well as adenine (Kao and Read, MBP 2007; Aphasizheva and Aphasizhev MCB, 2010; Zimmer et al. PLoS ONE, 2012; Decker and Sollner-Webb, Cell 1990; even Campbell et al, MBP, 1989). The description of tail addition in the review of which he is a contributing author reflects the reality of these tails much better.

Other minor details include information about transcription: since genes are encoded on BOTH strands of the maxicircle, we know there has to be a minimum of two transcriptional start sites. We know an additional one exists (a gRNA is encoded in the forward orientation). Might there be others? Finally, the treatment of trypanosome-caused disease is cursory. This is a basic science thesis and as a basic scientist myself I am fine with that. However, if disease is mentioned, proper context must be given for statements such as "The capacity of some Trypanozoon species to invade a host's tissues, such as nervous system, causes difficulties in disease diagnosis and results in pathogenic effects…". It is terribly vague. Indeed, the reference cited refers to porcine infection, but that is not apparent from the text. I would argue that the problem with human infection diagnosis is that the trypanosome infections are often initially assumed to be other, more innocuous infections, or are asympomatic at the disease stage when the parasite can be visualized. Also, we have to be careful to describe exactly what we mean here. I would not classify the nervous system as a "host tissue". I'm not sure whether intracellular trypanosomes are being referred to, or trypanosomes that obtain access to the CSF.

Chapter 3a is a peer-reviewed methods paper. Zhenqui has adapted fluorescence recovery after photobleaching (FRAP) for use in trypanosomes which are motile. Others in the field have also identified ways to utilize FRAP for trypanosomes; his contribution was the

development of a cell immobilization method that keeps cells alive and healthy during microscopic imaging. He validated his methods, and then illustrated an application of this methodology, drew appropriate conclusions and developed explanations when results were other than expected.

Chapter 3b is another peer-reviewed manuscript (in press). He characterizes a core protein of a large and poorly-defined complex that appears to control editing in *T. brucei*. His results are complicated; certainly analysis was challenging to Zhenqui and the other authors, but this displays his aptitude for understanding complex systems. The Contributions section states that Zhenqui contributed about 70% of the data, including techniques that pretty much span the world of trypanosome mitochondrial molecular biology. I would be interested in knowing whether he could see an application of his new FRAP methodology to answer outstanding questions apparent in the Chapter 3b Discussion.

Chapter 4 are unpublished data likely arising from experiments designed while analyzing and writing the Chapter 3 manuscript. As a trypanosome mitochondrial researcher, I can assess that these data are solid (appropriate controls/numbers of replicates) and personally very interesting to me. Thus I would unhesitatingly support their inclusion in the thesis. They are building blocks for further studies.

Chapter 5 and 6 are a brief reiteration of overall conclusions, plus the section of a review article that was written by Zhenqui. These minor contributions to major works are always difficult to fold into a thesis in a coherent way, which is why it stands alone here. I would rather direct my attention to Zhenqui's treatment of this same topic in his Introduction, where it fits much more seamlessly.

Zhenqui's thesis is comparable to those I have read at University at Buffalo- SUNY, and the University of Minnesota. The English usage is excellent throughout. As two full chapters and the review article in the thesis are already published in very solid journals, his thesis work is in the upper group in terms of acceptance by the larger scientific community.

I recommend Zhenqiu Huang for the doctoral degree in terms of fulfillment of the thesis requirements.

Sincerely,

Sara Zimmer

Sara Zimmer, Ph.D.
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In Prague 23rd of October 2015

The opponent review on the Ph.D. thesis of Mr. Zhenqui Huang "The dynamics of the MRP1/2 complex and the function of intact MRB1 core for RNA editing in *Trypanosoma brucei*".

The thesis consists of the Overview, two first-author publications, unpublished data, co-authored review article and the Conclusion.

The Overview contains a brief introduction to *Trypanosoma* taxonomy, medical importance and the characteristics of its life cycle. More detailed information is given on the current picture of the editing machinery in the kinetoplast, although the text remains rather general and does not bring the reader to the mechanistic details of the overall process. The final part mentions the importance of life cell imaging for the future research of the editing machinery.
While it is generally well written review it is not clear to me what is the purpose of the whole chapter as it contains just some selected aspects of the trypanosome biology with no clear single message. Also the part on the live cell imaging seems bit unrelated the preceding paragraphs. I understand that there is a need for an introductory part, which must prepare the reader for the coming publications but this could be done in more elegant way.

General questions to trypanosome biology:

1. What is the evidence for the insect transmission of *T. congolense*, even if, as mentioned in the thesis, it is a rare event?

2. What is behind "sleeping symptom" in sleeping sickness?

3. Is there a functional explanation for the morphology changes during the life cycle in Kinetoplastids? Why is it better to be a promastigote etc. in the insect stage?

4. Can you swap the media for PS and BS, i.e. will the parasites transform their mitochondria?

Specific notes (no need to respond to these during the defense):

- The reference list to the overview is at the end of the whole thesis, I would recommend to place it just at the end of the overview.
- The references to the figures in the text are in different formats

- The following sentences should be corrected:
    - "Thus, FoF1-ATPase of integrity possesses an essential, unique function in the mitochondrion (30)"
    - "In the case of the heterogeneous minicircles, it has been Overview 11 estimated number that is around 400 classes of these molecules are present in kDNA, each of which encoding 2-5 gRNAs in T. brucei (51,52).
- What is A) and B) in Figure 5?

Study #1 „Dynamics of Mitochondrial RNA-Binding Protein Complex in Trypanosoma brucei and Its Petite Mutant under Optimized Immobilization Conditions", Zhenqiu Huang, Sabine Kaltenbrunner, Eva Šimková, David Staněk, Julius Lukeš, Hassan Hashimi, Eukaryotic Cell 2014

The paper describes successful introduction of cell immobilization technique, which allows for the first time to observe the protein dynamics within *T. brucei* mitochondrion in live. By the use of YFP and YFP-tagged MRP1 authors were able to demonstrate different diffusion characteristics of the heterologous (YFP) and the "endogenous" (MRP1-YFP) protein. Different kinetics were observed for this RNA-binding protein in *T. brucei brucei* and *T. brucei evansi*, the latter of which is reminiscent of a rho0 yeast mutant. Although the study may appear simple and easy from the first sight, it is obviously of great importance to the field. There is very often a lack of straightforward and reliable techniques for different experimental models. It is a nice methodological paper, which introduces valuable cell biology tool for the trypanosome biologists.

Questions and notes:

1. Page 1235: I cannot agree with the statement: "The band intensities of the MRP1-YFP versus the endogenous MRP1 suggest that the stoichiometry of the former ranges from two to one copies." as no densitometry supports such claim and it is thus highly speculative.

2. Page 1238: "In contrast to the situation with cytosolic GFP-expressing L. major embedded in a CyGEL matrix (12), the photobleached ROI exhibited full recovery in PS, BS, and AK MTYFP trypanosomes." Why do you think there is a difference between these two studies?

3. Is there a RNA-tagging technique which would allow to follow the dynamics of RNA molecules in live?

Study # 2 Integrity of the core mitochondrial RNA binding complex 1 is vital for trypanosome RNA editing . Zhenqiu Huang, Drahomíra Faktorová, Adéla

Křížová, Lucie Kafková, Laurie K. Read, Julius Lukeš and Hassan Hashimi. RNA, in press

The paper summarizes the authors efforts to describe the molecular function of MRB8620 protein, the last uncharacterized component of the MRB1 complex. By silencing the expression of MRB8620 authors demonstrate the indirect effect of the protein on the editing of the mitochondrial transcripts. It is shown that the phenotype is likely caused by disturbed integrity of the overall MRB1 complex. It is further hypothesized that the resulting conformational defects may, in fact, prevent the editosome to come into contact with the target mRNA molecules. Moreover, the protein functions in both, the insect and blood-stream stages, which is shown for the latter by an elegant use of genetically manipulated parasites.
Although the paper does not provide decisive explanation for the function of MRB860 and the overall MRB1 complex, it truly provides very interesting insight into the complex editing machinery.
The paper involves number of complementary experimental techniques, which allow to articulate vey solid conclusions. Altogether, it is a very nice and high quality research paper.

Questions:

1. Can you think of a functional aspect of the transmembrane domain in MRB8620 and in other proteins involved in the mitochondrial RNA processing in T. brucei? Are there any BN-PAGE or microscopic data which would give the membrane association any functional meaning?
2. Still, the function of MRB1 complex remains rather unknown. Do you have any personal model of its function?
3. Also with regard of the unpublished data, which follow this study in the thesis, do you expect that there is some master regulator for the overall changes between glucose-rich and glucose-poor condition?

Notes (no need to respond to these during the defense):

- "While RNAi silencing of MRB8620 does not affect procyclic *T. brucei* fitness when grown in glucose-containing media, it is *somewhat* compromised in cells grown in ....." the expression "*somewhat* compromised" sounds strange, especially in the abstract to the paper.

- The following sentences need some correction:

    - "As shown in Fig. 4A, the ablation of MRB8620 results in affects the distribution of GAP1 and MRB3010 in the gradients (*cf.* Parental and MRB8620 RNAi)."
    - Nevertheless, when MRB8620 is downregulated, partner proteins GAP1, MRB11870 and MRB3010 exhibit a decreased associated with each other as a MRB1 core.

The final part of the Ph.D. thesis contains a RNA biology section of the review article "Malleable Mitochondrion of Trypanosoma brucei", 2015 in International Review of Cell and Molecular Biology, which was co-written by Zhenqui Huang. It is a nice and clear piece of text summarizing the current knowledge on the RNA editing and turnover in *T. brucei*.

To conclude, the Ph.D. thesis of Zhenqui Huang represents high quality experimental and intellectual work and as such rightfully deserves to be defended by the author on the 30th of October 2015.

Yours sincerely,

Pavel Doležal, PhD