Review of Lucie Hanzálková's Bc. Thesis

I will begin by briefly summarizing Lucie's thesis. The thesis is written in English, and it contains four major parts: Introduction, Materials and methods, Results, and Discussion. The goal of the thesis project was to characterize two related components of the mitochondrial RNA binding 1 (MRB1) complex in the protozoan parasite, *Trypanosoma brucei*. Utilizing tetracycline-regulated RNA interference, Lucie downregulates the levels of MP100 and MP102 separately in procyclic form of *T. brucei*, and characterizes the extent of knockdown and effect on cell growth. Lucie also characterizes a cell line generated by another Lukes laboratory member in which MP100 and MP102 have been simultaneously downregulated. Using quantitative RT-PCR (qRT-PCR), she analyzes the effects of MP100/102 depletion on the levels of several classes of mitochondrial RNAs. The studies utilize sophisticated molecular biology approaches and the discussion attempts to address some complicated aspects of the Results. In all, the work is of high quality for a Bachelor's student. Below I summarize my remarks and questions.

Remarks

1. Regarding the terminology used for various classes of RNAs (section 1.2.1): Lucie indicates that RNAs with limited editing (e.g. MURF2) are also referred to as "partially edited". My understanding is that "partially edited" refers to RNAs whose editing is not yet complete, such as pan-edited RNAs edited at their 3', but not at their 5', ends. Similarly, Lucie indicates that "never edited" RNAs can also be referred to as "unedited". My understanding is that "unedited" refers to the versions of edited RNAs that have not yet begun editing (also called pre-edited). Lucie should provide references for the terminology she uses, or alternatively, clarify this section of Introduction to be in line with generally used terminology.

2. Regarding Figs. 10, 11, and 12, Lucie should indicate to the left of each figure the sizes of at least some of the markers, so the reader can adequately judge the sizes of the relevant bands on the gels.

3. Section 3.3. This section should begin with a statement about why the focus was changed to the double knockdown.

4. Section 3.3.4. Lucie should include in the text a statement about bthe extent of knockdown of MP100 and MP102 as defined by qRT-PCR.

5. Lucie has generated a model to explain the decrease in the levels of pre-edited CO3 RNA. I found this description confusing, and believe it could benefit from a diagram.

Questions:

1. In her Introduction, Lucie states that the effect of downregulating the MRB1 component, GAP1/2, on RNA editing is due to a direct effect on steady-state levels of gRNAs. What is the evidence for this? Is it possible that the depletion of gRNAs in GAP1/2 knockdown cell

lines is an indirect effect – that is, that the primary function of GAP1/2 is not simply gRNA stabilization?

2. In Fig. 11, why are the inserts not visible in the gel?

3. By western blot analysis with anti-MP100 antibodies, Lucie identifies a reactive protein that is more than twice the expected size. Because this protein is downregulated upon tetracycline induction in both MP100 single and MP100/102 double RNAi lines, Lucie logically concludes that it corresponds to the MP100 protein. What are the possible reasons why the protein appears so much larger than expected? What experiments could Lucie do to address these possibilities?

4. Lucie speculates on a role for MP100/102 in **cleavage** of the maxicircle transcript, but also states the proteins likely affect "the **editing** that takes place on sites that are then recognized as cleavage sites". These ideas seem somewhat opposed. Does she hypothesize that the proteins are directly involved in cleavage, editing, or both? Or are some of the proposed effects indirect? Please clarify the model and approaches that could be used to test it.

In summary, Lucie Hanzálková's thesis fulfills all of the criteria for a Bachelor's thesis, and I recommend her for the Bachelor's degree.