Supervisor's Evaluation of Adéla Křižová

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Adéla decided to do her bachelor's thesis project in our laboratory October 2012. She immediately impressed me from this time by her work ethic and motivation, coming to the lab as much as possible to work on her project despite her very busy academic schedule here in České Budějovice. She willingly worked full time in the lab in the summer of 2013, before continuing her studies in the fall of that year. During this time she proved once again to be diligent and motivated in the lab.

Her project over the summer was to create a knockout of the gene *MRB8620* in *Trypanosoma brucei* in collaboration with her co-supervisor Zhenqiu Huang, a doctoral student in the lab. This was to test the hypothesis that the gene is essential for the viability of the parasite. Our weapon of choice for functional studies has been RNAi-silencing, and when applied to other subunits of the MRB1 complex has proven to be debilitating for the trypanosomes. Strangely, when MRB8620 was silenced, there was no such effect. After determining that the MRB8620 RNA was indeed degraded upon RNAi-induction, we thought that perhaps the protein is not essential for *T. brucei*.

However, Adéla and Zhenqiu's efforts to create the knockout were not successful, suggesting that the MRB8620 is not expendable after all. While this is not unexpected, it does not help to address the function of the protein. Luckily, Adéla and Zhenqiu decided to try a different route and try the RNAi-silencing again under glucose-poor culturing conditions. This was the breakthrough in the project as they did observe growth inhibition upon MRB8620-silencing. More importantly, this information was useful as it allowed them to choose an appropriate time point for phenotypic analyses of the cell lines. To this end, Adéla learned how to isolate RNA from cells, create cDNA and then apply real time PCR to this template to assay an effect on the RNA editing process, which the MRB1 complex plays an important role. This point is particularly important as it demonstrates another excellent quality that Adéla has, which is the motivation to learn new techniques. She has done so in collaboration with Zhenqiu for other aspects of the project that are not directly reported in her thesis, mostly in the summer of 2014.

To conclude, I am very satisfied with Adéla's performance in the lab. While I think Adéla could have started writing her thesis sooner, I admire that she was able to turn it in in time. This was not easy as close to the deadline, her laptop crashed and she was unable to work on it during a majority of this critical time. However, she refused to give up and insisted that she turn in her thesis on time. I agree with the reviewers comments about some mistakes in the thesis, but I trust that she will learn from them when she writes her master's thesis in the future. If she chooses to do her master's project in our lab, she would be most welcome.

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