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Evaluation Zhenqiu Huang

Zhenqiu was recruited by the head of the Laboratory of the Molecular Biology of Protists, Professor Julius Lukeš, who is an established researcher with experience advising many doctoral students. Thus, he had the misfortune of finding himself under my direct supervision when he came to the laboratory in August 2011. I have heard that a few times from others that the first doctoral student is painful for the advisor and especially for the student.

However, this situation did not dampen Zhenqiu's enthusiasm when he entered the lab and kindly accepted me as his supervisor. The project that he undertook was an ambitious one trying to YFP-tag nuclear-encoded proteins predicted to be targeted to the mitochondrion to further refine our picture of the *Trypanosome brucei* mitoproteome and also look for interesting localization patterns within the organelle. This approach had many problems: a hoped for high-throughput approach to creating these constructs for *in situ* tagging did not yield sufficient transformants; those proteins that were successfully tagged were so lowly expressed, they could not be visualized directly by YFP fluorescence. To make a long story short, the scope was reduced from the whole mitoproteome to those proteins involved in RNA editing, which I knew something about. Zhenqiu became interested in using FRET to map interactions among the protein involved in this process. Unfortunately, this approach was hampered by strange Western signals, decreasing confidence that the platform he developed would be tractable within the time of his doctoral studies.

So the focus of the project was further narrowed to developing FRAP to study the dynamics of mitochondrial RNA-binding proteins in live cells by FRAP. But of course, there were problems, not the least that *T. brucei* is a highly motile organism and this sensitive technique requires stationary cells; and if they stop moving, they die. Zhenqiu developed a simple and economical way to immobilize these cells in a viable state and was able to show in an elegant experiment that mitochondrial transcripts, the substrate of mitochondrial RNA processing also serves as a scaffold for keeping the machinery together. This simple technique generated a lot of interest from other trypanosome researchers when it was presented at a British Society for Parasitology conference.

Zhenqiu was in the unenviable position of establishing a completely new technique in a laboratory that lacked the know-how for doing this. So he had to tackle many of the enumerated problems at once more or less by himself. In my opinion, the outcome of his first first-author publication in his thesis is a testament that he is able to overcome what seemed like insurmountable problems.

After this paper was accepted he had a year and a half left in his stipend-supported doctoral studies. To have him do something in line with my knowhow, he resurrected a project studying the MRB8620 protein, which is part of the MRB1 complex involved in RNA editing. This project lay dormant for years since it was stubbornly intractable to RNAi, the bread and butter of our laboratory. He was able to not only successfully determine that it too is involved in the process, but was able to widen the scope of the project to show how the MRB1 core interacts with the GAP1/2 gRNA binding complex, which had been thought to be a more stable part of this core as well. He presents an interesting paradigm on how the core in conjunction

with GAP1/2 works in the RNA editing process. This was exclusively his idea, which initially met a lot of resistance by me his supervisor.

This brings me to what I think is one of the defining characteristics of Zhenqiu: he is a stubborn guy. It was quite a battle to convince him to focus on the FRAP studies that did yield and leave the other aspects of the projects so he would not fall into the abyss of what I admit know was a poorly-conceived project. He simply did not want to give up as he saw how potentially interesting such a study would be. He stuck to his guns when he suggested the coolest experiment in his second first-author manuscript: the RNA IP. I thought it would not work and this turned out to be critical in creating the nice and creative model that concludes the paper. And he persevered in including that model into the paper, which turned out to be an excellent decision.

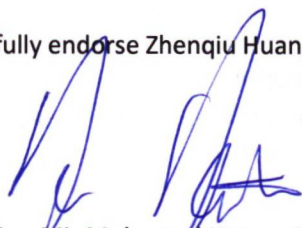
So his stubbornness was in the end excellent quality that allowed his vision to come through his work. The main barrier for me in accepting his ideas more often and readily was his lapse in attention to certain details. While he often point out things I had overlooked in our conversations, he sometimes also based certain thoughts on factual and procedural errors. My advice is for him to check over his work when it is finished and also to pay more attention when he gets information from papers, presentations and conversations with colleagues. However, I freely admit, this is advice I should also take as well.

Overall, I think that Zhenqiu was a great collaborator and a hard-working person who does make the effort to think about the research he is performing instead of just going through the motion of pipetting. He got along well with his colleagues in the lab, and was the co-advisor of two bachelor's students, Sabine Katlenbrunner and Aléla Křížová, whom he supervised in the lab directly. They both praised his supervision.

Because he is not one to wait around, he is now trying to optimize iCLIP to characterize RNAs bound to a mitochondrial Nudix Hydrolase, which appears to also have a role in RNA stability in the organelle. However, due to the very preliminary nature of this data so far, it was not included in his final PhD thesis.

I would also like to point out that he did his doctoral studies in a rather unique situation, where he was the only representative of his homeland in the vicinity. In my opinion, this is especially unique among his countrymen, where Chinese communities are often prevalent near academic institutions. I imagine doing a difficult doctoral study under such isolated conditions can be quite a hardship. Yet, as we see today, he persevered.

I fully endorse Zhenqiu Huang for the title Ph.D.



Mgr. Mir Mohamod Hassan Hashimi

In České Budějovice, October 30, 2015.