Biology Centre, Academy of Sciences
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Supervisor' review of dissertation thesis by Zdeněk Paris

It was quite surprising when I realized recently that Zdeněk is member of our lab for incredible 9 years. Called big Zdeněk, he arrived to our lab with his fellow small Zdeněk right after their enrolment into the 1st grade and stayed until now. I hope he does not regret it. When he joined, we were a small lab with very limited resources and with quite hazy vision of what to do. Consequently, in frame of his bachelor and master theses, Zdeněk participated in numerous projects in the lab, including mRNA stability and the influence of pre-ATG motifs on translation efficiency, functional analysis of the La protein and some other minor projects.

Again in tandem with small Zdeněk, they boldly went into the PhD with us and started to dissect the function of respiratory complexes. Zdeněk spend hell of a time by screening trypanosome RNA interference library obtained from the Englund lab with the aim of sorting knock-down cells with decreased mitochondrial membrane potential. Using reverse genetics, we hoped to identify novel components of the respirasome using this approach. This was a challenging project (at that time fancy too, but now we know that similar approaches led to failures in most labs) and Zdeněk eventually made it work, but the results were not worth the efforts, to put it nicely. In another project, he initiated functional analysis of knock down cell lines for several subunits of the *T. brucei* respirasome identified by Alena and co-workers at Seattle Biomed. This project fared much better and the results of Zdeněk and several co-workers in Budweis and Bratislava are now being wrapped up by Alena and will soon make a nice story.

However, a real breakthrough came when one day in the end of 2006 I picked the phone and on the other side of the line an English-speaking guy with heavy Spanish diction asked about our just published Nfs knock downs. As it immediately turned out, it was here present Juan Alfonzo, whose enthusiasm quickly started taking us in a direction no-one here knew anything about, namely tRNA metabolism, their import etc. I liked it from the start and had a feeling, confirmed now, that this will be an exceptionally pleasant and productive collaboration, but I was also not sure what to do next. For one reason or another, Zdeněk felt this was his chance, unhesitatingly stepped out of the line and threw himself fully into the project. He became the tRNA guy in the lab and we soon realized that it will take us ages to learn at least the basic yet still

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rather sophisticated methods in the field, and so we gladly nodded to Juan's proposal to teach Zdeněk in his and Mary Anne's lab in Columbus.

Zdeněk left with somewhat mixed feelings, since his English was still far from impecable and he was questioning his qualification for such an endeavor which, given the background he obtained in our lab, was more than justified. However, already few days after his arrival, it was clear it will work out just fine. Mary Anne and Juan gave him enormous attention both inside and outside of the lab and thanks to them, Zdeněk was able to follow individual tRNA species in various cell compartments, to track down their screwed modifications, and disentangle how these relate to localization, Fe-S cluster assembly and so on.

There was also, mainly thanks to Zdeněk and Juan, no shortage of ideas and even bold projects. This led us to investigate several possibilities which the rather complementary expertise of our labs allowed us to address. Consequently, Zdeněk investigated what happens with tRNA import and editing in knock-down cell lines made in our lab in frame of a different project and got some important discoveries that way. Numerous results obtained by an Indian group on *Leishmania* and published in top journals including Science unfortunately started for most of the community to slowly and steadily move into the hard-to-believe category, and Zdeněk embarked on a path to figure out what's going on. He was able to show, and publish well, that most of the claims made for *Leishmania* did not hold in *T. brucei*, which was rather suspicious, and so we were not that surprised when a few months ago an "Expression of concern" was published by the Editor-in-Chief of PNAS concerning some of the results obtained with *Leishmania* tRNA metabolism. This indicates that in the years to come, Zdeněk's work on the *T. brucei* tRNAs will likely be even more authoritative than it is now. In the most recent project, Zdeněk and Hassan used the unique features of the akinetoplastic *T. brucei evansi* cells to shed light on tRNA import and modifications in the bloodstream stage of *T. brucei* and in a smart way to put final nail in the coffin of the quite long-standing paradigm that the mitochondrial tRNA import in trypanosomes depends on respiratory complexes.

Following his first stay in the Alfonzo lab, Zdeněk wanted to spend the next summer there too, and I was encouraging that. And not surprisingly anymore, he wants to spend not only the next two to three summers but years in the same place, and as far as I know that may, depending on the outcome of today's defense, well happen.

During all those years, Zdeněk became an almost indispensable member of our lab. From a somewhat withdrawn and conservative position, he was

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slowly yet steadily moving into the forefront, and his performance matured with him like an old wine. Lately, almost each of his experiments yielded (and I hope will still yield in the remaining months in our lab) publishable results and it must be a joy to work so efficiently at the bench as he does. Moreover, as a senior PhD student in our lab, Zdeněk has been invaluable in helping other students in planning and execution of their experiments, data presentations etc. On a daily basis he supervised Piya Changmai, now another PhD student in the lab, was always willing to share his expertise and was a frequent contributor to our lab meetings.

In my opinion, Zdeněk is a gifted scientist and promising teacher, and I hope by arriving to a similar conclusion, he will sort in his mind out where his future lies.

I would like to state that the presented dissertation thesis fulfils, according to my opinion, all postulations and I recommend it to be accepted as a partial fulfillment of the requirements for the degree of Doctor of Philosophy at the Faculty of Biology of the University of South Bohemia.

Thanks Zdeňku for your friendship and great work.

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