

Posudek školitele specialisty na magisterskou práci Zdeňky Čičové

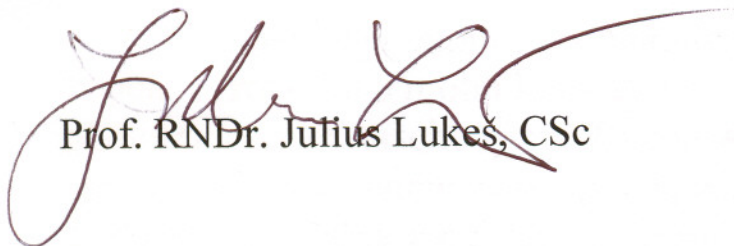
Zdeňka nastoupila do laboratoře molekulární parazitologie během 1. ročníku a mohu konstatovat, že se od svého příchodu stala platným členem našeho týmu. Rychle se naučila běžné metody a ve spolupráci s Hassanem se pustila do rozluštění funkce proteinu REAP-1. Po spoustě práce jsme zjistili, že publikované údaje, z nichž jsme vycházeli, jsou do značné míry chybné a navíc že jiná laboratoř publikovala prakticky totožné výsledky v době, kdy jsme studii uzavírali.

To ovšem nebyla jediná bolestná zkušenost Zdeňky. V dalším projektu se opět s Hassanem, s nímž založili dynamický tým, a ve spolupráci s oběma Luckami a Yan-Ze Wen rozhodli přijít na kloub funkci námi a jinými laboratoři čerstvě objeveného proteinového komplexu MRB. Výsledky, které za použití řady sofistikovaných molekulárních metod Zdeňka a spol. získávali, byly velmi zajímavé až vzrušující a podařilo se jim cíl splnit. Sen publikovat tato data ve velmi prestižním časopise se bohužel rozplynul ve chvíli, kdy si Zdeňka a Hassan loni vyslechli přednášku na podzimní konferenci ve Woods Hole, v níž byla kalifornskou laboratoří publikován příběh v mnohém se s naším překrývající. Po řádně stresujícím období se nám ale podařilo i tak slušně publikovat a Zdeňka má na přiloženém článku opravdu lví podíl.

V současnosti sepisuje Zdeňka ve spolupráci s několika z nás vyžádanou knižní kapitolu o kinetoplastu a bude spoluautorkou ještě nejméně jedné silné publikace o podjednotkách MRB komplexu. Zdeňka byla úspěšná v hledání PhD pozice a bude ve své velmi slibně se rozvíjející vědecké kariéře pokračovat v Mnichově. Předtím je ale ještě připravená nám velkoryse pomoci s Y2H systémem na již zmiňovaném MRB komplexu.

Zdeňka je jednoznačně nadprůměrná studentka jak svým zájmem o vědu, rychlým chápáním problémů, navrhováním řešení a vynikající mluvenou angličtinou (v té písemné už je také na dobré cestě) a schopností psát kvalitní vědecké texty již v magisterském stupni studia. Její účast v naší laboratoři považuji za skvělou a přeji jí to nejlepší v další vědecké kariéře.

V Č. Budějovicích 27.5. 2009

A handwritten signature in dark ink, appearing to read 'Julius Lukeš', with a long, sweeping horizontal stroke extending to the right.

Prof. RNDr. Julius Lukeš, CSc



Supervisor's evaluation of Zdeňka Čičová's master's project: "Functional analysis of subunits of mitochondrial RNA binding 1 in *Trypanosoma brucei*"

Zdeňka joined the Laboratory of the Molecular Biology of Protists in October 2004 at the beginning of her bachelor's studies. Thus began her scientific career: partnered with an inexperienced grad student (me) working in the obscure field of RNA editing in trypanosomes on what turned out to be a doomed project working on a protein called REAP1. Her bachelor's project uncovered that the data presented in previous publications describing its discovery and central role in editing were actually bogus, which was little reward for a tremendous amount of solid work.

Despite this outcome, Zdeňka decided to continue with her master's studies in our laboratory, with the same green supervisor (me) working in the same obscure field. We just hoped that the new project would be less disappointing. We had isolated a putative protein complex, which was eventually dubbed the mitochondrial RNA complex 1 (MRB1), and had absolutely no idea what its function was. Zdeňka was assigned the task of generating RNAi knockdown cell lines of two of the putative MRB1 subunits, a predicted RNA helicase and NUDIX hydrolase. She designed primers for amplifying gene fragments to be cloned into constructs for generating dsRNA *in vivo*, making sure they would silence only the intended target. She used sequencing data to verify the identity of the fragments as well as Northern analysis on RNA harvested from pilot experiments to select clones exhibiting the most robust downregulation of the targeted transcript and/or dsRNA expression. After meticulously choosing the appropriate clone and experimental time-point, based on when growth is inhibited in the knockdowns, she performed a real-time PCR-based assay of RNA editing to determine silencing of these genes influences this process. The simple bar graph presented in the thesis belies the work of RNA isolation, DNase treatment, verification of the effectiveness of said treatment, cDNA generation and the wrist-melting amount of pipetting needed to perform the PCR as well as the intricate steps to extract and analyze the data. These efforts led us to the exciting conclusion that a subset of this complex was involved in the biogenesis of small guide RNAs, required for RNA editing.

But there were some open questions: what exactly was the function of the complex? What was the actual composition of the complex, since not all the subunits appear to have a role associated with gRNAs? If we



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answered these questions, we could aim for a top journal. Zdeňka agreed to the next task, considerably more ambitious task, which was to map protein-protein interactions within MRB1 by yeast-two-hybrid analysis. She poured her energy and enthusiasm into diligently researching the literature about this procedure, collecting reagents and strains, learning protocols and began to PCR amplification of the 14 putative MRB1 subunits for cloning into the bait and prey vectors. However, things took a turn for the worse: certain ORFs were not easily amplified, most were not easily subcloned, and many proved to be difficult to sequence. And her supervisor didn't help much since he too preoccupied, struggling with experiments tackling the function of MRB1.

It was a frustrating and unfruitful summer that led into fall, when Zdeňka and I went to the annual Molecular Parasitology Meeting where we presented the data we had generated thus far about MRB1 and a some time to refresh ourselves. At her first international conference, we made the rather shocking discovery that another group has not only arrived to a similar conclusion, but had their article already in press to the very same top journal we were aiming for. Needless to say, this was a heartbreaking experience for the both of us, and we had to deal with the unpleasantness of being scooped during the whole conference. Zdeňka handled this huge disappointment by keeping her head up even with a heavy heart, and even coaxing data from the rival group. Upon returning from the conference and rushing the story we had to another journal, she was instrumental in putting together figures for the article that comprises the core of her thesis.

I remember when I first met her at one of the lab parties that Julius hosted in his home, my first impression was that of a shy, quiet girl. When we next met in the lab to talk about her project I was surprised to discover her incredible facility with the English language. At the ill-fated Woods Hole meeting, as we walked along the beach-lined streets, she revealed to me that she acquired her English by overcoming her shyness and harsh, unsupportive teachers. This is why I remunerate the gauntlet of struggles she went through since entering the lab, to underscore the qualities that she posses that cannot be assessed in her thesis or during her defense. Despite all the demotivating setbacks, she remained engaged in her project and efforts for self-improvement. This attitude requires perseverance and fortitude to overcome unpleasant obstacles, and was something that even helped me get over these difficult times. She has experienced the harsh realities of science--pouring considerable efforts into an unsuccessful project, getting scooped--before even being a doctoral student. In the fall, she will be matriculating into the prestigious Max Plank Research School for Molecular and Life Sciences in Munich, Germany, where she will pursue her PhD.



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Our frequent discussions about the state of the field and recent papers, as well as her tenacity in defending her opinions, were also refreshing and enlightening for me. Thus, she has demonstrated a drive to keep up with the literature as well as an ability to think about the enclosed data. Although, I suppose I should mention that she has problems synthesizing the information, although I suppose this is understandable in a field with such disparate nomenclature and data. I have no doubt this will improve in during the course of her PhD work thanks to the qualities I have just described. On a positive note, the valuable information she obtained on improving the construction of the MRB1 yeast two hybrid library is now being implemented as we currently have resurrected this project in collaboration with an American group.

I would like to conclude by thanking Zdeňka for her efforts as well as patience with an inexperienced supervisor. Teaching her has been one of the most rewarding parts of my scientific career. I wish her much success in her future endeavors and it has been a real pleasure and honor to work with her for these five years. I feel that not only has she fulfilled the requirements for being awarded with a Master's degree, she also possesses those intangibles that gives her the potential to be an independent researcher if she so chooses.

In České Budějovice, the 29th of May, 2009.

Hassan Hashimi, PhD