



Faculty of Science

University of South Bohemia

Branisovska 31, 37005 České Budejovice, Czech Republic

Tomas Dolezal, Ph.D. – Department of Molecular Biology

Phone: 387772229, E-mail: tomas.dolezal@prf.jcu.cz

Mentor's statement to Ph.D. thesis of Milena Nováková

Milena Nováková joined our group in 2006 - right in the beginning when the laboratory was established, and took up an ambitious Ph.D. project aimed to study the regulation of extracellular adenosine by adenosine deaminase in vivo using *Drosophila* as a model. To do this, Milena was supposed to create an expression reporter for the main *Drosophila* adenosine deaminase called ADGF-A. The easiest and therefore the most common way to create an expression reporter is to place a coding sequence of a reporter (for example GFP) behind a promoter sequence of the studied gene and make a transgenic fly carrying this construct. However, with this way you can not be sure that the reporter truly marks the endogenous expression since the regulatory sequence might not be complete. Therefore we decided to undertake a different procedure using homologous recombination to precisely replace the endogenous ADGF-A coding sequence with the reporter to preserve all regulatory sequences intact. This procedure is of course much more laborious. Milena spent lot of time just cloning quite complicated construct which she finally injected in the fly embryos by herself to create a transgenic fly. The construct was large and she was able to make the fly – the commercial services actually do not guarantee for the money spent a successful transgenesis with constructs of this size. After preparing the transgenic line, another laborious part followed – to induce homologous recombination by multistep genetic crossing and molecular verifications of the results. Milena went through all this successfully - not without numerous obstacles – and she prepared the reporter line exactly as she was supposed to. I should stress here that she did it because she is undoubtedly a hard worker and she would never give up although it certainly “shortened her telomeres significantly” since she is not always the calmest person. One would probably ask here if it was really worthy to go this way – one of the most complicated one taking nerves of my student – it is slowly becoming typical for my lab. I just do not want to go always the easiest way, especially if it is predetermined from the beginning to be too far away from the reality. I think it paid off in case of Milena's work – at the end we got exciting results with this reporter which are described in her publication in respected PLoS One journal and which, I am convinced, are reflecting the reality. Her work nicely connects other results from our lab and shifts the whole project studying the extracellular adenosine in flies to a new level. I am proud of her work and I am really glad that we undertook it although my telomeres are most likely shortened little bit too.

In České Budějovice, April 28th 2011

Mgr. Tomáš Doležal, Ph.D