



Supervisor's evaluation on PhD student Lenka Bučinská

Lenka's PhD studies started in 2011 and her project was focused on the biogenesis of chlorophyll-binding proteins (core components of photosystems) in cyanobacteria. During the project, the original topic was extended on the biogenesis of the whole thylakoid membrane, as we have been more and more convinced that availability of chlorophyll in the cell and the production of certain chlorophyll-proteins (core subunits of photosystem I) is tightly linked to the abundance of thylakoid membrane. Lenka came to laboratory already as a specialist on electron microscopy and, from the beginning, the project was designed to employ microscopy techniques in combination with molecular biology and genetics. Such plan was indeed bold as I'm well trained in molecular biology or biochemistry, but my knowledge of electron microscopy was completely poor. Here, I have to thank Jana Nebesářová, who served as a consultant supporting Lenka's work a lot and helping with technical issues.

The main scientific question we have been interested throughout the Lenka's PhD project sounds: at what place in the cell chlorophyll-binding proteins are produced and how such a site is organized on the molecular level. The original strategy was based on using of nanogold labelling to localize enzymes in chlorophyll biosynthesis and various protein factors involved in the biogenesis of photosystems. Apart from this, Lenka analyzed mutant cells with broken or misregulated chlorophyll biosynthesis, which brought her three strong co-author publications. At the molecular level we tried to isolate unassembled chlorophyll-proteins to see what are their protein partners. And it was a successful strategy. Lenka has identified a number of putative factors involved in the biogenesis of chlorophyll-binding proteins. We selected one such candidate, a protein named Pam68, and used it as a bait for a pulldown assay. The obtained results were beyond our expectations because with Pam68 we co-isolated the whole SecY translocon system with ribosome attached, with nascent chain of chlorophyll-protein

CP47 and many other protein components. These results provide a first direct evidence that chlorophyll molecules are loaded into apoproteins immediately in the vicinity of translocon and it is a first real insight of how this machinery is organized. On the quality level, my ranking of this particular Lenka's work is very high, perhaps belonging to best five publications produced in my group (in 15 years). We have been less successful with nanogold labelling though Lenka developed a reliable protocol. Nonetheless, we have later focused on the biogenesis of thylakoid membranes in *Synechocystis* cells during recovery from long term dormancy and the results, which are included in the thesis as a manuscript, look as a potential breakthrough.

Overall, it is a nice thesis and I like it because it presents more than just publications put together. However, there was a problem I have to point out - it took so long. Seven and half year is too much. I have my own part of responsibility because the resubmission of Pam68 story into Plant Cell journal has not been apparently prepared well enough and finally rejected. We lost half year. However, Lenka's PhD studies would take long anyway. First, Lenka has been writing her first author manuscript as well as the thesis so incredibly slow that it was a constant source of frustration. The second point is that Lenka is very friendly person, willing to help and collaborate with people around with such a passion reaching self-destructive altruism. It is difficult to be focused on the own thesis than and things go slowly. On the other hand, Lenka is author or co-author of eight publications and another first-author manuscript is ready for submission. In addition, Lenka is already a respected expert on microscopy but she is also skilful in many techniques of molecular biology and biochemistry; it is quite rare combination and, all together, it is an excuse for being late.

The list of Lenka's achievements is surely long enough to meet the criteria for the PhD degree and I'm looking forward to meet her tomorrow in the laboratory as a new postdoc.

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Roman Sobotka

