



Supervisor's report on Petra Skotnicová and her PhD Thesis

From the beginning, the subject of the Petra's Thesis was "Assembly of photosynthetic complexes and regulation of the tetrapyrrole biosynthetic pathway" and apparently the subject was broad enough so in a course of her study we did not have to modify or change it.

Petra's PhD study started quite a long time ago, in the fall of 2011. It was about that time when it became clear that the last missing protein in the tetrapyrrole pathway in most cyanobacteria and many other organisms is HemJ. Unlike other groups trying (and failing) to express this hydrophobic protein heterologously, she isolated the tagged version of the protein expressed homologously in our work-horse model organism *Synechocystis* 6803. She was the first to show that this protein is binding heme and that this heme is probably involved in enzymatic activity. Unfortunately, we could not measure the enzyme activity, indicating that something from the picture is still missing.

Interesting was the complementation of the HemJ mutant by a nonhomologous enzyme from *E.coli*, by HemG, non-homologous PPO from *E.coli*. Accumulation of high levels of the intermediate of the previous reaction showed a functional coupling between the two enzymes. This part of Petra's work has been concluded in the JBC paper. Despite the collaborative parts dealing with identification of the unknown metabolite and HemJ structure modeling, this is primarily Petra's paper.

Another project connected to the tetrapyrrole pathway was Petra's study of the BtpA protein previously (wrongly) characterized as a membrane biogenesis protein. Indeed, the BtpA mutant did not accumulate any thylakoid membranes, but as Petra showed, the primary reason for the lack of membranes was the lack of chlorophyll. By employing pull-down assays, Petra showed that BtpA interacts with GluTR, an enzyme at the beginning of the tetrapyrrole pathway. This finding was nicely complemented by an approach very close to my heart, localization of suppressor mutations complementing the phenotype of the original BtpA mutant. Here, several suppressor mutations again pointed to GluTR. Overall, this is a very nice story changing our view of the role of the whole protein family. Writing this chapter surely helped Petra to organize her data and by the time of defense this part should be in the form of a manuscript.

The connection to the assembly of photosynthetic complexes is provided by the third project dealing with curvature thylakoid protein (CurT) involved in thylakoid organization and probably also in formation of biogenesis centers where photosynthetic complexes may be assembled. Again, using pull-down assay Petra showed that some proteins important for Photosystem II assembly are associated with CurT. Connection between CurT and Photosystem II assembly was further strengthened by the fact that this indispensable protein can be smoothly deleted in the mutants not assembling Photosystem II. Interesting finding was that CurT was co-purified with several enzymes of the tetrapyrrole pathway, showing that logical and expected colocalization of chlorophyll synthesis and delivery into the assembling Photosystem II may be real.

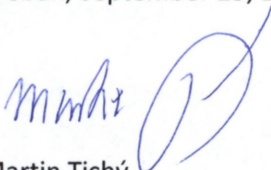
Besides this, Petra spent quite some time in constructing and testing various fluorescent protein tags and in our group she is the expert in confocal microscopy. Also if you need a particular strain, you ask Petra, because there is a good chance she is keeping it on a plate. From all this it should be clear that Petra loves to work in the lab. Most experiments she designs makes good sense. Petra is independent, determined, persistent, relentless, in one word stubborn. If the experiment makes little sense, it is generally easier to let her do it than to persuade is that she should not.

Similar stubbornness is apparent also in Petra's scientific writing. For some reason, probably based on plagiarism issues she thinks that each sentence needs to be an original. I am definitely not a person who should dare to criticize anybody else on writing but sometimes it was a battle to produce standard readable and understandable text. I hope that Julian's review will point out if we succeeded.

Apart from last few sentences, Petra clearly deserves the PhD degree as I am pretty sure that she on the right track to become a great scientist.

Thank you Petra for your work and I wish you great success in your future career.

Třeboň, September 25, 2019


Martin Tichý