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Review on the doctoral thesis

"Evolution of euglenid plastid proteome"

submitted by Mgr. Anna Novák Vanclová

Although in the center of several scientific projects, euglenophytes are still enigmatic unicellular organisms. In case of the phototrophic taxa, they harbor a plastid of most likely secondary origin, which is, as one of the enigmatic characters, surrounded by three membranes. Unfortunately, only incomplete data in respect to genomics, transcriptomics and proteomics were so far elaborated, which especially holds true for the plastid of euglenophytes. This changed by the work of the Hampl group and Anna Novák Vanclová contributed a lot with her excellent work.

Anna Novák Vanclová submitted a cumulative doctoral thesis, in which four high impact papers are central. In the time of submitting the thesis, three of the manuscripts were already published and one was under review. However, the latter is now accepted for publication. As therefore all of the manuscripts are peer-reviewed by international scientists and accepted, it is obvious that the quality of the manuscripts and therefore that of the doctoral thesis is very high.

The thesis starts with a very informative introduction on euglenophytes and the evolution, morphology and functions of plastids in general, and describes the knowledge of these fields in a very complete manner. By reading that, I had the impression that this high-quality introduction could be easily "transformed" for an

informative review, especially in respect to the general characteristics of primary and secondary plastids. Moreover, a chapter on the general methods of proteomics is part of the introduction, again composed in a very elegant manner.

The next major parts of the thesis are the four already mentioned publications to which Anna Novák Vanclová contributed significantly.

The first manuscript, in which Anna Novák Vanclová is the first author, has review character and provides a state-of-the-art recapitulation of the knowledge on the evolution, structure and especially the physiological capacity/biochemistry of the plastid of euglenophytes.

In the second paper, to which the candidate contributed as an author, the transcriptome of a secondarily non-photosynthetic euglenophyte, *Euglena longa*, was determined, analyzed and compared to existing data on euglenophytes. Important results are, beside others, related to the plastid protein import apparatus and plastid division as well as to a very interesting case of horizontal gene transfer.

In the third paper, a "multi author paper", the phototrophic *Euglena gracilis* was in the focus. Here the authors published the transcriptome and partial genome for *E. gracilis* Z1. Having this as the basis, detailed analyses on the capacities of *E. gracilis* were possible and Anna Novák Vanclová was involved in the *in silico* prediction and annotation of the plastid proteome from transcriptomic data.

The fourth paper, now being published as final form Early View, has the wonderful title "Metabolic quirks and the colourful history of the *Euglena gracilis* secondary plastid". Anna Novák Vanclová is the first author and was mainly involved in the "annotation of the mass spectrometry-based plastid proteome, reconstruction of metabolic pathways, and analysis of the plastid-targeting signals" (according to the description in the thesis). In this manuscript subcellular fractionations in combination with mass-spectrometry helped to elucidate the composition of the plastid proteome (1345 plastid protein groups) of *E. gracilis*, which was the basis for their characterization. By this strategy extremely important data were generated, which includes, beside others, the non-expected finding of at least 100 proteins that were

acquired by horizontal acquisitions from non-green algae or prokaryotes. Additionally, my favorite findings are, beside many other important ones, the analyses of Fe/S cluster biosynthesis systems and the detection of Derlin counterparts, which might have replaced a TOC system.

In summary, a lot of very important data were created by the Hampl-group and Anna Novák Vanclová contributed substantially to that. As in addition the doctoral thesis is exceptional and very well written, Anna Novák Vanclová met all the criteria to assign her with a PhD. Moreover, at my university, I would award her thesis with an excellent grade and additionally assigning it with the honor "summa cum laude", which is the best grade that can be achieved.

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