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Evolution of euglenid plastid proteome

Evoluce proteomu plastidu euglenidů

Summary of the doctoral thesis

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ABSTRACT

Endosymbiotic gain and transfer of plastids is a widespread evolutionary phenomenon and a major driving force of eukaryotic evolution. The integration of a new organelle is accompanied by changes in its structure, gene content, molecular mechanisms for biogenesis and transport, and re-wiring of the host and organelle metabolic pathways. To understand the course and underlying mechanisms of plastid evolution, it is important to study these processes in variety of secondary algae and notice their differences and similarities.

Euglenophytes gained their plastids from green eukaryotic algae after a long history of heterotrophic lifestyle. In my thesis, I participated in analyses of newly generated sequence datasets: transcriptomes of *Euglena gracilis* and *Euglena longa* and mass spectrometry-determined proteome of *E. gracilis* plastid with especial regard to the potential novelties associated with plastid gain and incorporation. In the resulting publications we particularly focus on plastid protein import machinery and targeting signals and report extremely reduced TIC and completely absent TOC in euglenophyte plastid. Using the proteomic dataset, we predict potential novel plastid protein translocases recruited from ER/Golgi and re-analyze plastid signal domains, characterizing previously overlooked features. Protein inventory of *E. gracilis* plastid suggests complex, in some cases redundant metabolic capacity. Chlorophyll recycling is one of the sources of phytol for reactions not connected to MEP/DOXP pathway. Plastid contribution to amino acid metabolism is very low, if any. We screen the proteome for proteins of other than green algal phylogenetic affiliation and report substantial contribution from "chromists" as well as several cases of LGT from bacteria, including an acquisition of additional SUF pathway.

In summary, the work presented in this thesis provides a solid contribution to plastid proteomics, resource for both basic and applied *Euglena* research and potential foundation for various follow-up studies.

1 INTRODUCTION

Plastids *sensu stricto* arose through one primary endosymbiotic event but diversified and spread horizontally from eukaryote to eukaryote. There were at least three instances of secondary endosymbiosis between a heterotrophic eukaryote and green or red alga (giving rise to chlorarachniophytes, euglenophytes, and likely cryptophytes), followed by tertiary, quarternary, and possibly even higher-tier transfers (resulting in haptophytes, ochrophytes, chromerids and apicomplexans, and many different lineages of dinoflagellates) [1–8]. Such complex evolutionary history leaves substantial traces in structure, biogenesis, and functional capacity of these organelles. By studying similarities and differences between plastids originating from independent endosymbiotic events, we try to deepen the knowledge on global evolution of eukaryotic organelles and, by extension, eukaryotes as a whole.

Euglenophytes represent a group of widespread freshwater photosynthetic organisms bearing secondary green plastids derived from pyramimonadalean alga [9] and surrounded by three membranes [10]. Diversity [11,12], ultrastructure [13], plastid genomes and genetics [14–24], and biochemistry [25–32] of these organisms is well-studied but difficulties in obtaining genome sequence [33] and genetic transformants [34–36] hampers the efforts to understand details of their molecular and cell biology and metabolic capacities. For instance, while the general pathway importing nuclear-encoded protein to plastids through ER and Golgi is understood [37,38], protein translocases mediating this route are unknown, although the process is believed to be similar to other complex plastids [39–42], especially those of peridinin dinoflagellates (also bound by three membranes), as suggested by the N-terminal signals plastid-targeting proteins possess [43–45].

In this thesis, we present broad reconstruction of putative molecular and metabolic capacities of euglenophyte plastid based on newly generated comprehensive datasets of transcripts of *E. gracilis* and *E. longa*, and mass spectrometry-determined plastid-localized proteins of *E. gracilis*.

2 AIMS

1. To annotate plastid proteome of *E. gracilis* and estimate its metabolic potential.
2. To reconstruct protein import pathway of euglenophyte plastids using transcriptomic data of *E. gracilis* and *E. longa* and proteomic data of *E. gracilis*.
3. To analyze characteristics of euglenophyte plastid-targeting domains based on the proteomic dataset determined by mass spectrometry.

3 MATERIALS AND METHODS

Materials and methods are described in detail in the respective publications. In general, DNA and RNA sequencing and liquid chromatography tandem mass-spectrometry (LC-MS/MS) proteomics were used to obtain large sequence datasets which were subsequently investigated using bioinformatic tools for homology detection, signal prediction, metabolic reconstruction, and determination of phylogenetic affiliation.

4 RESULTS AND DISCUSSION

The review chapter by Vanclová *et al.* [46] summarizes the state of knowledge regarding the evolution, structure, biogenesis, and biochemistry of euglenophyte plastids as of 2017.

E. gracilis draft genome assembly reported in Ebenezer *et al.* [47] is 300-500 Mbp in size, highly fragmented and suggesting extensive expansion of non-coding sequence (>99%). *E. gracilis* transcriptome assembly is 38 Mbp with 87.9% CEGMA recovery. Many protein families involved in signalling are highly expanded by paralog duplication. Differential transcriptomic and proteomic evidence as well as the existence of polyprotein-coding transcripts [22,48,49] suggest that gene expression regulation takes place at protein level, similar to kinetoplastids [50]. The transcriptome was used for *in silico* prediction of plastid proteome of around 1900 proteins and drawing a map of chloroplast metabolic pathways.

This was followed by LC-MS/MS of the isolated plastid and mitochondrial fractions. Chloroplast to mitochondrion (CP/MT) ratio of label-free quantified [51] proteins is used

as indicator of credibility of their organellar localization. The resulting plastid proteome reported in Novák Vanclová *et al.* [52] contains 1,345 protein groups, 43% of which could not be assigned a clear functional annotation or lacked homologs in other organisms whatsoever, suggesting a considerable potential for functional novelty and/or plasticity The reconstruction of *E. gracilis* plastid metabolism supports some enzymatic processes described or proposed previously based on biochemical evidence [26,53–55], but also brings a number of novel findings. We propose chlorophyll recycling as one of the sources of phytol for tocopherol synthesis which, as our data confirms, is not connected to plastidial MEP/DOXP pathway in *E. gracilis* [25,56]. Our data suggest very low, if any participation of plastid in amino acid metabolism of *E. gracilis*, which is unusual in phototrophic organisms. We identify additional, plastid-localized set of SUF pathway for FeS cluster assembly which is present in several other euglenophytes and likely represents horizontal acquisition from Chlamydiae or related bacteria. We also note a large proportion of proteins phylogenetically affiliated with "chromists" which supports "shopping bag" and "red carpet" model of plastid evolution [57,58].

Transcriptome was also generated for *Euglena longa*, close relative of *E. gracilis* which is secondarily non-photosynthetic but retains reduced plastid with genome. The assembly reported in Záhonová *et al.* [59] is 75 kbp with 89.1% BUSCO recovery and represents important context to the analyses of *E. gracilis* and other phototrophic euglenophytes.

Most remarkably, we report highly derived plastid protein import machinery in *E. gracilis*, *E. longa*, and the early-branching, marine *Eutreptiella gymnastica*. While the machineries importing proteins to thylakoids are generally conserved in the phototrophs and absent in *E. longa*, the expected translocases of plastid envelope, namely TOC and TIC components, are largely absent in all organisms. The only conserved subunit is Tic21 which likely forms an inessential channel in plant plastid and which is present in three isoforms in *E. gracilis* plastid proteome while TOC subunits and other outer membrane proteins are completely absent from all euglenophyte transcriptomes, suggesting the existence of an alternative pathway. Taking advantage of the mass spectrometry-determined plastid proteome we identified several plastid-localized proteins recruited from ER/Golgi molecular machinery (Rab5, GOSR1, and two derlin-like rhomboid pseudoproteases) which we propose mediate protein-transporting vesicle fusion on the outermost membrane and possibly protein import across the middle membrane. If confirmed by molecular and/or imaging methods, the latter could disprove the presumed cyanobacterial-like origin of the membrane.

We used a set of 375 highly credible plastid proteins determined by MS and re-evaluated the topology of their N-terminal targeting domains and amino acid composition of their transit peptide-like (TPL) region. We report that a non-negligible cohort of proteins does not possess the typical hydrophobic domain motif [44] and that euglenid TPL exhibit a unique pattern of amino acid frequencies, most notably substantial enrichment in proline which could greatly affect their secondary and tertiary structure.

5 CONCLUSIONS

We bring protein-level support for conclusions of previous studies as well as numerous novel findings based on a new comprehensive set of *E. gracilis* plastid proteins determined by mass spectrometry and newly generated transcriptomes of *E. gracilis* and *E. longa*. Our proteomic dataset represents only a second proteome of a photosynthetic complex plastid (the first being the one from chlorarachniophyte *B. natans*, [60]) and one of the few full plastid proteomes of unicellular algae [61,62]. Based on these data, we report some metabolic peculiarities of the euglenophyte plastid, including very low contribution to amino acid metabolism and additional SUF system of chlamydial origin. We describe an extensive reduction in plastid import machinery of the inner two plastid membranes, propose novel candidate protein translocases, and re-evaluate the characteristics of plastid-targeting signal domains.

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EDUCATION

- 2014 – 2019: **PhD study of Parasitology**, Faculty of Science, Charles University, Prague. Thesis: Evolution of euglenid plastid proteome.
- 2012 – 2014: **MSc study of Parasitology**, Faculty of Science, Charles University, Prague. Thesis: Membrane proteome of euglenid plastid.
- 2009 – 2012: **BSc study of Molecular Biology and Biochemistry of Organisms**, Faculty of Science, Charles University, Prague. Thesis: Transport of proteins into secondary plastids.

TEACHING

- 2017: Invited seminar talk: Colorful history & peculiar protein import of *Euglena gracilis* plastid, University of Warsaw.
- 2014 – 2019: Practical course of Protistology.

FUNDING & AWARDS

- 2019: **Holz-Conner Travel Award** *(VIII European Congress of Protistology – ISOP Joint meeting, 28/7-2/8/2019)*
- 2019: **Živa Award** for the best popularization article in the respective age category (*Losers Finders: Life Without Semiautonomous Organelles, Živa 2018/1*)
- 2018: **Charles University Mobility Fund** – research stay at Dalhousie University, Halifax, NS, Canada.
- 2016: **Gordon and Betty Moore Foundation** – Development of Transformation Protocols for a Spectrum of Marine Protists, (co-investigator).
- 2014 – 2018: **STARS** – Supporting Talented PhD Research Students, Charles University.

MEMBERSHIPS

- 2016 – Present: **International Society of Protistologists**.

OTHER EXPERIENCE

- 2018: Research stay at Dalhousie University, Halifax, NS, Canada; acquired basic skills in Oxford Nanopore method for genome sequencing.
- 2018: TATAA Biocenter course: Hands-on qPCR.

8 LIST OF PUBLICATIONS

Vanclová AMG, Hadariová L, Hrdá Š, Hampl V. **Secondary Plastids of Euglenophytes**. In: Y. Hirakawa (ed.), Advances in Botanical Research, Academic Press 2017. doi: 10.1016/bs.abr.2017.06.008

Záhonová K, Füssy Z, Birčák E, Novák Vanclová AMG, Klimeš V, Vesteg M, Krajčovič J, Oborník M, Eliáš M. **Peculiar features of the plastids of the colourless alga *Euglena longa* and photosynthetic euglenophytes unveiled by transcriptome analyses**. Sci Rep. 2018 Nov 19;8(1):17012. doi: 10.1038/s41598-018-35389-1.

Ebenezer TE, Zoltner M, Burrell A, Nenarokova A, Novák Vanclová AMG, Prasad B, Soukal P, Santana-Molina C, O'Neill E, Nankissoor NN, Vadakedath N, Daiker V, Obado S, Silva-Pereira S, Jackson AP, Devos DP, Lukeš J, Lebert M, Vaughan S, Hampl V, Carrington M, Ginger ML, Dacks JB, Kelly S, Field MC. **Transcriptome, proteome and draft genome of *Euglena gracilis*.** BMC Biol. 2019 Feb 7;17(1):11. doi: 10.1186/s12915-019-0626-8.

Novák Vanclová AMG, Zoltner M, Kelly S, Soukal P, Záhonová K, Füssy Z, Ebenezer TE, Lacová Dobáková E, Eliáš M, Lukeš J, Field MC, Hampl V. **Metabolic quirks and the colourful history of the *Euglena gracilis* secondary plastid**. Under review.