Leibniz-Institut DSMZ Deutsche Sammlung von Mikroorganismen und Zellkulturen, GmbH

Mikrobielle Ökologie und Diversitätsforschung

PD Dr. Jörn Petersen

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To the Head of the
Committee for PhD studies
Dr. Tomas Dolezal
University of South Bohemia
Ceske Budejovice

Review

of the PhD thesis of **Jan Michálek** with the title:

Genomes of Chromerid Algae

Jan Michálek has conducted the work for his PhD thesis in the group of Miroslav Obornik in the Institute of Parasitology at the University of South Bohemia (Ceske Budejovice, Czech Republic). The cumulative thesis is written in English and essentially based on three published peer-reviewed publications with two shared first authorships and one co-authorship. The three papers are framed by (i) an informative introduction about alveolates with a special focus on the chromerid algae *Chromera velia* and *Vitrella brassicaformis*, the origin and distribution of complex plastids, the genome structure and respiratory chain composition of myzozoan mitochondria and a chapter about endosymbiosis and HGT, followed by (ii) a very short paragraph about the aims and scope of the cumulative PhD thesis and (iii) a conclusion that summarizes the main novelties of all three publications.

Background and aim of the work

The discovery of photosynthetic alveolates that are closely related to apicomplexan malaria and toxoplasmosis parasites was a scientific sensation (Moore, Obornik et al. 2008). The chromerid alga *C. velia* represents a ‘connecting link’ in alveolate evolution, and thus serves as an ideal model to investigate the transition from a photoautotrophic to a parasitic life style. The second chromerid alga that has been described to date, *V. brassicaformis* (Obornik et al. 2012), exhibits conspicuous differences in the life cycle, gene organization and biochemical properties. Nevertheless, phylogenetic analyses and synapomorphic traits such as the presence of a proteobacterial form II Rubisco provided strong evidence for a common origin of complex plastids from chromerids and dinoflagellates (Janouskovec et al. 2010), even if

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different evolutionary scenarios have been proposed to explain the different number of diagnostic plastid membranes (Petersen et al. 2014; Füssy & Obornik 2018). *Chromera* and *Vitrella* are due to their easy cultivation and their (in relationship with peridinin-containing dinoflagellates) comparably small genome sizes (<200 Mb) predestined model organisms for the investigation of the biology of apicomplexans. Based on the nuclear draft genomes of both algae, which have been sequenced and analyzed by a large scientific consortium [Paper II], the two major aims of the current PhD thesis were (i) the investigation of the respiratory chain as key component of aerobic algal mitochondria [Paper I] and (ii) the understanding of the mode of fatty acid biosynthesis in chromerids [Paper III].

Spectrum of methods & contribution

I must admit that it was difficult for me to completely estimate Jan Michalek´s contribution to the three published papers. It is obvious that he was responsible for the bioinformatic analyses in all three papers based on comprehensive genome searches, targeting predictions, multienzyme structure analyses, comprehensive phylogenies, reconstruction of pathways and the development of the draft scheme of the respiratory chains. However, based on the statement regarding the "*List of papers and author´s contribution*" it remained unclear for me which wet-lab experiments he performed for Paper I (in order to confirm the absence of the mitochondrial complexes I and III in *Chromera*). In the light of the general bioinformatic focus his contribution for the eLife Paper II is also somehow cryptic ("*JM, Performed fatty acid biosynthesis*"). Finally, in the cumulative PhD thesis I would have preferred to read at least some more selfish statements like "*I investigated*" or "*my aim was*" instead of rather collective declarations such as "*we have aimed*" (Aims and scope), "*to support our findings we have investigated*" and "*we also described*" (Conclusion). However, my open questions regarding the specific contribution of Jan Michalek can easily be answered in the defense of the thesis.

Results of the thesis

The thesis of Jan Michalek provided a bunch of fascinating insights into the biology and evolution of *Chromera velia* and *Vitrella brassicaformis*.

(1) The comprehensive analysis of transcriptome and genome data provided in combination with wet-lab experiments (Northern blot, electron microscopy, growth with inhibitors) the basis to (i) identify the crucial mitochondrial proteins involved in respiration, to (ii) infer their phylogenetic relationships, to (iii) reconstruct the respiratory chains of both chromerid algae and to (iv) present an overview about the evolution of mitochondria in alveolate key species (Flegontov, Michalek et al. 2015). I am aware of the challenging and laborious *in silico* analyses, because the ambiguous mitochondrial genome organization in *Chromera* with heterogeneous linear DNA fragments caused some intellectual headaches in our former

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work (Petersen et al. 2014) and the unambiguous assignment of the transcripts to the subcellular eukaryotic compartments is also not trivial (Ludewig et al. 2017). Accordingly, the reconstruction of the respiratory chains is a great achievement that allows to understand the basis of aerobic ATP generation in these algae in the light of a nearly complete organellar genome reduction. The absence of complex III in the respiratory chain of *Chromera* was a striking observation. It exemplifies lineage-specific evolutionary adaptations in alveolates and in turn documents the relevance of the quite divergent close relative *Vitrella* for general conclusions about the biology of chromerid algae.

(2) The manuscript about the draft genomes of *C. velia* and *V. brassicaformis* was a pioneer study that paved the way for future analyses of these model organisms (Woo et al. 2015). Based on a solid phylogenetic reference tree it nicely illustrated the gene content changes in the evolution of alveolates. The comprehensive comparison of various metabolic capacities including the important isoprenoid biosynthesis, which is essential for the maintenance of the heterotrophic plastid in apicomplexan parasites and was investigated by Jan Michalek, showed for *Chromera* and *Vitrella* a nearly absolute degree of completeness of the pathways. The same result was observed for the presence of endomembrane components, which hence impressively confirmed the suitability of both chromerid algae as apicomplexan model systems. The considerable number of 43 authors reflects that analytic depth of the eLife paper.

(3) The manuscript about the biosynthesis of fatty acids in chromerids has very recently been published in the journal 'Biomolecules' (Tomcala, Michalek et al. 2020). Fatty acids that serve as molecular fingerprints for the differentiation of bacterial species are the building blocks of biological membranes. The understanding of their formation and composition in *Chromera* and *Vitrella* is a great task especially due to the endosymbiotic ancestry of their complex plastids that are surrounded by four membranes. The identification of the crucial enzymes for different FA synthesis pathways and their subcellular localization in the plastid, cytosol and endoplasmatic reticulum was a complex biochemical puzzle, which included the experimental detection of the fatty acid composition via gas chromatography and comprehensive *in silico* analyses of candidate genes. The presented model of FA biosynthesis proposes that short-chain saturated fatty acids (C14:0 to C18:0) are produced in the complex plastid via the FAS II pathway and will subsequently be elongated and desaturated in the cytosol and the ER. Many identified proteins with ketoacyl synthase domains, which are homologous to type I fatty acid synthases (FAS I), might represent polyketide synthases of unknown function. The new insights provided the basis for promising follow-up studies.

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Evaluation of the thesis

I enjoyed reading the thesis, could refresh many aspects about the evolution and biology of chromerid algae including their mitochondrial respiration and gained new insights into the interesting field of fatty acid biosynthesis. The thesis of Jan Michalek, which is dealing with the two very exciting strains *Chromera velia* and *Vitrella brassicaformis*, comprises a broad analytical spectrum of state-of-the-art bioinformatics and documents the intellectual depth in the field of molecular genomics and evolution. The number of two shared first author publications and one co-authorship in very renowned journals (MBE, eLife, Biomolecules) is absolutely convincing. Accordingly, and addressed to the Head of the Committee for PhD studies of the Faculty of Science, University of South Bohemia, Czech Republic, **I explicitly recommend the thesis to be defended**. I am looking forward to the defense of Jan Michalek on November the 16th 2020. A list of questions that came up during reviewing the thesis is provided below.

Braunschweig, den 06.11.2020 PD Dr. Jörn Petersen

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List of questions that might be discussed in the defense

(01) Page 3, first paragraph

When I saw the first time a culture of *Vitrella brassicaformis* CCMP 3155 the conspicuous green color was quite surprising for me. How can the - on the first glimpse - irritating color be explained? Which relevance has the color of algae with respect to the understanding of higher order endosymbioses?

(02) Page 4, second paragraph

The lack of a plastid genome in the heterotrophic green algal genus *Polytomella* is very interesting. Which (i) bioinformatic and (ii) cell-biological proofs would be required to convince a very critical reviewer from the presence of a non-photosynthetic plastid in *Polytomella*? The absence of a plastid genome could alternatively also be explained by a complete loss of the organelle.

(03) Page 4, second paragraph

You mentioned two independent losses of the apicoplast in *Cryptosporidium* and *Gregarina niphadrodes*. Could the absence of a heterotrophic plastid in these lineages not also be explained by to independent endosymbioses, one in the common ancestor of chromerid algae and the second one in the LCA of apicoplast-containing sporozoa? Is there any evidence that *Cryptosporidium* and gregarines ever harbored a plastid?

(04) Page 8, second paragraph

Your statement "*Similar to mitochondria, plastid genomes have been gradually reduced with the consequent transfer of endosymbiont genes into the host nuclear genome*" is generally true, but also seems to be oversimplified. Which two additional genetic mechanisms beyond the "*endosymbiotic gene transfer*" can explain the successful establishment of essential plastidial genes in the nucleus of the host cell?

An early review about the evolution of the Calvin cycle from Martin & Schnarrenberger (1997; Curr Genet 32: 1-18; doi: 10.1007/s002940050241) and a publication of your co-author Patrick Keeling (2003; MBE 20: 1730-1735; doi: 10.1093/molbev/msg195.) might be helpful to answer the question, even if the concept of "chromalveolates" has meanwhile been falsified.

(05) Page 10, last paragraph

Your wrote that "*the nuclear genome of C. velia also encodes an outstanding number of enzymes, which are rather specific for anaerobic protists but not for most sporozoans*" and conclude "*that the common ancestor of chromerids and apicomplexans was much more metabolically versatile*". Would you determine the genetic repertoire of the LCA of a specific eukaryotic lineage by summarizing the complete set of genes that has been detected in genome-sequenced representatives? How would you deal with possible HGT and cryptic endosymbioses?

(06) Page 12, second paragraph

Your found a large spectrum of genes resembling type I fatty acid synthases (FAS I) in

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chromerid algae and speculated about their functional role as polyketide synthases for the production of algal toxins. Which toxins or other secondary metabolites have yet been identified in *Chromera* and *Vitrella*?

(07) Page 12, second paragraph & Biomolecules paper, Figure 4
You mentioned that "*The phylogenetic analyses showed that many genes encoding enzymes involved in fatty acid biosynthesis display various origins reflecting an endosymbiotic evolutionary history of chromerids and sporozoans*". Which genes are diagnostic for the common plastid origin of myzozoa?
How would you interpret the left part of the ketoacyl synthease domain phylogeny in Figure 4? What is your explanation for the close relationship with animal sequences in the more basal part of the tree shown on the right hand side?

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