Phd Thesis Review

in Třeboň, 22.10.2021

PhD candidate: Mgr. Jitka Richtová
University of South Bohemia, Faculty of Science. 2021

Reviewer: Jan Janouškovec
Address: Institute of Microbiology of the Czech Academy of Sciences
Centre ALGATECH, Novohradska 237, 379 01 Trebon, Czech Republic
Phone: +420 704 101 754, Email: janouskovec@alga.cz; janjan.cz@gmail.com

Summary:
Jitka focused her PhD work on determining the subcellular localization of enzymes of the tetrapyrrole pathway in the microscopic alga *Chromera velia*. *Chromera* that represents an independent lineage of photosynthetic eukaryotes and is specifically related to parasites from the group Apicomplexa, with whom it shares a tetrapyrrole pathway with several unusual features. A key among them is a mitochondrial-like ALA synthase, an enzyme not previously seen in oxygenic phototrophs.

By combining heterologous expression in *Phaeodactylum* and *Toxoplasma*, and direct immunogold-TEM localization in *Chromera*, Jitka's work conclusively suggested that the *Chromera* ALA synthase is indeed localized in mitochondria. This is the first experimental evidence in a photosynthetic organism that tetrapyrroles can be made from mitochondrial succinyl coenzyme A and glycine, two compounds directly involved in core cellular metabolism (TCA cycle, amino acid turnover).

Jitka localized several other enzymes of the tetrapyrrole pathway by using xenotransfection and she interpreted their more or less conclusive results in the light of protein targeting principles and computational localization predictions based on the enzyme primary sequences. She published the results in a molecular biology journal, and extensively, objectively discussed them. She contributed to four other publications, which include a major experimental contribution to a paper about chromerid life cycles.

Jitka's work does not conclusively solve where and how *Chromera* synthesizes its tetrapyrroles but it is a welcome step forward in understanding an organism that is exceedingly difficult to work with. With the benefit of hindsight, one can question some experimental choices the candidate made, directions she took when experiments failed, perhaps even the research impact of following the very question she started with. Yet, one's virtues are tested not by tackling questions that are easy. Jitka showed extraordinary determination in following through with the project. She mastered working with distantly related algae and parasites, used diverse methods ranging from cloning and protein work to advanced microscopy and TEM, visited and collaborated with research groups abroad, was persistent and innovative when challenges appeared. She ended up writing a round and well readable thesis on a topic that was initially new to her, and showed she can independently and successfully conclude a difficult project – fundamentally the experience one would ask for in fulfilling the requirements for a doctoral degree. Thus, I recommend Jitka's thesis for a formal defence before an examination panel.

Detailed feedback and questions:

1) I think the three-chapter Introduction is well done and I particularly appreciate that the structure was easy to follow. The amount of information is well balanced and I was happy to learn a number of new things that I was not aware of or have long forgotten (e.g., some papers on early apicoplast

research, apicomplexan vs. algal symbiosis in the McFadden and Waller 1997 paper). The candidate demonstrated an extensive background knowledge by writing, e.g., about guanine crystals in eukaryotes, energy transfer in photosynthesis and the early histories of protein targeting, tetrapyrrole research and apicoplast discovery. My principal suggestion is to have less peripheral information and, more importantly, always highlight how such information specifically links to the topic of the study (e.g., how iron uptake or fatty acid synthesis in *Chromera* relates to tetrapyrroles).

2) It is a generally good idea to write out your Aims. Not a rigid requirement, but if you avoid doing it your aims must be clearly understood from your Abstract and Introduction. Chapter 4 does this to an extent, though not in a detail and clarity I'd like to see, and your Abstract unfortunately fails to do it. "This thesis aimed to describe C. velia as an organism of interest in the perspective of its heme pathway localization." is vague. I think Chapter 4 (Conclusions) is a perfect place for you to tell us about your work in more detail and with clarity; you could easily expand there on expense of some more peripheral background information in Chapters 1-3.

3) You did a very nice and difficult-to-write section on the tetrapyrrole pathway localization and regulation. I only really missed a scheme that would summarize the information in different organismal models. I know that overviews of localizations are in your paper and earlier papers. But considering how central this is to your thesis aims you should one in your Introduction - and I'd love to see the regulation in there too!

4) Language: Good in general. I could image the candidate did more proof-reading herself or asked others to re-read individual sections or chapters for her. The issues I have are the usual suspects: word order, word choice ("specific disorder for each step"?) and form ('understand' function → understood function), articles (many), cumbersome expressions ('wealthy' culture; 'fulfilling' the phylogenetic gap), typos (chlorphylls in Fig. 1 of your paper), and so on. You could use active speech more often and simplify formal, complicated language (is equipped with → has), and also write a bit more concisely. Notice that with directness comes simplicity – "heme can be involved" → "is involved"; "appears to be essential" → "is essential" – we can summarize without presumption of the unknown. The quality of the text varies: some sections of the Intro are better than other; the language in your paper is surprisingly rough in parts.

5) Literature: Broad, balanced and made me very happy in general. Two exceptions to this. Firstly, there are missing citations: Tortorelli et al., in press; Gonepogu et al. (unpublished data – how to cite this; personal communication?); Treitli et al., 2018 – I'd love to know these. Secondly, you could cite original primary literature instead of reviews more often, especially about the biosynthesis of porphyrins and localization of tetrapyrrole enzymes, both of which are central to your thesis (e.g., see your use of the Tanaka et al., 2011 citation).

6) You say: "The overall metabolic profile of C. velia suggests its extraordinary adaptability, effectively working as a free-living phototroph capable of switching to photoparasitism when necessary or when environmental conditions are suitable." What is the evidence for this? Can you present arguments for and against the idea of photoparasitism?

7) Residual body: Curious to know more about what the *Toxoplasma* work showed about it.

8) Chromerosome: Is the chromerosome a real structure? Is it really as omnipresent in the TEM images of older cells as your statement implies - "takes about half of the cell space"? How does the chromerosome resemble the ejectisome and trichocysts in detail – surprising to hear for me (p. 5)?

9) Trophic transition: You keep bringing this up in relation to parasitism. Can you justify and discuss what was the original state of the transition, evolutionary steps, and how feeding and life cycles fit in? Where does *C. velia* fit in the spectrum of strategies and how can it serve as a model?

10) Your paper – congratulations, a major achievement after all the challenges you have gone through. Good discussion, despite all the interpretational difficulties, but omitting conclusions and implications from the Abstract was a unfortunate move - it makes the relevance of your work fuzzy.

11) What tetrapyrrole enzyme localizations would you expect in a mixotrophic organism and why?

12) How could the pathway be regulated in Chromera and apicomplexan given the precedent evidence in plants/yeast/animals?

13) How did you check for gene model correctness, especially their N-termini, in enzymes not investigated by 5' RACE in Koreny et al., 2011 – illumina transcript read mapping?

14) What is the basis for you saying that SignalP/TargetP have "good prediction performance"?

15) Can you relate the information about charge variation and distribution (p. 5 of paper) to comparing net charge values across the peptides?

16) To me, the ER staining was not presented clearly enough in the results. Can you summarize it together with information on whether your stained peroxisomes and/or how we can do it?

17) Why do the UROS cells look different from the other and is this explained in the paper (Fig. 5)?

18) Why does the mito stain varies between red and yellow? Why are the 'cytosolic' localizations spotty?

19) Why did you choose anti-ATPasebeta as a control if there is another variant in the plastid? Are the two proteins and their specific epitopes similar? What is the expected size of the plastidial variant and does it differ from the mitochondrial – would we expect to see the cross-reaction on your Western blot? What other control could you use instead?

20) The discussion in your paper is very rich and I appreciate the depth of discussion. It could be written more concisely and I find it difficult to follow without having subheaders. But good job.

21) What do you think is the significance of having several enzyme variants to catalyze a single enzymatic step in porphyrin biosynthesis – how would it work if they are localized in different organelles, specifically considering the need for intra-organellar targeting of potentially toxic intermediates?

22) What is the structure of periplastidial SP/TP targeting signals in diatoms and apicomplexans?

23) Is the CAB domain actually present in all secondary plastid FECHs? Useful for context...

24) Can you discuss the possibility of dual protein targeting – did you actually discuss this in your paper and how likely do you think it is in the individual enzymes? Could there be another part-complete pathway in the *Chromera* mitochondrion and how about, say, a parasite like *Amoebophrya*?

25) “… two ferrochelatases in a single cell could be protection of the cell under stress conditions” – Can you please explain in more detail.