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Review of the PhD thesis entitled

„Biogenesis of the cyanobacterial photosystem II complex: involvement of selected accessory factors with emphasis on the novel Psb35 protein"

submitted by Guillem Pascual Aznar

(Supervisor: Prof. RNDr. Josef Komenda, DSc)

The thesis deals with the role of three selected accessory factors involved in photosystem II (PSII) biogenesis/repair. In particular, the thesis reveals structural information on Ycf48 protein and gives new data about its role in the biogenesis of chlorophyll (Chl) binding protein complexes. Furthermore, it investigates the role of RubA in PSII building and characterizes a newly identified accessory factor, the Psb35.

The subjects of the thesis are of great interest in the field of photosynthesis research. The thesis is based on 3 already published articles, one with first authorship of the Candidate. Since the results are published indicating that they were already reviewed by international juries and found to be important and up to date. The first chapter of the thesis is a detailed introduction that gives an overview about the concerned themes, providing a good background indicating the Candidate has satisfactory knowledge of the field. He justified well his choice of the model organism. The articles represented in the 2. Published results chapter of the thesis. The 3rd chapter discusses the main findings and describes some unpublished results, while the 4th Conclusions chapter also summarizes the main results. The results and the conclusions represented in the articles are well argued. The methods - molecular biological, biochemical and biophysical techniques - applied by the Candidate are up-to-date and relevant. The candidate cited 132 references in this thesis proving his scientific knowledge about his topic. The quality of English and the general presentation are good.

In the following, I give my list of critical remarks and questions.

Unfortunately the thesis misses a chapter of the objectives of the work.

Some abbreviations are not listed, such as: PSII[1], PSII[2], PSII(1) PSII(2), PSI(3) etc.

Page 2: The sentence "Almost all cyanobacteria……as thylakoid membranes, which expands greatly the available surface for the photon absorption by the membrane embedded antennae" is not fully correct since the main light harvesting antenna of cyanobacteria, the phycobilisome is located on the membrane surface.

TM is not explained in the Figure 1 description.

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Page 3. The *T. elongatus* abbreviation was not introduced.

Page 6: Figure 2.: The *T. vulcanus* abbreviation was not introduced. The quality of the picture in the pdf file is poor.

Page 8.: high light-inducible proteins.

Page 9.: Figure 3.: It would be better to make a full page figure, because the letters are hardly visible. Was the picture taken from somewhere? If yes then the reference is missing.

Page 12.: "Although the primary site of damage is still under debate,....", since the preceding sentence deals with PSI it would be better to state that it is about PSII.

Page 13.: The Candidate lists the first line defence members, then without any explanation gives a thorough introduction of Hlips. It should be justified by few sentence why just the Hlips.

Page 14.: It needs to be explained why the Candidate doubted in the data represented by Wang and co-workers (Wang et al., 2018) regarding the binding of Hlips to PSI.

Page 15.: It sounds strange that after describing the role of FtsH proteases in D1 removal, the next sentence is about completing the repair.

Page 16.: I think it would be better to use a more general sentence at the end of chapter 1.6 and not a sentence about the aim of the thesis. I would prefer the aims in a separate chapter.

Ycf48 should be written in the first sentence of chapter 1.6.1. It is not enough that it is the title of the chapter. The fluorescence-based screening should be explained.

Page 109.: I think it would be better to describe the subjects of the thesis in the introduction part of the Discussion chapter and not just refer to the published articles.

In the sentence " We proved that , as in the spinach ....and it is hypothesized that the..." the "it is hypothesized that" would better to phrase in 1st person plural.

Page 110. The RubA should be written in the first sentence of chapter 3.2. It is not enough that it is the title of the chapter.

I think it is not really appropriate to use the phrase "In the present work, " when it refers to the results of the published article.

Page 112.: The layout of the page is different from the other pages.

Page 117.: The summary could be a separate (3.4) chapter.

Page 118.: Figure 8. It would be better to make a bigger figure and represent in a landscape orientation.

Page 119: Deletion or replacement of Arg residues?

Page 126: The reference " Hollingshead et al. 2016" is not well formatted.

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Additional questions:

If the hypothesis regarding that the PSI(3)-CP47m' complex is involved in the delivery of the freshly synthesized Chl from CP47 to PSI(3) is true , what could be the reason for introducing the newly synthetised Chl first into CP47 and not directly to PSI(3)?

The Candidate suggested that the primary reason for low Chl biosynthesis in the RubA-null mutant seems to be the insufficient delivery of electrons that are required for some redox reactions connected with Chl biosynthesis pathway. Which other ways can the decreased PSII content affect the Chl biosynthesis?

How can the relationship between photosystem biogenesis and synthesis of tetrapyrroles influence the adaptation capability of cyanobacteria?

On the basis of the presented results, that published in international journals of good standards, and the nicely written thesis, I suggest to the Doctoral Committee the admission of the dissertation to public defence and in the case of succesfull defence awarding of the PhD degree to Guillem Pascual Aznar.

Szeged, 2021.08.27

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Review of the Ph.D. thesis by Guillem Pascual Aznar „Biogenesis of the cyanobacterial photosystem II complex: involvement of selected accessory factors with emphasis on the novel Psb35 protein"

Biogenesis of photosynthetic multi-subunit pigment-protein complexes, like photosystem II and photosystem I, is a very sophisticated process, which requires a high degree of co-ordination of individual steps with involvement of various assembly protein factors. The thesis deals with a biogenesis of photosystem II in cyanobacteria and it is specifically focused on elucidation of structural and functional roles of accessory factors such as Ycf48, RubA, and newly discovered Psb35 protein in this process. Experimental results of the thesis significantly contributed and extended our knowledge in this scientific field.

This work is based on three excellent papers, which were published in highly impacted journals. This clearly implies that the scientific topic is up-to-date, it is of interest of a broad scientific community and published work is of a very high quality. Guillem Pascual Aznar is the first author of one paper, which indicates his major contribution in experimental part of the work, data analysis, compiling and writing the manuscript.

The thesis starts with Introduction, where the author provides readers with a brief introduction to photosynthesis and details of structural and functional roles of photosystem I and photosystem II in this process. In line with the subject of the thesis, the author describes a current stage of knowledge about the biogenesis of photosystem II in cyanobacteria. The author pays a special attention to the three selected assembly factors, Ycf48, RubA, and Psb35, which are subjects of the experimental part of the thesis. This part is very-well written and informative.

Experimental part of the thesis, which contains three published papers together with supplemental material, is followed by Discussion. In this part, individual papers are further discussed at a more general level together with unpublished data. Author also included a scheme of photosystem II assembly in cyanobacteria (Figure 8.), which clearly summarizes the current view of biogenesis of photosystem II with help of assembly factors described in the thesis.

The thesis ends with Conclusions, where the main characteristics and functional roles of the PSII accessory factors, Ycf48, RubA, Psb35, are summarized. It leaves the reader with a clear picture of the presented work.

I have several questions which I would like to ask at the thesis defense:

1. Co-crystallization of Ycf48 with C-terminal of D1 peptide provided details of their mutual binding. What is known about the mechanism of their dissociation?

2. The binding of Ycf48 to YidC insertase and an involvement of Ycf48 in chlorophyll biosynthesis indicates its role in insertion of chlorophyll into the nascent chlorophyll-binding polypeptides. Is it known more specifically which ones?

3. Single particle electron microscopy revealed that Psb35 protein binds to CP47 and trimer of photosystem I. Do you have any indication (e.g. from image analysis) about a multiple binding of Psb35+CP47 to photosystem I trimer?

4. In the Figure 6 (page 86), there is 2D analysis of membrane proteins of WT and mutant lacking Psb35. You claim the band CP47m´ detected in WT was missing in the separation profile of the mutant ΔPsb35. But I can see almost identical band in ΔPsb35 sample. What is the origin this band? Can you comment on that?

5. A basic photosynthetic characterization of the ΔPsb35 mutant (Fig. 5, page 85) did not reveal significant differences compared to WT, thus it seems cyanobacteria can somehow cope with the absence of Psb35. How do you see the relevance of Psb35 in cyanobacteria?

6. An involvement of assembly cofactors in both biogenesis and repair mechanisms of photosynthetic apparatus implies their permanent presence in a cell. Do you have an idea about the abundance of Ycf48, RubA, and Psb35 and their ratio to e.g. photosystem II and photosystem I in during a cell life under different growth conditions?

Finally, I would like to congratulate the author on the considerable scientific contributions to the field of biosynthesis of the cyanobacterial photosystem II complex and I strongly **recommend, in case of a successful oral defense, the award of Ph.D. to Guillem Pascual Aznar.**

In Olomouc, August 25, 2021.

RNDr. Roman Kouřil, Ph.D.
Palacky University, Olomouc