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Review of the PhD thesis entitled
„Role of Psb28 proteins in the biogenesis of the Photosystem II complex in the cyanobacterium *Synechocystis* sp. PCC 6803"
submitted by Martina Bečková
(Supervisors: Prof. Josef Komenda)

The thesis deals with the role of Psb28 proteins (Psb28-1 and Psb28-2) in the biogenesis of the cyanobacterial photosystem II (PSII). In particular, the thesis reveals that Psb28 proteins exist in different oligomeric states and provides evidences for the binding of Psb28-2 preferentially to PSII monomers whereas Psb28-1 to RC47 complexes. Furthermore, it investigates the phenotype variability of various wild types.

The subjects of the thesis are of great interest in the field of photosynthesis. The thesis is based on 4 already published articles (one still in an unedited manuscript format), 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 articles represented in the Results chapter of the thesis. The first chapter of the thesis is a detailed introduction that gives an overview about the concerned themes, providing a good background for the results chapter indicating the Candidate has satisfactory knowledge of the field. The second chapter draws up the aims of the thesis. The 3rd chapter summarizes the main findings and the 5th Conclusions chapter also highlights the main results. The results and the conclusions represented in the articles in the 4th chapter are well argued. The methods - molecular biological, biochemical and biophysical techniques - applied by the Candidate are up-to-date and relevant. The quality of English and the general presentation are very good.

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

There are only few small mistakes in the text e.g. page 5: ligation – ligation of; oxygen evolving complex – the oxygen evolving complex; page 11: lipidic – lipid; page 88: 300 µL is in different format; page 114: CP47 synthesis: – CP47 synthesis.; page 115: high light conditions – high-light conditions.

Chapter 1.1.1: Water splits not only into oxygen but also hydrogen.

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Chapter 1.1.2: I think when the Candidate wrote that the protein composition of the photosystems is conserved across the photosynthetic organisms, she meant across oxygenic photosynthetic organisms.

Chapter 1.1.3: What does cover the unique combination of physiological, morphological and molecular genetic characteristics of *Synechocystis* and how this contributes to be a good model organism?

"GT is widely used for its easy cultivation under a wide range of physiological conditions in both liquid and solid media, its spontaneous natural transformation, DNA homologous recombination ability, loss of motility allowing better manipulation with single colonies and the biggest advantage, the possibility to study mutants with disrupted photosynthesis (Williams, 1988)." This sentence suggests that PCC WT is not suitable for studying mutants with disrupted photosynthesis, is this true?

The Candidate wrote that *Synechocystis* 6803 contains up to twelve copies of a single 3.57 mega bases chromosome, while it was shown that it could be more than 12.

Chapter 1.2.1: It would be better to rephrase the following sentence: On either side are inner antennae CP43 and CP47 each with 6 transmembrane α helices and cofactors (Chl *a* and β-carotene).

I also suggest to write "comprise around 70% of thylakoid lipids" and not exactly 70%.

In the sentence "The glycolipids DGDG (digalactosyldiacylglycerol) and PG (phosphatidylglycerol) were shown to be important for the function of PSII in particular (Boudière et al., 2014; Hagio et al., 2002; Sakurai et al., 2007)" glycerolipids should be written and not glycolipids.

Chapter 1.2.2.1: In the figure title of Figure 3 it would be nice to explain that Cyt b-559 is composed of a heterodimer of the PsbE and PsbF subunits.

Chapter 1.3: It would be better to rephrase the following sentence: "Not all auxiliary factors from *Synechocystis* 6803 are known but compared to number of factors known from plants they are just few (Lu, 2016)".

It would be nice to give information about which protein were His-tagged, because it also could give information about what kind of complexes could be pulled down: "Another set of protein factors, the HliA/B/C, PAM68, Psb27, Psb28, and Psb29 (encoded by the ssl2542/ssr2595/ssl1633, sll0933, slr1645, sll1398 and sll1414 genes, respectively) mostly identified as components of His-

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tagged PSII preparations isolated from *Synechocystis* 6803 (Boehm et al., 2011; Kashino et al., 2002) were proposed to function in later stages of the PSII assembly."

Chapter 1.3.2.1.1: I have to note that it was a very good idea to put this chapter in the thesis and highlight the confusion in the nomenclature.

The reference is missing from the end of the following sentence: It was first described as a 6.1 kDa polypeptide associated with the reaction centre core complex of the photosystem II from spinach (*Spinacia oleracea*).

Chapter 1.3.2.1.2: It would be better not to start the first sentence with "For the first time".

Chapter 1.3.2.1.4: It would be good to represent this chapter in more detail.

Chapter 2: In the first point of the list of main aims the names of the proteins should be written and not just mention as "both proteins".

Chapter 4: Insertion of the supplemental data and figures at the end of the subchapters facilitates the understanding.

Chapter 4.4: Incorporation of figures into the text would help reading of this chapter. (This chapter represented as an unedited manuscript.)

In the sentence: "When isolated from cells exposed to high irradiance, the preparation additionally contained a significant amount of the PSII-PSI supercomplex (Figure 4, right panel)." would be nice to use the designation RCCS for the easier identification on the figure. Also it might be good to note that the amount of PSI(3) also increased. In the next sentence "both" is not needed before PsaD, also "and 2D blots" is not necessary when referring figure 4.

Based on the information given on Supplemental figure 5 the delta-Psb28-2 strain contained higher amount or RCCS even under normal condition that is not mentioned and explained in the text. (Even latter stated: the accumulation of PSII supercomplexes is not dependent on the presence of Psb28 proteins.)

It might be better to explain a little bit more what does cover "In our original study", because it could be a little bit confusing and hard to follow which WT was used in the earlier explained FLAG-tagged and the following studies in this chapter.

They wrote in this article that: "This result indicates that the decreased level of Chl in the Psb28-1-less strain seen by Dobáková et al. (2009) was most probably related to the Chl- deficient phenotype of the WT strain originally used for mutant construction." which might suggest that in

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the article published in 2009 they compared the mutant not to its original WT, or the mutation decreased the Chl content compared to the WT that was used for the mutation? If the mutation caused additional decrease then what could be the explanation?

Figure 8 is not referred in the text.

It would be nice to give the sequences of the used primers in a supplemental table.

How specific was the Psb28 antibody to Psb28-1 since it was raised against the C terminal that showed quite high homology to Psb28-2?

Chapter 4.4.1: It would be better just write "Figures" and not "Figure legend" for this chapter.

On Figure 1 the deletion also indicated but not on the other figures.

The CN-PAGE is hardly visible on Figure 2.

Figure 7 could be better organised without several repetitions and for easier comparison of normal, intermittent- and high-light conditions.

On supplemental figure 3 not all complexes are indicated.

Additional questions:

What could be the physiological role of the Psb28-1 dimer, since it seems the dimeric structure of the protein is not needed for binding to PSII?

What could influence the ploidy level of *Synechocystis*?

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 Martina Bečková.

Szeged, 09.11.2016

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Referee comments on Ph.D. thesis

Title: Role of Psb28 proteins in the biogenesis of the Photosystem II complex in the cyanobacterium Synechocystis sp. PCC 6803

Ph.D. candidate: Mgr. Martina Bečková

Ph.D. thesis comprises the most important results obtained on the role of Psb28 protein in the biogenesis of PSII in cyanobacterium *Synechocystis sp.* PCC 6803. This hydrophilic PS II assembly protein was previously studied by the group and the current Ph.D. thesis brought more insight into the understanding its role. The thesis includes a well written introduction which describes and discusses the current knowledge on PSII biogenesis with the focus on the PSII auxiliary proteins. In result section, four articles published in peer-reviewed journals are listed. Published papers are focused predominantly on the structure, localization and function of two Psb28 homologues, Psb28-1 and Psb28-2. In particular, different oligomeric states, location of binding to CP47 and role in PSII and PSI assembly were precisely addressed. In general, it is concluded that the topic of thesis is up-to-date and questions answered in the thesis are very important for the understanding of PSII biogenesis.

All four papers were published in peer-reviewed journals highly ranked in the field of plant science. One paper was published with the first authorship and other three papers as co-author publications. The Ph.D. student´s contribution to the published papers is clearly stated by the corresponding authors. There is no doubt that Ph.D. student contributed significantly to the obtained results. As the most conclusions made in Ph.D. thesis were already published, I have no serious objections to the content of Ph.D. thesis. I have just several questions and comments for discussion:

1) PSII core complex lacking CP43 (RC47) is considered as intermediate in the assembly of the monomeric PSII core complex. Is there any reason why CP47 is bound to PSII reaction center prior to CP43?
2) It is known that RC47 complex is vulnerable to light. Based on the observation that RC47-His complex was found to be photochemically active, the authors proposed that redox potential changes of Q_A eliminates singlet oxygen formed by charge recombination in PSII reaction center. Can Ph.D. student suggest how RC47 complex is protected against singlet oxygen formed in the antennae complex C47 via photosensitization Type II reaction?
3) It was shown that Psb28 protein is bound to CP47 in the vicinity of the PsbH and Scps. It was proposed that the binding sites of these proteins might even overlap. What will be physiological consequence, when these proteins compete for the same binding site?
4) Crystal structure of Psb28 protein obtained at a resolution of 2.3 Å showed that Psb28-1 likely exists as a dimer *in vivo*, whereas Psb28-2 protein is solely monomer. Even if physiological relevance is unknown, might Ph.D. student propose potential physiological relevance of dimeric structure?
5) Psb28-1 protein bound to RC47 promotes PSII and PSI assembly by supporting the synthesis of CP47 and PSI. Contrary, Psb28-2 which prevents the binding of Psb28-1 to RC47 avoids PSII and PSI assembly by inhibiting synthesis of CP47 and PSI. Can Ph.D. student explain how two structurally similar proteins might have antagonistic function?
6) Can Ph.D. student propose why there are two Psb28 homologues in *Synechocystis*, whereas in other cyanobacteria exist solely one homologue?

Ph.D. candidate managed to handle a number of experimental methods, provided ability to perform research and achieve scientific results, showed the ability to extract the information from experimental data and made appropriate conclusions. Thus, I recommend Mgr. Martina Bečková for receiving Ph.D. degree.

Pospíšil

In Olomouci, 9th November 2016 Doc. RNDr. Pavel Pospíšil, Ph.D.