Institute of Microbiology
Centre Algatech
Academy of Sciences of the Czech Republic
379 01 Trebon
Opatovicky mlyn, Czech Republic

Review of the doctoral thesis of Miloš Duchoslav 'Function of PsbO isoforms'

The thesis of Miloš Duchoslav is focused on the function of PsbO subunit of Photosystem II (PSII) and, more specifically, on the role of the second isoform of PsbO protein (PsbO2), which can be found in many plants species. The thesis is very carefully written and is provided with a nice and detailed introduction that really helps to understand the topic. Author summarizes and critically discusses the current knowledge about the PsbO including the enigmatic PsbO2 isoform.

Miloš Duchoslav is the first author of the article providing phylogenetic analysis of plant *psbO* genes and the co-author of publication describing biochemical analysis of recombinant PsbO proteins. These papers clearly demonstrate author's experience in the data collection, processing and writing the articles. In addition, the thesis contains unpublished data in a form of manuscript. As two mentioned articles were already published in peer-reviewed journals, I focus my review only on the enclosed manuscript entitled 'Functional differences between PsbO1 and PsbO2 proteins of *Arabidopsis thaliana* are smaller than anticipated '. This is a carefully performed research leading to a conclusion that the ability of both Arabidopsis PsbO isoforms to support PSII activity is comparable; at least during relatively mild stress conditions. Apparently, the level of PSII depends strictly on the PsbO availability and it is the total level of PsbO what matters. Author did not find a phenotypic difference between Arabidopsis lines possessing a similar level of PsbO1 or PsbO2 as the only PsbO isozyme. The main message of the work thus might sound a bit negative because no qualitatively new information about a specific role of PsbO isoforms is provided. I think however that these results have merit since unambiguously rejects the 'photoprotective role of PsbO2' reported previously by some authors.

I agree that the PsbO2 is hardly just a second (backup) copy of PsbO. As author has showed in his first publication, there is no explanation for the fact that the modified C-terminus of PsbO emerged repeatedly during plant evolution. Given the low level of PsbO2 in chloroplast, this protein could preferentially associate with a specialized or modified PSII, which can however accommodate also PsbO1 and a subtle advantage of PsbO2 is difficult to detect in laboratory conditions. It is pity that author did not test a broader spectrum of conditions, I believe that there is a good chance to detect a significant difference between *psbo1* and *psbo1*psbo2* lines. High light conditions are not a big challenge for Arabidopsis but the PsbO2 might be more important during e.g. rapidly fluctuating light, a nutrient limitation (low manganese), cold and high light stress or etiolation. Generally conditions inducing massive damage of PSII or its degradation. Proteomic analysis of *psbo1* and *psbo1*psbo2* lines would have much more sense if they show a phenotypic difference. The proteomic analysis presented in the thesis thus cannot help much as both lines were phenotypically almost identical. Indeed, it revealed a very different pattern between both PsbO mutants and the wild type control; however it is what one would expect for a poor, low-PSII mutant. For Western blot and immunodetection (Fig. 4.2) either less material should be loaded or antibodies much more diluted. The signal is too strong for quantification (it is looks too saturated to be linear) and with no signal control (e.g. 25 and 50% loading of the control sample).

I would like to emphasise that all my comments do not reduce the solid scientific quality of the presented thesis written in very good English and containing almost no formal errors. The thesis fully complies with all general demands for the doctoral thesis and I fully recommend it for the defence.

Questions:

1. The GTPase activity of PsbO appears extremely slow. Is there any example of biological relevant but very slow GTPase? What is the concentration of GTP in chloroplast lumen? Is there any?

2. The introduction of the thesis indicates that a structural change in PsbO induced by PsbO protonation might regulate PSII activity (page 20). Is there a model of how (when) such a regulation works. Could be the acid–base hysteresis in PsbO, described in publication 2, connected to a regulation of PSII?

3. How to interpret the observation of Fischer et al 2008 (now, after more than decade) that a spontaneously tuberizing mutant potato lacks one isoform of PsbO (PsbO2?). Does the PsbO (PSII activity?) really affect tuberation?

4. In contrast to plants, cyanobacteria (*Synechocystis* 6803) can grow photoautotrophically without PsbO protein. What is the level/activity of PSII in the *Synechocystis* PsbO-less mutant. What is known about the stability of cyanobacterial and plant/algal PSII complexes lacking the PsbO subunit?

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Doc. Roman Sobotka, PhD

☎ +420 384 340 491 Email: sobotka@alga.cz