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Cc.: Thesis review for Ms. Vendula Krynická

Title: „Role of FtsH proteases in the cyanobacterium *Synechocystis* sp. PCC 6803 "

Photosystem II (PSII) of the photosynthetic apparatus is one of the most important bioenergetics complexes in nature, which is responsible for light induced splitting of water into electrons, protons and molecular oxygen. This process utilizes water as a practically unlimited source of electrons, which together with protons and atmospheric CO_2 are utilized in the production of hydrocarbons during photosynthetic conversion of light energy into chemical energy. Due to the high complexity of the PS II complex and its inherent light sensitivity, which results in the rapid degradation of the D1 reaction center subunit and other PSII components, PSII requires a strict quality control. This process ensures the removal of damaged PSII subunits and the restoration of active PSII centers under different light environments via de novo protein synthesis. Key elements of this quality control process are the so called FtsH proteases, which belong to the ATP dependent transmembrane metalloprotease family. These proteases are present in mitochondria, bacteria and chloroplasts and have important function not only in PSII but also in protein processing and turnover in general, and also in maintaining cellular homeostasis.

The work of Ms. Krynicka deals with a comprehensive study of the cellular function of FtsH proteases using the model photosynthetic organism *Synechocystis* sp. PCC 6803, which encodes four FtsH homologues, FtsH1-FtsH4. The focus of her interesting work is the detailed understanding of the quality control role of FtsH2 in PSII, the importance of interactions

among the FtsH homologues, as well as clarifying the role of the other FtsH (1, 3, 4) homologues.

In the first part of her thesis, Ms. Krynicka performed a thorough analysis concerning the role of FtsH2 in quality control of PSII. In this approach she used mutant systems, which either lack the extrinsic oxygen-evolving subunits, or unable to process completely the D1 precursor protein. In the absence of the extrinsic proteins, which make the water oxidizing complex partly inactive, D1 turnover was accelerated and the amount of assembled PSII complexes was decreased. Additional elimination of FtsH2, by deleting its ftsh2 gene, induced a significant slowdown of D1 turnover. These data provide evidence for the important role of FtsH2 in the degradation of D1 in PSII complexes under conditions of donor side photoinhibition, which is induced by the decreased water oxidizing activity. In addition, the data also showed that the lack of FtsH2 did not affect the degradation of unassembled D1 protein in the mutant in which PSII assembly was blocked in an early step of the process. This interesting result shows that other proteases than FtsH2 are involved in the degradation of D1 subunits, which are not incorporated into larger complexes.

In the second part of her thesis work Ms. Krynicka analyzed the structural relationship among the FtsH homologues including the composition of FtsH complexes in vivo. By using 2D electrophoresis in combination with specific antibodies three oligomeric FtsH complexes were identified. Among those the FtsH2/FtsH3 hetero-oligomeric complex was purified, and the hexameric structure of the complex was demonstrated by single particle analysis, which showed alternating FtsH2/FtsH3 subunits. It was also shown that the absence of FtsH3 impairs D1 protein degradation. Therefore, it is not the FtsH2 homologue alone, but the FtsH2/FtsH3 hetero-oligomeric complex, which is responsible for the main quality control of PSII.

In the third part of her thesis work Ms. Krynicka studied the role of the other hetero-oligomeric complex, FtsH1/FtsH3. Both of these FtsH homologues are essential for the survival of the cells, therefore neither of them can be permanently deleted. In order to overcome this problem conditional knock-down mutants were created in which it was possible to down regulate the level of the targeted proteases. The interesting results show that the mutants, which were conditionally depleted of FtsH1 or FtsH3 are unable to induce the expression of the IsiA chlorophyll binding protein and of the FutA1 iron transporter under conditions of iron deficiency. It was also shown that the FtsH1/FtsH3 complex regulates iron-depletion induced genes by controlling the level of the Fur transcriptional regulator. By using

GFP tagged FtsH homologues it was demonstrated that both FtsH1 and FtsH3 are localized in the cytoplasmic membrane.

In the final part of her thesis work Ms. Krynicka studied the dark stability of PSII subunits in *Synechocystis* mutants, which are blocked in specific stages of assembly. It was found that the D1 subunit becomes degradable in the dark by the FtsH2/FtsH3 complex even in photochemically active PSII complexes, which lack the CP43 subunit, which normally shield D1. Similarly the removal the CP47 subunit, which shields D2, was shown to increase the FtsH mediated degradation of D2 in the dark. These data demonstrate that protease accessibility in partially assembled PSII complexes is an important regulator of D1 and D2 degradation by FtsH.

The results of Ms. Krynicka have been published in four articles in leading journals of the field including one BBA, one Plant Cell, one Molecular Microbiology and one (accepted manuscript) Nature Plants. This excellent publication output impressively documents the high scientific quality of the work of Ms. Krynicka and her significant contribution to the improvement of our understanding the function of the FtsH protease family in the quality control of PSII in cyanobacterial cells.

The thesis contains these four articles and provides a well written introduction into the topic, as well as a clear summary of the results. It reflects the capacity Ms. Krynicka to carry out high quality and internationally competitive scientific work.
Therefore, I strongly recommend Ms. Krynicka for a PhD degree.

Yours sincerely,

Dr. Imre Vass
Director, Plant Biology Institute, BRC-HAS

Review of the Ph.D. thesis by Vendula Krynická „Role of FtsH proteases in the cyanobacterium *Synechocystis* sp. PCC 6803"

The thesis deals with the functional and structural characterization of FtsH proteases in cyanobacterium *Synechocystis* PCC 6803. This work is based on four excellent papers. Three of them were published in highly impacted journals. The last paper was recently accepted for publication in Nature Plants. Publication list 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. Vendula Krynická is the first author of the last two papers, which indicates her major contribution in experimental part of the work, data analysis, compiling and writing the manuscript.

The thesis starts with the Overview, where the author gives an introduction to proteases and their role in adjustment of a protein level inside the cell. In line with the subject of the thesis, the major part of the Overview is devoted to FtsH proteases. The author describes a current stage of knowledge about the structure and function of FtsH proteases in different organisms and organelles. Although this part is well-written, I would appreciate a better description of open scientific questions related to FtsH proteases and how the published work by Vendula Krynická and co-authors contributed to answer those questions. In my opinion, the author did not sufficiently emphasize the impact of published work in the context of the Overview. Nevertheless, this aspect is not completely omitted in the thesis. The impact and contribution of the published work becomes more evident from the following chapter, the Summary and the last chapter, Conclusions.

Regarding the experimental work, the content of the Results part indicates that Vendula Krynická had to master a broad spectrum of experimental techniques from the fields of microbiology, molecular biology, biochemistry and biophysics, which is impressive.

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

1) The FtsH2/FtsH3 hetero-complex is involved, among others, in a degradation of the D1 protein under high-light conditions. Is it known how the concentration of the FtsH2/FtsH3 complex is controlled? Is it constant or does it also depend on environmental conditions?

2) FtsH protease forms a supercomplex with a membrane bound HflK/C complex, which was proposed to be involved in a degradation of membrane proteins. How is it in case of the degradation of D1 protein by the FtsH2/FtsH3 hetero-complex? Does it also require a presence of the HflK/C complex?

3) In the Conclusions chapter, there is stated that the FtsH4 homo-oligomeric complex is located in well-defined spots within the thylakoid membrane (unpublished data). Can you be more specific about the localization of the FtsH4 complex? What is the role of the FtsH4 homo-oligomeric complex?

4) FtsH1/FtsH3 is involved in the acclimation of cells to iron deficiency. It regulates transcription of iron depletion-induced genes by controlling the level of the transcriptional regulator Fur (Sll0567). It was suggested that the synthesis of IsiA proteins is related to the level of oxidative stress. Is there any relation between reactive oxygen species formation and the action of the FtsH1/FtsH3 under conditions of iron deficiency?

5) Page 2, the first paragraph: "... cyanobacteria are likely more than 3.5 million years old." Is this information correct?

Finally, I congratulate the author on the considerable scientific contributions to the role of FtsH protease in cyanobacteria and strongly **recommend, in case of successful oral defense, the award of Ph.D. to Vendula Krynická.**

In Olomouc, December 7, 2015.

RNDr. Roman Kouřil, Ph.D.