

Localization of the Small Chlorophyll *a/b*-like Proteins in the Cyanobacterium *Synechocystis* PCC 6803

The Ph. D. thesis of Kamoltip Promnares, M.Sc. deals with SCP proteins – small members of the LHC protein family known from cyanobacteria and eucaryotic plants. Little is known about their function and exact localization in the thylakoid membrane and this work is a substantial contribution to the knowledge in this area.

The thesis is based on two papers accepted by the Journal of Biological Chemistry, which makes the reviewer's task much easier. The acceptance by such high-impact journal is a proof of certain level of quality, already tested by others, allowing to read much of the work as an interesting information source rather than a text to be critically reviewed.

The enclosed papers contain most of the results. The rest of the work consists of a twenty page Introduction, ten page Results and Discussion and a list of about eighty (my rough estimation) references, cited in the two mentioned parts. There is no experimental part, all the methodology is described in the enclosed papers.

The Introduction briefly treats the basics of photosynthesis, photosynthetic electron transport machinery and some cyanobacteria specials. Then a nice review follows, introducing to the LHC protein family and particularly it gives an overview of its less known members distant from the light-harvesting antenna complexes.

The Results and Discussion part contains some additional results, however, its main purpose seems to be to discuss transparently, more in details, topic by topic and in one place, the results described in the enclosed papers. Here I miss a brief summary which – anywhere in the thesis – would outline the main results. This part discusses the results, citing the enclosed papers as well as many others, and one can hardly distinguish between the results of the author and of the other groups. Moreover, some of the subchapters here cite only other groups' papers.

So if I extracted well the information, the author draws the following main conclusions:

- Confirmation that ScpD is localized in the thylakoid membrane and associated with PS2.
- In the PS2 complex, ScpD is in close contact with CP47 and PsbH proteins, oriented with the N-terminus to the stromal side of the complex.
- ScpD is present both in PS2 dimer and monomer, in a number from zero to several copies.
- ScpD does not form large complexes similar to that of iron-deprived cyanobacteria or prochlorophyta.
- ScpC associates with PS2 as well, likely in a way very similar to that of ScpD, with functional complementarity to ScpD.
- ScpE does not seem to be involved in any large thylakoid membrane complex.
- ScpB may be associated with PS2, probably not in its dimeric form.

As already mentioned, the results are discussed in detail both in the enclosed papers and in the accompanying text.

To achieve these results, the author had to master a broad range of methodology, starting from construction of the mutants, continuing with growing the cyanobacterial strains (not always easy with mutants), isolation of thylakoid membranes, extraction of membrane protein complexes, their separation and purification by means of various

electrophoretic methods or affinity and ion-exchange chromatography, immunological methods like western-blot detection or gold labeling and finishing with data analysis and the ability to write a good scientific paper.

After reading the thesis, I am convinced it meets all the conditions for a Ph.D. thesis I know to be required at the Faculty of Sciences of the Charles University in Prague.

To answer the other questions that your faculty study department (probably) asks me:

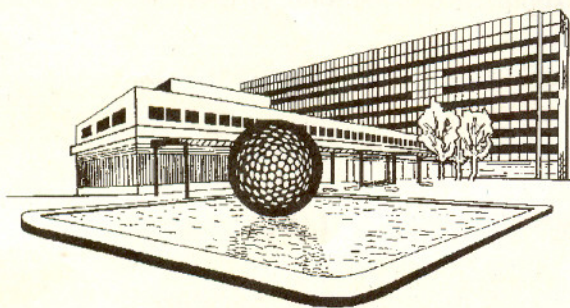
- 1.,2. *Is there an apparent original intellectual contribution of the student? — Is there an apparent self-reliance of the student in the experimental work and in the interpretation and presentation of the results? —* Yes, the author's original intellectual contribution, as well as her self-reliance in experimental work and in result interpretation and presentation is visible from the thesis: To successfully write a scientific paper with a contribution of 80 %, an intellectual contribution and self-reliance is indispensable.
3. *Are the results original? What is their main benefit? —* Yes, the main results are summarized above. They are very important in understanding the function of Scp proteins and potentially also of their higher-plant homologues. They contribute substantially to the knowledge on the structure of PS2 and particularly on its structure-function relationship, the different structure possibilities needed for regulation and protection of PS2 under different conditions. They may also help to elucidate some of the evolutionary relations between the various members of LHC protein family from functional point of view.
4. *Would this work be sufficient as a Ph.D. thesis at your institution? Yes or No. —* Yes, see above.
5. *How would you evaluate this student's work among the other Ph.D. students you have known: among the best 10 %, among the best 25 %, average, among the worst 25 %. —* According to the thesis, I am sure the author belongs to the better half of the Ph.D. students I have known. However, their number is still insufficient to allow me for any percentage estimation.
6. *Do not rate the work, please give only your opinion whether it meets the requirements for Ph.D. or not. —* Yes, see above.

Finally I would like to ask the following questions to the author:

- In my experience, the resolubilization of the first dimension gel lane and its proper loading on the second dimension SDS gel are often critical steps for successful 2-D separation. Have you observed different solubilization efficiency for different first dimension gel concentrations? How do you assure the tight contact of the two gels?
- Have you ever encountered, in your work or in literature, any relationship between the incorporation of Scp proteins into PS2 and the various types of PS2 functional heterogeneity known for quite a long time?

Prague, November 7, 2006

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OPPONENT'S COMMENT

The Ph.D. thesis written by Kamoltip Promnares entitled „Localization of the small chlorophyll a/b-like proteins in the cyanobacterium, *Synechocystis* sp. PCC6803” is a well-constructed and grammatically correct thesis. I could hardly find any even minor mistakes. These mistakes did not disturb the understanding of the thesis. I shall give my general and specific comments in the order of chapters in the thesis according to the requirements of the university.

I recognized the results written in the chapter „Results and Discussion” as original and new results about the localization of the proteins of SCP family, which indicate that some of these proteins are located in the photosynthetic thylakoid membranes and in the close vicinity of PS II reaction centres. These results could suggest the important role of these proteins in the structure and function of photosynthetic apparatus.

1. General comments.

- a) The subject of the thesis is in the front of the candidate's research area.
- b) The thesis is well constructed and the individual chapters are proportional. Since the logic of the thesis is clear it can be easily followed and it is easy to read.
- c) The number and the quality of the referred publications demonstrate that the candidate knows the main lines of her subject and she is able to summarize the results of her field.

- d) The used techniques and methods are modern, appropriate and well illustrated in the text of thesis or in the attached figures and papers.
- e) The style and the language of the dissertation are excellent and I could find just a few mistakes that I indicated in the text. The figures are demonstrative and well constructed.

2. Specific comments.

- a) The "Introduction" is a nicely written chapter.
- b) The "Aim of Study" is a very important part of the doctoral thesis. I think she focused on the main point of the thesis so I could accept that aim what she wrote.
- c) The "Results": Here she listed several important new findings concerning to the localization of SCP family in the thylakoid membranes.

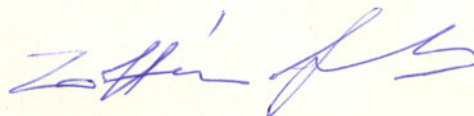
I missed a part, which would conclude and summarize the results. It would give a frame of this nice work. All my critics do not question the main goals and importance of the thesis. The candidate generated an enormous number of very useful data. I found that results, summarized in the thesis, are important achievements of the candidate's research area. I could recognize the results as new and important for the understanding the localization and functions of the proteins of SCP family.

1. The results of the students are new and original findings, which supports the localization of the proteins of SCP family in PS II and in photosynthetic membranes.
2. According to the papers published with the results, the student performed autonomously of her results and she could interpret the experimental results. She could present her results in papers and at scientific meetings.
3. The results and the project are original which generated several new findings concerning to the localization of the proteins of SCP family. These results could suggest the role of these proteins in regulation and protection to light stress. It also

suggested the involvement of the proteins in the biosynthesis of photosynthetic pigments.

4. Yes. This thesis is adequate for PHD degree in my country.
5. Among the best 10%. The two papers that are accepted in Journal of Biological Chemistry underline that the candidate did her job at very high quality.
6. I find that her thesis meets the requirements of the Ph.D. process, therefore I suggest to be awarded the Ph.D. title.

30, 10, 2006, Nagoya



Zoltán Gombos D.Sc.

The PhD Thesis Evaluation

PhD applicant: **Kamoltip Promnares**

Title: **Localization of small chlorophyll a/b – like proteins in the cyanobacterium *Synechocystis* sp. PCC 6803**

Range: 87 p. including the Abstract, Introduction, Results and Discussion, and two enclosed papers, one in print, another submitted

Referee: RNDr. Helena Synková, CSc.

Ústav experimentální botaniky AV ČR, Praha

The main aim of the thesis was clearly declared in the title and it was the localization of small CAB-like proteins (SCPs) in cyanobacterium *Synechocystis* sp. PCC 6803.

The first part of the thesis introduced readers into the world of light-harvesting proteins and their closer and distant protein relatives that have been identified in higher plants, green algae, and cyanobacteria. This part is very informative, it is well written and easy to follow. It gives an excellent overview of this field of study. The following part of the thesis summarizes all valuable results, which were obtained during this study, and discusses them with respect to available literature. To localize SCP proteins in the cells of cyanobacteria, the author chose highly sophisticated approach that included a construction of several deletion mutants expressing His-tagged proteins, which enabled easy localization using commercially available anti-His antibody. The author proved that she is able to utilize advanced methods of molecular biology for the construction of mutant and transformant strains, various methods of isolation and purification of pigment-protein complexes, and methods of protein separation and analysis (such as e.g. radiolabeling, 2D electrophoresis and immunoblotting). Two enclosed papers confirm that the aim of the thesis was realized, because they fully cover the topic studied in the thesis. Thus, several members of SCP protein family were found to associate with high-light damaged PS II and to be localized on the periphery of this photosystem.

The author demonstrates her ability to combine her theoretical and practical knowledge and use advanced methods of molecular biology and proteomics to obtain new and interesting

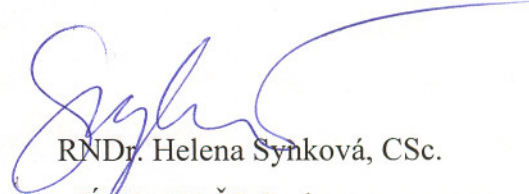
results that contribute to our basic knowledge concerning processes of reparation and/or turnover of pigment-protein complexes in photosystems.

The most important results are included in Paper I, which was reviewed and accepted in the Journal of Biological Chemistry (IF 2005 = 5.854). Paper II was prepared and submitted in the same journal and it will be hopefully accepted soon, because it is (according to my opinion) of the same quality as the previous one. This is the reason why I do not have any serious objections against the presented thesis and my remarks are only marginal. The work is well written and I have found only a few typographical errors. I would appreciate a separate list of basic abbreviations, which would be helpful particularly for the Introduction reading, even when the abbreviations can be found in enclosed papers. In legends to Fig. 1.9 (p. 19) dashed lines are mentioned, but they are hardly to be recognized at this size of the scheme. It was mentioned (p. 30-31) that variable chlorophyll fluorescence and non-photochemical quenching was studied in several SCP-less strains. I would like to know if the applicant herself was involved in such a study and if yes, then whether any differences in photosynthetic performance of several *Synechocystis* mutants were found under stress conditions. Have been such results shown or published elsewhere?

The PhD work of Kamoltip Promnares meets demands set to PhD thesis, because she proved her ability to work independently, to draw original conclusions from experimental data obtained by advanced scientific methods, and to summarize all in the paper that can be accepted in renowned international scientific journal. Although only one paper has been accepted so far, another one would surely follow. This should be enough to reach PhD title, because her work brings new and important information in this field.

Taken into account of all above mentioned, I recommend this PhD Thesis to be accepted and the PhD title be conferred upon the applicant.

Praha 31.10. 2006


RNDr. Helena Synková, CSc.
ÚEB AV ČR Praha