EBERHARD KARLS
UNIVERSITÄT
TÜBINGEN

Mathematisch-Naturwissenschaftliche Fakultät

PD Dr. Iris Maldener

Interfakultäres Institut für Mikrobiologie und Infektionsmedizin
Organismische Interaktionen

Auf der Morgenstelle 28
D-72076 Tübingen
Tel.: +49 7071 29-78847
Fax: +49 7071 29-5843
iris.maldener@uni-tuebingen.de

To the Committee for PhD studies
Faculty of Science
University of South Bohemia
37005 České Budějovice

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Evaluation of the PhD thesis from Lenka Bučinská

With the title: „Biosynthesis of chlorophyll-binding proteins in cyanobacteria"

This thesis deals with the biogenesis of the complex thylakoid membrane and its relation to the embedded protein-Chl*a* complexes in the unicellular cyanobacterium *Synechocystis* PCC 6803, which serves as model organism for photosynthesis and cyanobacterial metabolism. Cyanobacteria are the only bacteria, which perform oxygenic photosynthesis using two different photosynthetic complexes, PSI and PSII. By endosymbiosis, this important energy producing system was introduced to plants, in which chloroplasts evolved from the symbiosis partner. Hence, in higher plants, a similar photosynthetic apparatus exists, and the study of cyanobacteria can help to solve important questions on the structure, biogenesis and mechanism of plant photosynthesis.

The thesis deals with the link between the formation of the inner thylakoid membrane (TM), which bears all components of the light reaction, except the phycobilisomes serving as PSII antennae, and the biosynthesis and assembly of Chlorophyll (Chl) –binding proteins of the photosystems.

Four publications with the contribution of Mrs. Bučinská and unblished results (manuscript in preparation) are included in the dissertation:

1. In Koečná et al. 2012, Mrs. Bučinská preformed the TEM analysis of *Synechocystis* cells, grown under different light conditions. She could show that under high light conditions, the thylakoid membranes were much less and less compact compared with cells grown in low light. Also high amount of glycogen granules were clearly visible in ultra-thin sections of the cells, which were prepared after high pressure freezing and freeze substitution. This was in line with the observation that PSI was extensively down regulated under high light, and suggested that content of TM and PSI complex are linked and commonly regulated. This was a valuable contribution to this topic.
Q: With respect to the methods described in the paper I wanted to discuss the following: the cells have been shifted from normal/low light conditions to medium or high light and then incubated for 24 hours. This corresponds to approximately one generation time. Is this enough growth time to speak about acclimation? Are those cultures after 24 hours still in a process of adaptation or already acclimated to the new conditions?

2. In Hollingshead et al. 2016, published in the reputable journal *Frontiers in Plant Science*, Mrs. Bučinská had performed the ultra structural analysis by TEM of mutant cells, which were deleted in the *ycf54*-gene. This gene encodes a protein with unclear function in the Chl*a* biosynthesis. The detailed characterization of the mutant provided insights in the relation of de novo Chl*a* biosynthesis and PS complex formation. A reduced chlorophyll level prevented PSI synthesis and formation of the PSII inner antennae. In addition, Lenka Bučinská´s ultra structural study of thin sections of the wild type and the *ycf54*-mutant showed that formation of TM highly depends on the presence of PS complexes, even in presence of the other TM bound proteins, which are not affected by lack of Chl*a* biosynthesis. This was an important contribution to this analysis of TM formation and PS biosynthesis.
Q: Different wild type background strains were mentioned and used in this study. What are the characteristics of these strains and why are some useful for this study and the other was not?
Q: Can the *ycf54*-mutant grow, like under mixo-photrophic conditions, and does it then still perform photosynthesis?

3. The third publication, Mrs. Bučinská co-authored, was published in *Current Biology,* a high-ranking journal of bioscience. Here, she investigated the ultra structure of *Synechocystis* cells during chlorosis and after re-greening. In this paper, a detailed investigation of the metabolic, transcriptional and structural levels was performed. The authors could demonstrate that this cyanobacterium has a highly sophisticated adaptation mechanism and that it is after a prolonged dormant state immediately prepared for resuscitation.
TMs were almost completely absent in chlorotic cells, which lacked chlorophyll and hence photosynthesis. This is in accordance to the study No 2, which showed that without PS complex formation, the TM membrane system is not built up. Hence, this process allowed the investigation of TM formation in TM free cells.
Besides TM formation, Mrs. Bučinská focused on the presence of storage compounds, which accumulate during chlorosis in granules of glycogen and PHB. Glycogen was used for respiration during resuscitation and the granules reduced after 24h. The TM re-appeared, concomitant with recovery of pigments and PS-activity. PHB granules persisted for a longer time, in line with the chemical measurement. Also in line with the transcriptional activity, the development of ribosomes was observed. Fully recovered cells were visible after 66 hours and represented the exponentially grown cells. This was a thoroughly performed study, which added valuable data for this interesting phenomenon of awakening cells.
Q: Concerning the method, I was wondering whether the cells used for TEM imaging came from the same culture as the cells used for the metabolic and transcriptomic study? If not, was the cell density and growth as well as starvation and recovery conditions the same in all experiments? This also related to point 5., in which a faster timescale was described.

4. In the 4th publication, contributing to this dissertation, Mrs. Bučinská is first author and describes the function of a ribosome bound protein involved in chlorophyll insertion into the CP47 protein. This is a very fine study of PSII complex formation, using state-of-the-art biochemical methods to purify interacting proteins, and mutational analysis. In this work, part of the complicated process of PSII assembly has been studied: the membrane insertion of the antenna protein CP47 with the participation of SecYEG translocon,

Pam68 protein and ribosomes. By FLAG-tag pulldown of Pam68, the interacting proteins were identified. Investigation of the *pam68*-mutant showed that this protein is important under high light stress conditions, when CP47 synthesis is affected. In a resulting working model, the CP47 assembly in the membrane and with Chl*a* and the PsbH subunit is depicted. A membrane stabilizing function of Pam68 during chlorphyll insertion in the PSII antenna is demonstrated by this study.

5. Following the work from publication 3 (Klotz et al., 2016), Mrs. Bučinská investigated in more detail TM biogenesis and PS assembly (unpublished results in chapter 3). The appearance of PS subunits and pigments was studied in parallel to the ultrastructural changes during resuscitation after chlorosis. An interesting star-like TM structure was identified and studied by a modified sample preparation protocol in order to preserve the native structure much better. 3D modeling using serial sections showed that the star structures were the origins of TM synthesis, where a high amount of ribosomes were present.
In this part, she describes the uninduced culture as WT, for wild type. Since no mutant is used in this study she should change this to uninduced culture/cells, or similar. The legend of Figure 11 **C** is missing.
Q: What new method could provide an even more native and detailed image of the cells and their membranes and inclusions?
Q: How could protein candidates be localized to the star structures?
Q: Which strategy is used by diazotrophic cyanobacteria to adapt to lack of nitrogen?

In summary, the dissertation of Mrs. Lenka Bučinská is a nice piece of work adding new results and knowledge in the field of PS and TM biogenesis in cyanobacteria. She clearly demonstrated by ultrastructural and biochemical studies that both events are strongly interdependent. The experiments are well designed and performed showing the high scientific quality of her work. They are published in peer reviewed journals with high reputation in the field. The own contribution to the publication is clearly described and there is no sign of disregard of proper scientific work. Except that the text is clearly written in good English, I had problems when reading the chapter 2, called "Introduction" and to identify which part was actually done or published by her. The chapter rather is a combination of introduction, result and summary.

Despite this I consider this dissertation as a very good scientific work, which matches all criteria for a PhD thesis.

Iris Maldener

Review of the Ph.D. thesis by Lenka Bučinská „Biosynthesis of chlorophyll-binding proteins in cyanobacteria".

The thesis deals with a biosynthesis of chlorophyll-binding proteins and biogenesis of thylakoid membranes in cyanobacteria. This topic represents a very complex process in cyanobacteria. Experimental results of this thesis shed light on several aspects of the process of biosynthesis of photosynthetic proteins, formation of thylakoid membrane and their close relation.

This work is based on four excellent papers, which were published in highly impacted journals. 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. Lenka Bučinská is the first author of one paper, which indicates her major contribution in experimental part of the work, data analysis, compiling and writing the manuscript.

The thesis starts with the Preface, where the author provides readers with a general description of photosynthesis and the aims of the thesis. The Introduction part deals with details of architecture of the thylakoid membrane and a composition of the photosynthetic apparatus in cyanobacteria. In line with the subject of the thesis, the author describes a current stage of knowledge about the biogenesis of photosystem II, photosystem I and thylakoid membrane. The author emphasizes the impact of published work in the context of the Introduction. This part is well written and informative.

Experimental part of the thesis summarizes so far unpublished results related to biogenesis of thylakoid membrane during a recovery of cyanobacteria from chlorosis. This part is well described and discussed. As the author performed all experimental work, it indicates a very good theoretical and practical knowledge of all used methods (e.g. electron microscopy and specimen preparation, electrophoresis and immunoblotting, absorption spectroscopy, culture cultivation).

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

1. What is your opinion about interaction between plasma membrane and thylakoid membrane in cyanobacteria? Are there any connections between these two membranes?

2. In the part 9.3 (publication Bučinská et al. (2018) Plant Physiology), there is a CN-PAGE separation of solubilized protein complexes (Figure 4) (page 98). There is one unassigned high-molecular band at the top on the CN-PAGE. The presence of the band correlates with a presence of both PSI trimer and PSII dimer. Do you have any idea about the composition of the band?

3. Thylakoid membrane in cyanobacteria contains various microdomains with segregated Photosystem II and Photosystem I, which resembles the situation in higher plants. As thylakoid membrane in cyanobacteria does not form grana, what is the segregation factor, which keeps Photosystem II and Photosystem I separated in the microdomains?

4. At the early stage of the recovery of cyanobacteria from chlorosis, monomers of Photosystem I are synthetized. You prosed that they could be involved in cyclic electron transport (page 34). Do you have any experimental evidence that the cyclic electron transport is functional at this stage? How is the cytochrome b6/f complex affected by chlorosis?

5. Structural studies using transmission electron microscopy were performed on ultra-thin sections of cyanobacterial cells prepared after high pressure freezing and freeze-substitution. What are the benefits of this approach compared to conventional method of fixation at room temperature?

Finally, I would like to congratulate the author on the considerable scientific contributions to the field of biosynthesis of chlorophyll-binding proteins in cyanobacteria and I strongly **recommend, in case of successful oral defense, the award of Ph.D. to Lenka Bučinská.**

In Olomouc, February 19, 2019.

RNDr. Roman Kouřil, Ph.D.