VU VRIJE UNIVERSITEIT AMSTERDAM

Evaluation of the Ph.D. thesis of Eliska Kuthanova

Committee member: Roberta Croce, professor of Biophysics of photosynthesis, Vrije Universiteit Amsterdam.

The thesis focuses on the mechanisms of photoprotection, especially NPQ, of two interesting secondary endosymbionts algae, *Chromelia velia* and *Rhodomonas salina*.

The thesis is composed of an introduction providing the background to understand the regulatory mechanism of photosynthetic light reactions, a description of the methods used, an overview of the thesis, and a general discussion. The introduction is followed by five research articles which are all already published. The first research article focuses on the effect of violaxanthin on antenna quenching. In the second article, acclimation of *C. velia* to high light is studied by combining physiological and biochemical measurements. In chapter 3, a detailed study on the effect of pH-induced aggregation on the lifetime of the antennae of *C. velia* is presented. Finally, the last two chapters are dedicated to the study of the photosynthetic components of the alga *Rhodomonas salina*, with emphasis on the Chl a/c-binding antennae and on the PSI supercomplex (chapter 4) and on the photoprotective strategies of this alga (chapter 6). The thesis is multidisciplinary and presents a combination of biochemical and biophysical approaches applied to the understanding of a fundamental biological process: photosynthesis.

The work is original and interesting and helps in shedding light on the large diversity of photoprotection mechanisms in algae. I particularly appreciate the use of non-model algae that permits to explore biodiversity. This is very challenging since all methods need to be optimized, which can be hard, especially for the biochemical work. The candidate did an very good job. The thesis is, in general, well written, and the candidate gives the impression to master the topic well as can be seen by the research articles and the discussion. It is also important to highlight that she is an author of several papers that are not part of this thesis. The choice of what to present in the thesis is well justified and has permitted her to deliver a coherent manuscript, focused on high light responses.

Based on the arguments above, I recommend admitting Eliska Kuthanova to the thesis defense to obtain the title of doctor in Plant Physiology and Developmental Biology.

Since due to Covid-19 I am unable to attend the defense, I am adding below a few personal words for the candidate and some questions for the defense.

With my best regards

Roberta Croce

Amsterdam 10 June 2020

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Dear Eliska,

First, I would like to congratulate you on your very nice thesis. I enjoyed reading it, and I learn a lot about these very interesting algae that you have studied. I would have loved to be there to discuss your results in person, I hope we will have another occasion. I am writing below some questions in case there is the time during your defense. Enjoy your day!

In the research **chapter 1** you are attributing the difference in lifetimes between the three antenna bands to the difference in their violaxanthin content. However, these bands seem to differ in the protein composition (see gel) and in their molecular weight, since they have different mobility in the sucrose gradient. Their absorption and fluorescence spectra are also different, indicating changes in the protein and pigment organization/composition. In addition, fraction 1 seems to contain some free pigments. Considering all those differences between the bands, could you please explain how you can conclude that the main effect on the lifetime is directly due to the change in the violaxanthin content?

Regarding the aggregation experiment, did you check the effect of other xanthophylls added to the mixture compared to the effect of violaxanthin (inset in figure 4, chapter 2)?

In **chapter 2** you show that *C. velia* acclimated to high light decreases the number of PSII, while keeping a large antenna. We observed the same effect for the green alga *C. reinhardtii* in photoautotrophic conditions (Polukhina et al. 2016), suggesting that this is a more general strategy of algae. However, in *C. reinhardtii* NPQ is very large and relaxed very fast, while in *C. velia* it does not seem to relax (figure 1). This is puzzling because you also show that the photosynthesis rate of high light acclimated *C. velia* is high at light intensities in which sustained quenching should be present (figure 4a). Could you please help me to understand how I can combine the results in figure 1 and figure 4a?

In **Chapter 3** you are looking at the quenching by pH-induced aggregation comparing LHC with CLH. The difference in pI well explains the experimental results in isolated complexes. However, while the pI is calculated on the whole structure, in vivo, the protonation is most probably only possible at the lumen exposed residues. Do you think that the protonation of the three acidic residues of the luminal exposed loop is sufficient to explain aggregation *in vivo*?

In **Chapter 4** the first decay component for PSI is very short (13 ps) compared to other organisms with similar red form content. The spectrum also has a deep around 715 nm, which might suggest that this component is a mixture of trapping and excitation energy transfer from blue to red Chls. Could you comment on it? Did you try to fit the decays with more components to see if it was possible to disentangle the two contributions? How do you explain the large difference in amplitude between the two components (13 and 80 ps)?

Oponentský posudek disertační práce
Autor: Mgr. Eliška Kuthanová
Název: Regulation of photosynthetic activity of pigment protein complexes in thylakoid membranes

Význam tématu disertace v kontextu rozvoje oboru:
Disertační práce je zaměřena na studium fotoprotektivních mechanismů u dvou doposud z tohoto hlediska nepříliš prozkoumaných řas (Chromera velia a Rhodomonas salina). Téma práce je aktuální a práce jednoznačně významně přispěla k poznání variability (specifických charakteristik) nezářivé disipace absorbované excitační energie (NPQ) ve světlosběrných anténách a aklimace fotosyntetického aparátu na zvýšenou úroveň FAR u fotosyntetizujících organismů, v souvislosti s jejich evolucí.

Obsah práce a obecné hodnocení:
Práce má klasické členění (1. Teoretický úvod; 2. Hlavní a dílčí cíle práce, 3, Materiál a metody, 4, Přehled vlastních výsledků; 5, Závěr a další perspektivy; 6, Reference; rozsah 80 stran).
Stručně by se dalo říci, že celá práce je kvalitní, je napsána přehledně, je čtivá a nebyla odbyta stručným úvodem a přepisem abstraktů a závěrů publikací, jak tomu někdy bývá u disertací, které jsou podloženy bohatým souborem publikací. V této souvislosti bych obzvlášť ocenil kapitolu 2 (Hlavní a dílčí cíle), která jednoznačně a podrobně vysvětluje koncepci výzkumu a začleňuje do ní jednotlivé dílčí cíle (subprojekty). V práci je citováno téměř 200 relevantních publikací, včetně nejnovějších prací.

K práci je přiloženo 5 publikovaných článků (z toho 2x 1. autor, 1x sdílený 1. autor) v kvalitních impaktovaných časopisech. Z prvoautorských článků bych chtěl vyzdvihnout článek „Antenna proton sensitivity determines photosynthetic light harvesting strategy", nejen protože byl publikován v J. Exp. Bot. (1. decil v kategorii Plant Sciences), ale především, že v tomto článku byla objasněna jedna z klíčových specifických charakteristik světlosběrných komplexů C. velia (CLH) spočívající v zvýšené citlivosti vůči protonům (protonaci), která vysvětluje usnadněnou indukci NPQ v CLH.
Autorka se, kromě uvedených 5 publikací, podílela na dalších 4 publikacích (jedna je velmi čerstvá), které nejsou součástí předložené disertační práce, byť se minimálně okrajově problematiky disertační práce týkají. Za 4 roky se tedy paní Kuthanová podílela na 9 publikacích, což je na doktoranda impozantní počet.

Formální hodnocení práce:
Práce je psána kvalitní angličtinou, je vhodně doplněna obrázky (schémata). Celkově se práce velmi dobře čte. Jediná možnost, která mne napadá pro ještě lepší orientaci v textu – zřetelně vyznačit v kapitole 1 citace prací, které jsou součástí disertační práce.
V práci se vyskytuje pouze několik nepřesných/nejednoznačných formulací (např.) :
Str 18: 1 věta kapitoly 1.5.1. „The most commonly accepted theory states that NPQ in higher plants and green algae resides in the light-harvesting antennas which change their conformation responding to ***this*** ΔpH ....." ***„This" používáte poměrně často, například v tomto případě je jeho použití matoucí.***
Str. 34: „C. velia NPQ shares similarities with higher plants mechanism (the presence of violaxanthin cycle) on one side (Belgio et al., 2018; Kotabová et al., 2011; Quigg et al., 2012), and with diatoms (***e.g. variable fluorescence below minimal fluorescence*** and the presence of "NPQ

lock" (Ruban et al., 2004)) on the other." ***Variabilní fluorescence je obvykle definována jako rozdíl mezi F_M and F_0, spojení „pokles variabilní fluorescence pod F_0", mi příliš nedává smysl.***
Str. 37: „Crocoxanthin and monadoxanthin are additional less abundant carotenes (Kaňa et al., 2012; Kuthanová Trsková et al., 2019) with ***one more triple bond.*** " ***V souvislosti s předchozí větou, by toto tvrzení znamenalo, že crocoxanthin a monadoxanthin mají 3 trojné vazby.***

Za mírný formální nedostatek lze považovat skutečnost, že tištěná verze a elektronická verze práce nejsou úplně identické: V tištěné verzi je na straně 9: „..... that can lead to the reduction of harmful products like reactive oxygen species – ROS. V elektronické verzi je již správně "... production ....". Navíc v tištěné verzi jsou „prázdné stránky" 26, 32, a 46, které v elektronické verzi chybí. Je to opravdu minoritní změna, ale správně by měly být obě verze identické.

<u>Komentáře a dotazy / Comments and questions</u> (vzhledem ke skutečnosti, že autorka plánuje vést obhajobu v angličtině, jsou uvedeny v angličtině). For better orientation questions are written in bold italics.

Page 4: „All light-harvesting antenna complexes share properties and fulfill the following crucial functions ..... ii) a widening of the spectral range of photosynthetically active irradiation by means of various pigments or proteins with different absorptions (see e.g. (Horton and Ruban, 2005; Horton et al., 2000))." ***I think this is a bit oversimplifying / inaccurate statement. Can you explain how proteins with different absorptions (I would rather use absorption spectrum) contribute to broadening of the spectral range of photosynthetically active radiation?***

Page 13: **„**For example, high light treatment in higher plants usually increases Photosystem I/Photosystem II ratio (Boardman, 1977; Kendrick and Kronenberg, 1994), **.....**" . ***According to my knowledge increasing PS I/PS II ratio is not a typical acclimation response of higher plants to high light (for review eg. Schöttler and Tóth, Frontiers in Plant Sci 2014). Can you comment on this?***

Page 20: Among different types of light-induced xanthophyll cycles in different organisms you have mentioned: „ iii) Lutein cycle involving lutein-5,6, epoxidase observed in cuscuta species (Bungard et al., 1999). ***Is lutein cycle limited to cuscuta genus? Is it true that lutein-5,6, epoxidase has been confirmed as the enzyme involved in this cycle? Is it clear which enzymes are involved?***

Page 38: „Culture cultivation**".** ***You have described that C. velia and R salina were cultivated under different light égime (continuous light x 12/12 h day/night cycle). Can you explain why?***

One of the main findings and conclusions of your work was that "CLH antenna are highly sensitive to protons, which makes them ready to quench (Kuthanová Trsková et al., 2018)." ***The quenching of CLH fluorescence in vitro was induced at higher pH as compared to LHCII, however still it was not saturated at pH 5.5. On the contrary for C. reinhardtii NPQ titration curve shows that the quenching is activated at pH 6.7 and saturates at pH 5.5 (Tian et al. PNAS 2019). Can you comment on the different pH (ΔpH) sensitivity of NPQ in C. reinhardtii and C. velia in relation to the evolution of NPQ mechanisms?***

Page 49: You have mentioned that "organisms with antennas not that efficient in light harvesting (i.e. antennas ready to be switched into the quenched state), such as CLH from C. velia, have to carry a high concentration of free (lipid phase) violaxanthin to improve their light-harvesting (Kuthanová Trsková et al., 2018, the model on Fig. 11). ***The potential role of lipid phase xanthophylls in regulation of CLH function is very attractive possibility, indirectly supported by***

lower relative violaxanthin content in isolated CLH as compared to intact cells of C. velia (Kaňa et al. 2016). However this should be taken with caution as washing out of loosely bound violaxanthin from CLH during isolation cannot be ruled out. Do you have another evidence for the violaxanthin/zeaxanthin presence in the bulk lipid membrane?

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Závěr

Jak teoretická část disertační práce, tak přiložené publikace jsou velmi kvalitní. Je zřejmé, že autorka odvedla opravdu velký kus poctivé experimentální práce. Jednotlivé prezentované výsledky svědčí o nesmírně koncepčním a nápaditém přístupu k řešené problematice s využitím biofyzikálních, biochemických i bio-informatických přístupů. Práci jednoznačně doporučuji k obhajobě a po úspěšné obhajobě práce doporučuji udělit Mgr. Elišce Kuthanové titul Ph.D.

V Ostravě dne 14.6. 2020

Doc. RNDr. Vladimír Špunda, CSc.
KF PřF OU