Reviewer´s opinion of diploma thesis

Reviewer of the thesis: Dr. Katarzyna Retzer

Date: 05.09.2017

Author of the thesis: Filip Linhart

Name of the thesis: Characterization of eukaryotic translation initiation factor 3 subunits (eIF3) in A. thaliana male gametophyte

Objectives of the thesis

1.Characterization of eif3b1, eif3b2 and eif3e knock-out mutant lines by phenotypic screening and transmission analysis.
2.Investigate promoter activities of eIF3B1, eIF3B2 and eIF3E by plant transformation and fluorescent microscopy as well as publicly available expression data.
3.Investigate subcellular localization of eIF3B1, eIF3B2 and eIF3E by plant transformation and fluorescent microscopy, colocalization with ER and PABP.
4.Analyze possible regulation of eIF3E by COP9 signalosome in the male gametophyte using knock-out mutant lines and fluorescent reporters.

Structure of the thesis

Size of the thesis (number of pages): 105 pages including cover page, acknowledgements and thesis content pages.
Are the English and Czech abstracts and keywords given? Yes

Formal level of the thesis (visual documentation, graphics, text, list of literature)

The thesis is written in logical and orderly manner and therefore the readability of the text is very good, the presentations of the figures is clear and of good standard and the author included a sufficient and up to date reference list.

Logical structure and language quality of the thesis

The thesis is well structured and the author used formal, scientifically precise language.

Literature overview:

Does it correspond to the topic and is it logically structured? Yes
Is it written comprehensibly? Yes
Are the literature sources used relevant and up-to-date? Yes
Are the literature sources used (including pictures) correctly quoted? Partially in the introduction and in some figure legends I am missing references.

Materials and Methods:

The extend of methodologies used. Yes
Do described methods correspond to results presented? Yes
Are methods comprehensibly described? Yes

Experimental part:

Are the aims of particular experiments explained? Yes
Is the documentation of the results adequate? Yes
Is the number of conducted experiments sufficient? Yes

Discussion:

Is it really a discussion, is it not just a repetition of previously mentioned results? Yes
Are the results related to the literature? Yes
Are there any hypotheses or suggestions for further research? Yes

Conclusions (Summary):

Are the main findings supported by the data? Yes

Are they formulated appropriately? Yes

Achievement of aims and overall assessment:

The student demonstrates understanding of the topic, and fulfilled the main aims. Although co-localization studies of the studied eIF3 subunits was not addressed completely, the student was able to collect a high amount of data about the distribution of the proteins of interest and proofed their importance for proper plant development. The total amount of done experiments extended by far the framework of an average master thesis.

He presented the results in a clear and understandable way, which supports the applicability of his research work in further studies.

The title and aims of the thesis are clearly formulated and sufficient addressed in the written thesis. The abstract is well structured and informative. The thesis is written in a logical and orderly manner, supported by well composed figures, which is reflecting the high amount of work invested into obtaining the data for the master thesis. The discussion summarizes and describes the results detailed and incorporated current knowledge from the literature. His work will allow to understand post-transcriptional regulatory mechanisms better and therefore has successfully fulfil its objectives.

Questions and comments of the reviewer (<u>mandatory part of the report!</u>):

Q1: You were using heterozygous eIF3 subunit mutants to study sporophytic phenotype, why do you thing you can't observe an obvious phenotype. Which approach would you choose to study the impact of their mutated variants during different developmental stages of plant growth and different tissues.

Q2: The semi qRT approach appeared to be challenging due to gDNA contamination. Why is this problematic for the evaluation of the result; which parameter are important for a successful semi qRT; and which methods can be instead done to check the expression of the gene of interest.

Q3: Promoter activity for the single eIF3 subunit were tested by fusing the corresponding promoter to GFP, which other method you would use to check promoter activity and why did you decided to use GFP. Why does gene activity and protein abundance don't always correlate?

Q4: Localization studies of the eIF3 subunits revealed intracellular clustering. Co-localization with an ER-tracker suggested that they are concentrated at the ER, for which proteins is this especially important. To further exclude co-localization with other compartments, which approach would you choose.

Q5: How can you determine the differences in translation for the single eIF3 subunit mutants compared to WT pollen.

Q6: If the eIF3 complex undergoes abundance regulation, which regulatory mechanisms beside protein degradation are possible. And if you want define which proteins are involved in eIF3 protein turnover, how would you test it.

Comments:

1. The semi qRT-PCR done with samples contaminated with genomic DNA and poor normalization will not allow a realistic analysis of the reduction in expression. Therefore, I recommend to repeat it with new samples or preferably by qPCR, which is more sensitive to detect expression differences.

2. Instead of T-DNA insertion lines, which are embryo lethal or showing a severe phenotype, to test sporophytic phenotypes or tissue specific regulatory effects, I suggest knock-down under inducible or tissue specific promoter.

Reviewer´s final classification proposal:

☒x excellent (výborně) ☐ very good (velmi dobře) ☐ good (dobře) ☐ unsatisfactory (nevyhověl/a)

Signature of the reviewer

Note: The usual length of a standard review is about 2-3 pages.

Instructions for the preparation and submission of the opinion:

- Use this form for evaluation of the thesis. The text in standard font serves as a guide.
- According to the University rules, the opinion must be made available **at least three working days prior defence**.
- You can submit the fulfilled form by yourself to the SIS or send it in advance electronically to: hana.konradova@natur.cuni.cz and lipavska@natur.cuni.cz. Furthermore, please, ensure the delivery of the signed original to the secretary´s office of the Department of Experimental Plant Biology, Faculty of Science, Charles University (Mr. Elena Kozlova), Viničná 5, 128 44 Praha 2. The signed printed copy of the opinion must be delivered in advance, without it the defence cannot start!