Ph.D. Thesis Review

The thesis of Mgr. Viacheslav Zemlianski „*Nitrogen source as a determinant of mitotic fidelity in fission yeast*" aimed, based on its preliminary scope as published on the online faculty information system, to

- Determine impact of different nitrogen sources on mitotic fidelity; elucidate the molecular mechanisms employed
- Define transcriptomic signatures of states promoting *vs.* suppressing catastrophic mitosis
- Test conservation of effects of nitrogen source availability in another fission yeast species.

The main part of the experiments employed mitotic fidelity analyses monitored by fluorescence microscopy approaches, complemented by RNA-seq analyses of selected mitotic mutants. The lipidome analysis performed by the Slovak cooperator was also an important complement, as it allowed to map the primary impact of the nitrogen source on lipid metabolism, whose defect seemed to be a potential cause of the observed errors in mitosis.

Form and elaboration

The thesis is written in a concise form, in a clear and error-free graphic layout, in a factual language with a minimum of typos. For example, the confusion of singular and plural occurs more than once: "*The term lipids represent a wide group of chemical substances…*" – p. 16, "*Disturbances of lipid metabolism leads to…*" – p. 18, etc. Similarly, the use of the words "*bond*" (noun) and "*bound*" (verb) is confused repeatedly – p. 17. A word "lipid" is probably missing in the sentence "*We showed that two main transcriptional regulators of non-sterol metabolism, Cbf1 and Mga2…*" – p. 41. Unfortunately, this type of oversight is not picked up by the standard spell checker and requires careful proof-reading. However, given the low frequency of these mistakes, this does not significantly affect the overall readability of the text.

The introductory section is the longest chapter of the thesis, and that's definitely a good thing in this case. The reader is gradually introduced to the various fields of cell biology that form the basis on which the thesis is built. I have only minor comments on the content of this otherwise comprehensive Introduction:

- Meiosis is not mentioned at all in section "*1.1.1 Cell cycle and mitotic entry*", but only much later in "*1.4.1 Role of nitrogen in the yeast cell"*. It's quite confusing, almost as if meiosis is a specialty of starved yeast among all eukaryotes.
- Section "*1.3.1 Overview of lipid metabolism*" seems inappropriately named. While the text therein is valid and valuable to the thesis, it bears little relation to lipid metabolism (except for the part on lipid droplets) and in any case does not provide a general overview of it. For example, it does not touch on biochemical principles, subcellular localization or regulation mechanisms of lipid metabolism at all.
- Source of the Figure 5B (which is Publication 1) should be indicated in the figure legend.

Unfortunately, the Results and the List of publications sections have been merged. While the information about the author's contribution is reasonable to give separately for individual publications, a summary of the results would be much clearer if it were divided with respect to the objectives of the thesis. Instead, main conclusions of individual articles are presented in the form of a bulleted list. The first idea of the logic of the experimental sequence is thus obtained only after reading the Discussion. I have also some minor suggestions to organization of Results:

- The Methods section contains only a description of the methods used in section 3.6. So wouldn't it be better to cancel the whole section 2 and make the text in it a part of 3.6?

- The inclusion of the section "*3.5 Publications not included in the doctoral thesis*" in the thesis seems to me exactly in the middle between the classical Epimenides' paradox and the Russel's paradox of the naïve set theory. My personal thanks!
- In Figure 10, you present only negative results - the absence of the expected nuclear envelope defects in the mutants studied. Wouldn't it be useful to include a positive control here? That is, a nucleus that does contain the defect in question? Just so the reader is sure what type of aberration was expected here…

The quality of the Discussion section is highly variable. It oscillates between two positions: while some parts of the Discussion are engagingly written and contain valuable arguments that go into the depth of the issues discussed (for example, 4.5 - TOR network), others are rather descriptive summaries of results that are not confronted with previously published data (4.6 - IFC). The latter ones thus become somewhat repetitive in relation to the otherwise well-conceived Summary.

Science

The thesis includes two original research articles published in Journal of Cell Science this year (Publication 1, Publication 3), one preprint of another original research article (Publication 4) and one preprint of a methodological paper (Publication 2). Besides these, two unpublished results are included, too (section 3.6).

The experimental data presented are well documented, correctly interpreted and provide answers to a large part of the stated objectives of the thesis. The fact that a significant part of the data represents negative results ("*ammonium does not restore decreased rate of NE expansion*", "*ammonium does not restore lipid homeostasis in cbf11Δ cells*" – Summary, p. 41) does not make them less valuable and important for understanding the molecular principles of the studied phenomena in further research. The observation that the monitored improvement of mitotic fidelity is not specific to ammonium, but that it is due to a different way of regulating the TOR network branches, is interesting in itself, as well as the postulation of the existence of Cbf11-Mga2 complex.

Conclusions

In terms of originality, the author's ability to conduct research and achieve scientific results, and the author's contribution to published studies, the thesis meets the requirements for a creative scientific work. Therefore, I think that the author should be awarded the degree of PhD.

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Questions

(answering these questions will not affect my evaluation of the thesis. It should only serve as a basis for discussion after the presentation of the candidate)

1. The decrease of total FA content detected in *cbf11*Δ cells (Publication 1, Fig. 4A) indicates in general lower amount of membranes in this mutant. How is this fact reflected in the morphology of *cbf11*Δ cells? In particular, are not the ER and/or mitochondrial network significantly reduced there?
2. Abnormalities in both mitotic and meiotic chromosome segregation were observed in *cbf11*Δ cells (PomBase). Does nitrogen supply also mitigate the meiotic phenotype?