

Review of the doctoral dissertation of MSc. Phu Nguyen Van

The doctoral dissertation of MSc. Phu Nguyen Van entitled *Antagonistic regulation by global transcription factors Tup1p, and Cyc8p of Flo11 and Flo11-dependent phenotypes in wild yeast* is based on two articles, which occurred in high-ranking international peer reviewed journals, and also contains a set of unpublished results. The dissertation is written in a traditional, extended form: the Abstract and General Introduction are followed by Literature Review, Materials and Methods, Results, Discussion and Conclusion. Copies of the two articles, in which Phu Nguyen Van is listed as the first author, are attached at the end of the thesis.

The Results section is divided into three chapters, two describing the results that have been already published, and one presenting the unpublished data. All the results presented in the thesis are rather novel, describing the role of Cyc8p and Tup1p transcription factors in a complex way, showing their role in regulation of metabolic genes, in morphological changes of the yeast colonies, and in the quality of biofilms formed on abiotic surfaces. The thesis thus brings a comprehensive analysis of the Cyc8p and Tup1p regulators, and a concise view on the current understanding of their physiological role in a broad context.

The Dissertation is well arranged and easy to read. This is, in fact, the second version of the Doctoral Dissertation submitted by the same author, and the improvement of the text in comparison with the first version is significant. While there has never been any doubt that the author worked hard and carried out impressive number of experiments, his performance during his last attempt to defend the dissertation raised questions on the extent of his intellectual participation in the projects. The PhD degree should reflect the ability to conduct independent scientific research, not merely the work, albeit hard, which can be performed by a qualified lab technician. The current version of the thesis submitted by Phu Nguyen Van indicates not only the author's writing skills, but also a deep understanding of the subject. Together with the first authorship of two articles, it provides a basis for awarding the PhD degree, provided the author addresses the questions, comments and reservations of the defense committee.

I recommend the Doctoral Dissertation of Phu Nguyen Van to be accepted.

I have following comments and questions:

1. The very first sentence of General Introduction (*Non-Candida yeast species, such as Saccharomyces cerevisiae, are increasingly being considered as opportunistic pathogens since they have recently been reported to infect immunosuppressed or critically ill patients*) is somewhat weird. The whole thesis is focused on *S. cerevisiae*, which is also a long-term model organism. While it is important to discuss the results obtained in *S. cerevisiae* in the context of pathogenic yeasts, and to acknowledge that *S. cerevisiae* may also act as opportunistic pathogen, taking non-*Candida* yeast species as the reference point to open this dissertation seems to me odd.
2. Figures together with their legends should form self-explanatory units. The legend of Fig.1.3. referring to the explanation in the text is not ideal.

3. Figure 3.2 shows expression of selected Cyc8p and Tup1p targets in the yeast colonies. It seems that in some cases, Tup1p and Cyc8p affects not only the level of expression of certain genes, but also the localization of resulting proteins within the colonies. The author should comment on this effect.
4. In the chapter 3.2.4 the author argues that Tup1p may regulate Flo11p not only on the level of transcription, but also on posttranslational level, affecting its half-life by inhibiting a protease, which degrades Flo11p. In the first chapter of the Results, the author shows new, unpublished proteomics data including a list of proteins whose expression was affected by Tup1. Based on these data, is it possible to hypothesize, which protease is responsible for Flo11p degradation?
5. Fig. 3.14. A and B shows growth curves of "44-h static cells" in two different media. The author explains the experimental approach, which led to these results. Despite that, the term *static cells* seems to me confusing in this context. The author also uses the term *static culture*, which, in my opinion, is more adequate.
6. In the chapter 3.2.5. the author mentions fibers interconnecting the cell. What is the chemical composition of these fibers?

Prague, February 3rd


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