Title: Antagonistic regulation by global transcription factors Tup1p, and Cyc8p of Flo11 and Flo11-dependent phenotypes in wild yeast

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The presented thesis of M.Sc. Phu Nguyen Van concerns the topic connected with the long-term interest of the host laboratory in processes related to the development of yeast colonies and biofilms. It is conceived as a full-version thesis presenting the results of two publications of which M.Sc. Nguyen is the first author. The papers are not attached, although it is stated so in the General introduction. The thesis contains all the necessary parts only the List of abbreviations is missing. However, the text is difficult to read in parts with results and discussion, part IV especially. There are uncomplete sentences without sense, missing words, sometimes without a noun, sometimes without a verb.

The Literature overview is the best-written part of the thesis and gives sufficient information on the studied topic. The author describes the importance of biofilms for human health and the environment, and genes affecting their formation. The structure and function of the FLO11 gene and pathways regulating its expression are also presented. A large part describes characteristics of Tup1 and Cyc8 proteins and their role in the regulation of various metabolic pathways.

My concerns about this part:
1/ A low print quality of many adopted images (i.e., Fig 1.1, 1.4, 1.5, 1.6) that are blurred at least in the pdf version I have received for the review.
2/ Figure legends are not distinguishable from the surrounding text since they are of the same font and size. The same states for figure legends in other chapters too.
3/ To describe mutants, small letters and italics is better to use, e.g., tup1, cyc8.
4/ Not consistent usage of protein names – Cyc8p x Cyc8, SWI/SNF x Swi/Snf

Questions to this part:
1/ Please clear up the two contradictory statements at the beginning of chapter 1.7.3. “... Neither Cyc8p nor Tup1p bind directly to DNA....“ and few lines below “...Tup1p independently controls transcription if it interacts with the target promoters via the DNA binding domain...“.
2/ What is the phenotype of the prion formed by the Cyc8 protein? Does the prion form affect cell morphology and growth?

Chapter Material and Methods is quite detailed, except the paragraphs describing microscopy of cells and colonies that do not contain appropriate information. There is no data on the type of microscope, objective type, magnification, and numerical aperture in the 2.2.4. section. No data on the filter wavelength of the GFP cube used, on objectives numerical aperture, camera type. Similarly, there is no information on a two-photon confocal microscope and its methodology, although its results are presented in Results and Discussion. Likewise, the details on electron microscopy and Northern blotting are missing; however, the author states that he did not perform these methods himself.

Other concerns:
1/ Centrifugation conditions are not sufficiently described – use either rpm with the centrifuge model or Rcf
2/ Dilutions of antibodies used for immunodetection of proteins and manufacturers of some antibodies are absent.
3/ Some untypical expressions are used like „wash with phenol“ – extraction would be better.

**Questions to this part:**
1/ Why do you use different induction conditions for mRNA and protein analyses?
2/ You prepared multiple deletions strains with cassettes containing one type of the Cre/lox site. Have you not met a problem with cassette replacement?

There are two chapters containing Results and their discussion divided according to published articles. The first paper is presented in Chapter III and deals with the inverse regulation of the **FLO11** gene expression and biofilm formation by Tup1 and Cy8 proteins. Wild yeast strains with various combinations of deletions and regulated expression of **TUP1** and **CYC8** genes were constructed, and their mRNA and protein content was analyzed by Northern blots and SDS-PAGE. The author also investigated the mutual roles of Tup1 and Cyc8 proteins in the regulation of flocculation and agar invasion.

Notes: Some figures are deformed (e.g., Fig 2), and some legends miss the information on cultivation conditions that are neither present in the text.
Fig 5D, 9A – SDS-PAGE images seem to be manipulated, which is unacceptable. Samples should be loaded on the same gel.
Fig 6A does not contain any loading control to be able to compare mRNA levels in various strains.

**Questions:**
1/ You state that the **CYC8** gene might be essential in the BR-F strain because you were not able to prepare a double deletion strain. Did you select transformants of BR-F strain on glucose- or glycerol-containing media? What was the result of deletions in the BRSnew strain? How can one monitor the essentiality of a gene in yeast in general?
2/ mRNA and protein analysis under non-inducing conditions did not show any **CYC8** mRNA or protein (Fig. 6A, B). Provided that the **CYC8** gene is essential as you state, then how the strain cye8Δ/pGal1-Cyc8 can grow in the absence of galactose? Interestingly, the strain with no functional Cyc8 protein grew better than that with unfunctional Tup1 (Fig. 7, not included in the publication). Can you comment on this?
3/ When you analyzed the impact of Tup1 and Cyc8 proteins on agar invasion (Fig 12), you concluded that „...cell morphology was not associated with adhesion to, and invasiveness into agar...“. However, you have analyzed cells in the „aerial“ part, as stated in the text. Have you had a look at cells at the agar surface?
4/ You have detected a negative effect of copper on transcription from the **GAL1** promoter. Might Cyc8 and Tup1 proteins also affect this promoter?
5/ Can you comment on differences in the **FLO11** mRNA level of the wild type BF-R cells with undetectable **TUP1** transcript and that in the strain with a high level of **TUP1** mRNA after induction (Fig 9A, columns 1 and 3) regarded your presentation of Tup1 as an inducer of the **FLO11** gene expression? Are there other activators of **FLO11** in the BF-R strain like Flo8, Ste12/Tec1?

Chapter IV brings the results on the regulation of biofilm formation and dispersal in wild **Saccharomyces cerevisiae** by glucose, Tup1, and Cyc8 proteins that were recently published. This part corroborates findings from the first paper and describes analyses of adhesion of various strains to plastic and the formation of biofilms in static and shaken cultures. Several figures were prepared by the author’s collaborators from the host laboratory. Although it describes an important topic, this chapter contains many sentences with misused or missing
words, making it very difficult to read, unfortunately. Also, some images seem to be deformed. In the discussion, part IV.B, there are some incorrect statements like …Cyc8p-GFP is produced at higher levels in nuclei of cells..“ – translation takes place in the cytoplasm, not in the nucleus. However, there is a good discussion of results regarding the spreading of pathogenic Candida and similar yeast infections in immunocompromised patients.

Questions
1/ How microscopy of 96-well plates with biofilms was performed?
2/ What is the reason for different morphology of the adhering cells with induced Tup1 in this chapter (Fig 19) compared to the experiment with agar invasion presented in the first part of Results, Fig. 12?
3/ Surprisingly, the author used in section 4.5 Cy8-GFP and Tup1-GFP fusions that were assigned as unfunctional in the first part of results (III.A) and followed the localization of proteins in cells. Why were the GFP fusions not used for quantification of Tup1 and Cyc8 expression in the first part of experiments when no specific antibodies could detect the proteins?
3/ What are the mechanisms by which Cyc8 inhibits Tup1? „…Cyc8p seems to exert an important effect on glucose presence, by inhibiting Tup1p functions, potentially via mechanisms as previously described (Nguyen et al., 2018)” – part IVB-discussion p. 93.

Altogether, M.Sc. PHU NGUYEN VAN proved his ability to conduct scientific research and to obtain good results. Two publications in high-impact journals have acknowledged their validity and importance. However, he did not pay enough attention to the preparation of this thesis. There are formal and language drawbacks, many unclear sentences, and vague statements. The reader can thus get a feeling that the author does not sufficiently master the scientific writing.

M.Sc. PHU NGUYEN VAN met the objectives stated at the beginning of his thesis and fulfilled the requirements of the Ph.D. program in Genetics, Molecular biology, and Virology by presenting two publications. After a successful defense, he can receive a Ph.D. degree.

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