PhD-thesis assessment

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Nitrogen source as a determinant of mitotic fidelity in fission yeasts

This dissertation aims to characterise the basis of ammonium's previously observed positive effect on *S. pombe* cells with impaired mitosis resulting from mutations in lipid metabolism and homeostasis. The thesis structure is classical and includes four papers/manuscripts. The Introduction chapter (15 pages) provides a detailed, easy-to-understand overview of all related aspects. I just miss comparing the situation of TOR networks between not only human cells and S. *pombe* (p. 23) but also the other yeast-model *S. cerevisiae* or pathogenic yeasts, e.g. *C. albicans*. This might be very relevant to the Discussion chapter (where some comparisons between *S. pombe* and *S. cerevisiae* are made, e.g. for VLCFAs, p. 34). Otherwise, I have just a few minor points related to this chapter: Figure 5 (p. 15) should state whether the picture belongs to the author's results or not and should introduce the *cbf11Δ* strain (it is introduced much later in the text), and Figure 6 (p. 18) is not referred to in the main text (?). I would also appreciate a clear definition of the aims of the work at the end of this chapter.

The chapter Methods is very short (p. 25); only two methods related to the results not included in the manuscripts are described. This is tolerable but should be explained in the text. The title of the second method (Autophagy assay, p. 25) is misleading. The autophagy was not assayed but inhibited, and mitosis was monitored.

The Results section briefly summarises the obtained results, which are included in the four manuscripts/publications, and adds two short chapters with unpublished results. All papers and unpublished results are well presented with highlighted conclusions. The main achievements of the candidate are covered in Publication 1 (manuscript but already published). The workflow is logical, the obtained results are well discussed, and the conclusions are justified. The same applies to the other two included manuscripts (both already deposited in the archives) and one publication. I have only two small, more-or-less formal comments on this section, which need some clarification. Publication 2: This manuscript presents the candidate as the first author, but the deposited manuscript in the archive has another first author. Second, could the candidate explain his contribution to Publications 3 and 4, pages 27 and 28, in more detail? Was it only the isolation of RNA, and what was the involvement in data analysis and image processing?

The Discussion (10 pages) is exemplary and well written; it unambiguously shows a broad range of knowledge of the candidate and, together with the Summary section, presents the amount of experimental work and analysis, as well as synthesis, of obtained results.

I would have several questions that might be answered during the oral presentation of the thesis:

1) Affecting sterol synthesis and homeostasis does not affect mitosis. How was this checked, and by whom?
2) Uracil was tested as a source of nitrogen. Its concentration was smaller than the other tested sources because it contains 2 N atoms. But uracil, due to its catabolic pathway that is not favourable, belongs to the poor sources of nitrogen. Why was glutamine not tested? It belongs to rich N sources and has two easily utilizable N atoms.
3) Several results show indirectly that Cbf11 and Mga2 work together or at least synergistically. Do they form a protein complex? If it is not known, would the candidate know some experimental approaches (there are at least two widely used in yeast cells) how to prove or disprove the physical interaction of the two proteins?
4) The main conclusion is that the effect of a rich nitrogen source on mitosis is not limited to mutants with affected lipid composition and metabolism but has a much broader impact. And there is a clear connection between mitosis and TOR signalling and regulation. Could the candidate speculate what might be the general basis of N-source involvement? What about the energy status of the cell? Mitosis is probably an energy-demanding process (is something known about it?), and spending less energy on metabolising nitrogen sources might be helpful for many types of mutants. Or something else?

Formally, the dissertation is very well written. I would just suggest writing the species' Latin names in italics in the list of references, similarly as it is in the main text and publications. Nevertheless, none of my comments on the formal level, above and here, are so severe that they would need any corrections in the thesis. The thesis may be accepted in its current form and layout. Altogether, in my opinion, the thesis fulfils all the requirements to be submitted and successfully defended.

Prague, 21.8.2024

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