Doctoral Thesis Review

Title: Function and localization of the SUN family proteins in yeast populations

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The presented thesis of Evgeny Kuznetsov concerns the topic connected with the long-term interest of the host laboratory in processes related to the development of yeast colonies. The thesis is conceived as a full version thesis and it is divided into classical parts, with the exception that Results are combined with Discussion. Two publications are presented in the thesis, the printed version of the first one is without the supplemental information and the second article is presented as a manuscript including supplements. List of used literature is placed at the end of the thesis. In my review, I will link my comments and questions directly to the description of each part of the thesis.

The Summary of the thesis (Autoreferát) and the Abstract is written in both languages, English and Czech, all other parts are in English. Abstract highlights the main topic and main results of the thesis. The Introduction part provides an overview of the knowledge existing on studied genes/proteins and processes related to their function are also outlined. In my opinion, there are many figures and graphs with a too detailed information for a literature overview. One would expect more general graphic documentation. Moreover, there are no references in the figure legends, so their origin is not clear and the reader does not know whether they come from a published article or are own images of the author of the thesis.

In the Materials and Methods section used strains, plasmids, equipment and methods are described. As concerns the used plasmids, it would be of interest to cite the original paper and then that it was obtained from Euroscarf. Also, an information about the purpose in which the plasmid was used (deletion, N- or C-terminal tagging) would be useful for a potential user since this type of information is not possible to find elsewhere in the thesis neither it is evident from the described genotypes of the constructed strains. I am not familiar with the *kan*MX18 version of the kanamycin-based selectable marker, what's its origin? In this section, I also missed the names of producers of antibiotics and of some equipment. Also, lists of used chemicals and abbreviations are not included. Concerning the methods, details on PCR mix composition and type of DNA polymerase would be useful for possible repetitions in future. Primers concentration used in the PCR mix seems to me to be quite high. A method used for verification of constructed strains by PCR is neither included.

The Results section is combined with Discussion. It is divided into two main parts according to enclosed publications and further divided into smaller sections and paragraphs copying the information published in the articles. Not all the results included in the articles are presented in this thesis, especially the information from supplements. The first part of results is devoted to the determination of the localization of the SUN family proteins in *Saccharomyces cerevisiae* cells and colonies. The thesis enlarged the current knowledge on the localization of these proteins for finding that three of the studied proteins Uth1, Sim1, and Sun4 can be detected outside the cells as secreted into the cultivation media or into the extracellular space within colonies although to a different extent. In contrast to the fourth protein Nca3 that was found entirely intracellular, the Uth1 protein was more intracellular than extracellular, Sun4 was found evenly in both locations and Sim1 was mostly secreted. The enhanced secretion of Sim1 was confirmed also in the analysis of yeast colonies when it was expressed only in young colonies but persisted in the extracellular space for 20 days. Do these observations

correspond to your data from microarrays on developing colonies? A better expression of the SUN family genes on non-fermentable carbon source than on glucose also suggests their potential relation to mitochondria function. Three of the SUN proteins Uth1, Sun4, and Sim1 were also found to be differentially regulated when cells were grown under hypoxia and anoxia and also during the development of yeast colonies. However, the absence of these three proteins did not affect the viability of cells within yeast colonies. Can the differentially regulated expression by oxygen levels be applied to some situations during the development and the life of a yeast colony? Of interest from the medical point of view is the finding that the absence of Uth1 leads to sensitivity to boric acid used as an antifungal drug. This sensitivity is more apparent on a non-fermentable carbon source and thus contradicts a higher resistance of this mutant to zymolyase. In the summary discussion after this part of Results, it is mentioned that three of the analyzed SUN proteins are almost not present in the cell wall fraction what is in contrast to published data from another laboratory and there are no experimental data with such an analysis presented in the thesis. Can the author comment on that? The author also hypothesizes on the reasons for secretion of the SUN proteins outside the cell and suggests that these proteins remodel cell wall from the "outside". What is the rationale behind this hypothesis when their glucosidase functions were not proved? What can be the reason for a rather long persistence of some SUN proteins in the extracellular space within colonies? What is the author's opinion about annotated intracellular functions and localization of most SUN proteins studied?

The second part of the Results and Discussion section is devoted to the study of cellular localization of the Sun4 protein and its interaction with proteins of the birth scar. Development of a new method of visualization of cell wall proteins is declared but there are no experimental data demonstrating it as it would be expected in the full-length thesis. Also, I am not sure that volumes of the buffers stated in the description of the method in the Materials and Methods section are correct. Surprisingly the author expressed doubts about the functionality of the C-terminal fusion of the Sun4 protein although all the results described in the first part that were published were obtained with C-terminal HA-epitope-tagged versions not only of Sun4 but also of other SUN proteins. Does the author have any data on the localization and functionality of C- and N- terminal fusions of other SUN proteins studied in the first part of the thesis? From the presented images, it is evident that the Sun4 protein localizes to the birth scar remaining on the daughter cell surface after its separation from the mother cell. In some mutants analyzed in this part of the thesis, the typical localization of Sun4 in the birth scar was no longer observable nor was Sun4 detected elsewhere on the cell surface. I think that another approach like live cell microscopy and/or indirect immunofluorescence would be necessary to understand and fully describe the localization of Sun4 in these mutants. In the discussion to this part, the author mentioned that Sun4 had an altered localization in the *swi5Δ* cells. Can the author comment on this altered localization? I cannot see it. The Sun4 protein stays localized at the birth scar but the birth scar becomes the old scar due to the inability of the *swi5Δ* cells to change the polarity. To visualize budding within the birth scar and in the old scars in these cells, staining with WGA would be preferable to CFW staining since the subsequent bud scars within the birth scar would be readily detectable.

Other two subchapters are devoted to the localization of daughter cell-specific proteins Dse2 and Dse4. They both were found in different parts of the birth scar when C-terminally tagged with the HA epitope. When tagged at their N-terminus, fluorescent signals of both proteins were found dispersed in the cell wall of the mother cell, mostly. The author considers both localizations correct but I do not share this conclusion. Dse2 and Dse4 are daughter-cells-specific proteins similar to glucanases and their encoding genes should be expressed from daughter cell nucleus at early G1 to ensure proper cell separation after cytokinesis. I suppose

that the constructed N-terminal fusions are expressed from the genes of interest own promoters. How would the author explain then the presence of both N-terminal fusions mostly on the surface of mother cells independently of the cell cycle phase? Did the author try to synchronize cells to monitor the expression and the correct localization of the Dse2 and Dse4 proteins? In this view, for comparison of localization of Sun4 in the birth scar with those of Dse2 and Dse4 only C-terminal fusions of both proteins should be considered. The same states for the experiment analyzing the Dse4 localization to the birth scar under the absence of septins Cdc11 and Shs1. How could that be judged when the N-terminal HA-Dse4 fusion does not localize to the birth scar even in the wild-type strain? Also to monitor a mutual localization of both Dse proteins, their HA-fusions at the same termini should be used. In the last sub-chapter, the impact of the absence of the protein Aim44 on the formation of the birth scar and the localization of Dse2 and Sun4 should be analyzed, however, there are no experimental data nor images shown, and just is it noticed that the Aim44 absence gives a result similar to the absence of Swi5. I do not consider this approach correct and sufficient for a full-length Ph.D. thesis. The final Summary discussion of this part of Results is very short and resembles more a summary than a discussion. A schematic depiction of localization of studied proteins Sun4, Dse2 and Dse4 brings an interesting idea about the differential orientation of the proteins termini within the cell wall. In my opinion, this model needs more additional experiments analyzing also an intracellular expression by indirect immunofluorescence and by live cell imaging using fusions with fluorescent proteins and especially many controls to be finally proven.

Taken as a whole I think that Evgeny Kuznetsov proved his ability to conduct a research and to obtain scientific results. However, this thesis was not carefully prepared. There are too many formal and language drawbacks, wrong genes and species nomenclature writing, many ambiguous expressions, unclear statements and mistakes in figures description. Since there is no summarizing Discussion chapter putting the obtained results into the context of current knowledge and suggesting further research directions and the short discussion paragraphs after each subchapter do not always contain appropriate references, the reader can get a feeling that the author does not sufficiently master the scientific writing. It is a pity since the results are interesting and paying attention to the discussion and to the connection with other projects and knowledge of the group on yeast colony development would highlight the importance and novelty of the results. Nevertheless, the main criticism from my part is that the full-length thesis should not only be a copy of published articles and that controls in all experiments are needed to be presented for the correct interpretation of the results. Especially controls in Western blots and in the microscopic visualization of cell wall proteins. Missing or incorrect controls typically result in false positive statements and incorrect data.

Despite all my comments, Evgeny Kuznetsov met the main objectives stated in his thesis and fulfilled the requirements for a Ph.D. thesis in the field of Molecular Biology by presenting two publications. After a successful defense, I recommend to award him with the Ph.D. degree.

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