Ph.D. Thesis Review

The thesis of Mgr. Jakub Masaryk „Trk1 potassium importers, key transport systems for yeast cell fitness and stress tolerance" aims to extend the existing knowledge of yeast potassium importers, with emphasis on Trk1 from *S. cerevisiae*:

- Detailed characterisation of the ability of Trk1 to switch its affinity and maximum velocity as a reaction to changes in the availability of external potassium.
- Elucidation of the putative mechanism of regulation of Trk1 through phosphorylation.
- Study of the role of Trk1 and Trk2 in the survival of glucose-induced cell death and high temperature.
- Characterization of potassium-uptake systems in non-conventional species *Kluyveromyces marxianus*.

(p. 37 of the thesis)

To do this, the author and his collaborators conducted a series of experiments, essential contributions of the author especially to those described in the first two parts of the Results are declared. Experimental approaches included up to date molecular biology methods, measurements of ion transport kinetics and membrane potential, fluorescence microscopy, cell viability and fitness monitoring, and many others. Original and valuable results were obtained, many of which have already been published.

Form and elaboration

The author's apparent hesitation in deciding between long and short form significantly influenced the final form of the thesis. The fact that the author did not make this decision in the end made it very difficult to read and understand the text as a compact entity, and significantly reduced the final quality of the thesis.

The problem is particularly evident in the Results section (Chapter 3), where, in addition to a short summary in each subsection, parts 3.1 and 3.3 both contain a facsimile of the published paper, part 3.2 is designed as a finalized manuscript, and part 3.4 presents the text with a decimal classification that does not appear in any other part of the Results.

The reader is especially bothered by the inability to search in copied parts of the text (incorporated as images) and the inconsistent numbering of images and references. While parts 3.1-3.3 each have their own list of references, part 3.4 does not, and while the numbering of figures in parts 3.1-3.3 always begins with the number 1, in part 3.4 it follows the end of the numbering in the general Introduction (Chapter 1). Finally, it is not clear why part 3.4, although it has its "Introduction" (3.4.1) and "Methods" (3.4.2) like all previous parts of the Results, completely lacks a "Discussion".

The overall embarrassing and enervating impression from the difficult orientation in the complex text of Results is not remedied even by the otherwise quite well-written general Discussion.

Minor comments:

1. The thesis is written in fairly good English with the usual frequency of typos. Terms such as "fluorescent microscopy" (3x), "fluorescent microscope" (1x) should be replaced by "fluorescence…", since neither the method nor the instrument itself emits light. The detected fluorescence signal comes from the biological sample, which is therefore the only "fluorescent" substance in the experiment. It is interesting how often the subtle difference in meaning between

the adjectives “fluorescent” and “fluorescence” is ignored among the Czech and Slovak scientific community, although the same pair also exists in both Slavic languages (e.g. Slovak “fluorescenčný”, “fluorescentný”).

2. Section 3.4.3: Figure 7 and Figure 8 contain identical panels. For the purposes of publishing these results, the affected panels of Figure 8 should be replaced for another biological replicate.

Science

Despite the aforementioned formal obstacles to joyful reading, the thesis contains valuable original findings that are worthy of the attention of a wider professional audience. So far, the results summarized in the thesis were published in two peer-reviewed publications. J. Masaryk is the first author of one of these. Another original first-authored paper can potentially arise from part 3.2.

Conclusions

The author has demonstrated the ability to conduct research and achieve original scientific results. The submitted thesis meets the requirements of a creative scientific work. Based on these reasons, I agree to award the author the degree of PhD.

Prague, Dec 16, 2022

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Questions

1. In Chapter 4.1 you present an extensive discussion of possible mechanistic models for the gradual changes of Trk1 affinity to potassium ions. You even admit that gradual changes in affinity of individual transporter might not be the only possible explanation for the measured data, but that binary switching of an increasing fraction of transporter molecules within a cell would lead to the same results. I like the idea, as it is simple enough to become a probable solution. However, the presented suggestion that a change in phosphorylation of the transporter molecule could be responsible for the affinity switch is not consistent with the proposed role of the membrane potential as a trigger for this switch.

   Instead, have you considered the possibility that the transporter could, for example, exist in the form of high-affinity oligomers that dissociate into low-affinity monomers upon cargo transport? This arrangement would be cargo concentration-dependent, high potassium-protective, with spontaneous oligomer restoration upon cargo scarcity characterized by a single association constant, and it would result in fine affinity tuning of the transporter population, fitting to your data. In addition, it is natural to imagine that some of the steps, such as the recovery back-flipping into the outward-open conformation after the transport event has been finished, will be directly

affected by the transmembrane voltage. Could you suggest an experiment to test the validity of the proposed model?

2. Have you considered the possibility that, being functionally interconnected with the plasma membrane ATP-ase, Trk1 could act as an important regulator of membrane potential? Haven't you detected, for example, hyperpolarized plasma membrane in Trk-defective, thermosensitive mutants?
3. To which intracellular compartment do you think the mutated Trk1 variants localized? In your manuscript (part 3.2), you speculate about cellular toxicity of misfolded insoluble protein aggregates, but at the same time, you sort of disqualify this possibility by the note that those are not toxic under high potassium. I think your note is correct. Have you considered other possible alternatives of S882A and T900A mutants localization?