

1. **Research Unit:** Laboratory of Plant Protein Homeostasis
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4. **Auxiliary supervisor:** dr Marcin Olszak
5. **Project title (English):** Characterization of the plant LSU family of small coiled coil proteins
6. **Project title (Polish):** Charakterystyka małych roślinnych białek z rodziny LSU posiadających strukturę zwinętej cewki
7. **Description of the project (up to 500 words):**

Background: Arabidopsis contains four *LSU* genes. They encode small proteins (about 100 aa) with an alpha-helical structure and the coiled coil regions [1]. These proteins have been postulated as stress-related hubs integrating abiotic and biotic stress responses, and they are involved in multiple protein interactions. The partners of LSU proteins are functionally diverse and located in different cellular compartments, suggesting that LSU proteins have complex regulatory role in various plant processes. In addition, an overlap in interacting proteins sets suggests that LSUs have partially overlapping functions, although some functional specificity exists. Exact function and importance of the LSU proteins, however, still remain unknown. Molecular modelling of LSU dimers, based on the coiled coil template structure, helped to understand and visualize difference between the isoforms [2]. Analysis of LSUs interactomes lead us to the hypothesis that these proteins somehow facilitate vesicular trafficking of their partners, what, in turn, indirectly affects plant response to stress. However, this hypothesis remain to be investigated. The *in silico* analysis of the LSU proteins structure reveals the possible (weak) bipartite nuclear localization signals, a potential transmembrane domain at the C-terminus and the LIR motif responsible for binding with ATG8 (the protein located in autophagosomal membranes). Indeed, our preliminary data suggest that some LSUs might be located in the nucleus and that they can co-localize with ATG8. Recently, human short coiled-coil proteins (SCOC) were identified as novel positive regulators of starvation induced autophagy [3]. Although LSUs demonstrate no sequence homology to SCOCs they could have a comparable role in plant cells. Especially that they interact with the selective autophagy cargo receptor, NBR1 [2].

Activity proposed: The aim of the project is to elucidate the function of LSUs in plants. The main activity will be the experimental dissection of the role of their domains and motifs by mutagenesis/deletion and verification of the effects on dimerization, interaction with various partners, localization and other properties. This part of work will be supported by structural modelling *in silico* (in collaboration). The modified LSU proteins will be produced in different systems. One of the routine techniques will be Arabidopsis plant transformation. We have a collection of *lsu* single and multiple mutants of Arabidopsis which will facilitate verification of the effects of introduced mutation on protein function *in vivo*, and on plant performance in different conditions. To monitor the spacio-temporal protein location the LSU-YFP fusion proteins will be expressed under native promoters in plants. Confocal fluorescent microscopy will be used to localize the proteins tagged with fluorescent markers, verify their interaction with partners and confirm the stability. Protein interactions will be also inspected in yeasts and *in vitro*. Besides, the factors affecting post-translational modifications (e.g. phosphorylation) will be identified. The project might be realized in collaboration with other scientists. Currently our lab has active collaboration with two groups from Germany, one group from Czech Republic and one from France. We would be happy to welcome an open-minded and dedicated person to work with us on this fascinating project.

8. **References related to conducted /planned research (maximum 3):**

[1] Sirko A, Wawrzyńska A, Rodríguez MC and Sęktas P. 2015. The family of LSU-like proteins. *Front. Plant Sci.* **5**:774.
[doi: 10.3389/fpls.2014.00774](https://doi.org/10.3389/fpls.2014.00774)

- [2] Niemiro A, Cysewski D, Brzywczy J, Wawrzynska A, Sienko M, Poznanski J, Sirko A. 2020. Similar but not identical – binding properties of LSU (RESPONSE TO LOW SULFUR) proteins from *Arabidopsis thaliana*. *Frontiers Plant Sci* 11:1246; doi: 10.3389/fpls.2020.01246
- [3] Wirth M, Mouilleron S, Zhang W, Sjøttem E, Princely Abudu Y, Jain A, Lauritz Olsvik H, Bruun JA, Razi M, Jefferies HBJ, Lee R, Joshi D, O'Reilly N, Johansen T, Tooze SA. Phosphorylation of the LIR domain of SCOC modulates ATG8 binding affinity and specificity. *J Mol Biol.* 2021 Apr 9:166987. doi: 10.1016/j.jmb.2021.166987
9. **Scholarship amount (net):** 3000 PLN for mid-term evaluation, after mid-term evaluation, change to 57% professor's remuneration (currently it would be 3242 PLN net).