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To the

PALACKÝ UNIVERSITY OLOMOUC

Faculty of Science

Report on the habilitation thesis by Tomáš Takáč

The thesis of Dr. Tomáš Takáč deals with the importance and perception of reactive oxygen species (ROS) in plants, with special respect to the role of mitogen activated protein kinases (MAPKs), vesicle trafficking and the cytoskeleton. Methodically he favours the proteomic-approach to deal with this topic.

The thesis opens with a very comprehensive review on the production, mode of action, and consequent signal transduction of ROS. It describes singlet oxygen ($^1O_2$), superoxide ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($OH^{\cdot}$) as the active forms of ROS in plants. Dr. Tomáš Takáč focuses on the signal transduction induced by these compounds. The entire text is very well written and compact in a positive way. He manages to combine and condense the important facts of this broad field from the old to the very recent literature.

After laying the foundations on ROS production, detoxification and signalling, Dr. Tomáš Takáč begins to integrate his own findings (Takáč et al, 2016a). This first chapter summarizes the work on antioxidant defence in plant development. He investigated the auxin-ROS interplay in flax. The beauty of this work is, that he has chosen a very simple and therefore a clear approach to study this subject in the development of lateral roots in flax. External Auxin (NAA) and H_2O_2 were applied, that simulated the outgrowth of lateral roots at low dosages. The effects were characterized by a histo-chemical, morphological and physiological approach. His work gave further evidence that ROS and peroxidases are major key players in the formation of lateral roots.

The next paragraph then focuses consequently on embryogenesis. Since the molecular switch to androgenesis is not well understood, Dr. Tomáš Takáč undertook a proteomic approach to characterize the changes in the maize anther proteome during cold pre-treatment and after their transfer to the androgenesis-inducing media (Uváčková et al, 2012). Technically the approach was driven by the usage of 2D-PAGE and MALDI-ToF-MS based identification of the proteins. About 100 proteins were found to show differential abundances upon treatment in the induced microspores. Beside a subunit of the ATPase and actin, the small GTPase Ran-2 showed an increased abundance, in accordance with its role in cell division.

He subsequently analysed, using a proteomic-approach, the changes within the Arabidopsis root proteome on treatment with the PI3K-inhibitor “LY294002”, interfering with the endocytotic pathway of the cells. Most interestingly, a number of enzymes involved in the detoxification of ROS were affected (Takáč et al, 2013). A critical comment regarding a detail of this work involves the quantification of the proteins. It is questionable if a 0.34-fold change of NP_001031710.1 (Table 1) is really significant. Beside this minor comment, the work proves a highly interesting connection between endocytosis and ROS metabolism.

A part of this second chapter concentrates on the enzymatic antioxidant defence by MAPKs. A double mutant on the layer of the MPKKKs of Arabidopsis, namely *anp*2*anp*3 versus the wild type was used in a 2D-nLC-ESI-MS/MS approach (Takáč et al, 2014). He could show by this state of the art proteomics analysis that a couple of ROS-related proteins, particularly SODs were upregulated in the mutant versus the wild type. These aspects were further analysed in a publication focussing on the importance of MPK4 and MPK6-2 in Arabidopsis roots (Takáč et al, 2016b). After H2O2 treatment, CAT activity increased in the mpk4 mutant, but not in the mpk6-2 mutant.

In summary, the work described in this first chapter makes a very good contribution to the understanding of the biological effects of ROS in plant development. The approaches by Dr. Tomáš Takáč combine plant developmental biology with cell biology and proteomics. This combination is well suited to the broadening of our understanding of the basic mechanisms in plant development.

The second chapter deals with the subsequent signal-transduction downstream of the MMPKs. Again a proteomic-approach was chosen to analyse the functions of MMPKs in Arabidopsis lines deficient of specific MAPKs. At the beginning Dr. Tomáš Takáč points out that ANP signalling diverges into at least two different cascades controlling oxidative stress and immune response as well as plant cytokinesis. For this reason, all results obtained by these studies require a complex interpretation. This requirement is fulfilled with great effort by Dr. Takáč.

Shotgun proteomics of the Arabidopsis *anp*2*anp*3 mutant revealed a complex adaptation of the signal-transduction elements and other regulatory proteins in this situation. Here the

experiment was carried out on a 2D-nLC-ESI-TOF-MS/MS system. The setup was complex and demands high skills on the technical- as well as the bioinformatical-side. The analysis of Arabidopsis seedlings of yda1 and DNyda1 mutants reveals a complex role of YODA a MAP3K4 in plant development (Takáč et al, 2014; Smékalová et al, 2014b). As expected the underlying protein-protein interaction network is complex. The detailed analysis carried out by Dr. Takáč opens a systems biological view of the plants signal-transduction machinery. This view was further extended by the analysis of salt-stress sensitive SIMKK-YFP plants. As before, again a demanding nLC-proteomic approach was chosen. Oxidative stress related proteins like CAT, PRX and others turned out to be less abundant in these plants.
Taking all data together Dr. Takáč suggests that SIMKK overexpression negatively regulates the key decomposers of H_2O_2 and facilitates water loss, which were crucial for the changed sensitivity of the transgenic plants to salt stress (Ovečka et al, 2014). Dr. Takáč delves deep into the complex network addressed by the MAP-Kinases and related proteins. His systems-biological view on the level of these proteins make an important recognized contribution to a better understanding of the complex signal-transduction network. Highly interesting is the finding that the phospholipase D seems to be a phosphorylation target of MAPK (Takáč et al, 2016b). Dr. Takáč has contributed significantly to a better understanding of this important part of the plants signal transduction machinery.

The signal-transduction network has to be seen in context with the entire cell and its compartments. Dr. Takáč takes this into account and sheds light on the vesicle trafficking system with a focus on the signal transduction network. Most of the vesicle trafficking processes are so fundamental, that mutations in proteins that control these processes are not vital. For this reason he applied chemical inhibitors on these processes and reapplied a proteomic approach to analyse the system.
Wortmanin, LY294002 and Brefeldin A are three very well established inhibitors interfering with vesicle trafficking. Dr. Takáč used these inhibitors on Arabidopsis roots and analysed the outcome by nLC-ESI-MS/MS. Interestingly he found the small GTPase RabA1d up regulated. RabA1d is of special interest since it is involved in the morphogenesis of growing root hairs and is localized to the cell plate in dividing cells. Using GFP-RabA1d fusions and confocal laser scanning microscopy he could localize the fusion-protein to the trans-Golgi network (TGN). Other GTPases, and PI3/4Ks are also affected by the treatment with inhibitors. This pioneering work indicates a complex interplay of the signal-transduction machinery and vesicular trafficking.

The last chapter takes the consequent step from vesicle trafficking to the compounds of the plants´ cytoskeleton. The two major proteins of the cytoskeleton are actin and tubulin. Dr.

Takáč used two KATANIN 1 mutants of Arabidopsis, which are defective in a regulatory ATPase that is involved in microtubular organisation. These mutants link the hormonal signalling to the cytoskeleton. Again a proteomic approach was chosen to study the function of microtubular organisation on cell biology (Takáč et al, 2017a). Actin was the second target studied within this context. Actin-depolymerization was induced by the drug Latrunculin B and the plants were analysed by 2D-PAGE-MALDI-ToF-MS/MS and by nLC-ESI-MS/MS. Dr. Takáč found important proteins linking microtubule severing to the actin cytoskeleton, cell plate formation and nuclear shape control. He also detected proteins connecting actin polymerization in Arabidopsis roots with ABA signalling and vesicular trafficking.

Dr. Tomáš Takáč is responsible for a significant scientific breakthrough in linking signal-transduction, vesicular trafficking and cytoskeletal organisation of plant cells. The beauty of his approach is, that he used multiple focal points to shed light on this complex topic. His chosen field is plant proteomics. But he is aware of the context specificity. This is what he did to perfection. One expects the coverage of a field to a certain extent within a habilitation thesis. Dr. Tomáš Takáč did this with bravery, stating ROS- and MAPK-signalling, covering central aspects of vesicle trafficking and integrating cytoskeletal functions. No scientist in this field can work without citing one or the other of his publications.

Therefore, I suggest that the reputed PALACKÝ UNIVERSITY OLOMOUC, Faculty of Science to accept the habilitation thesis by Dr. Tomáš Takáč.

With kind regards,

Karsten Niehaus

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