Annex No. 11 to the MU Directive on Habilitation Procedures and Professor Appointment Procedures

PUBLIC LECTURE EVALUATION

Masaryk University	
Faculty	Faculty of Science
Procedure field	Genomics and Proteomics
Applicant	Assoc. Prof. RNDr. Jan Hejátko, Ph.D.
Lecture date	Dec 3rd, 2024
Lecture topic	Mechanisms of Signal Integration and Specificity in Plants
Persons present (number)	39
Designated evaluators (board members)	Prof. RNDr. Jiří Fajkus, Ph.D., *CEITEC, Masaryk University, and Fac. Sci., Masaryk University* Prof. RNDr. David Honys, Ph.D., *Institute of Experimental Botany of the Czech Acad. Sci., member of the Academy Council, Czech Acad. Sci.* Prof. Dr. Dirk Inzé, *Ghent University and Center for Plant Systems Biology of the VIB, Ghent* Prof. RNDr. Eva Zažímalová, CSc., dr. h. c., *President of the Czech Acad. Sci., Institute of Experimental Botany, Czech Acad. Sci.* Prof. Dr. Dorothea Bartels, *Institute of Molecular Physiology and Biotechnology of Plants, University Bonn, Germany*

Lecture of Jan Hejatko in front of the professional audience entitled "Mechanisms of Signal Integration and Specificity in Plants" took place on Dec 3 at 2:30 pm in the lecture hall B11/205 at the University Campus Bohunice.

The lecture was focused on the sophisticated mechanisms through which plants integrate multiple signaling inputs to regulate growth, development, and stress responses. Central to this discussion is the Multistep Phosphorelay (MSP) signaling pathway, which was initially described to be involved in cytokinin response but has since been found to mediate a broader range of plant processes. The lecturer highlighted recent research of his lab into how ethylene, light and other signals are integrated into the MSP pathway and provided structural insights into the mechanism underpinning fidelity of the signaling interactions. The following sections summarize the key points from the talk, focusing on the mechanisms behind signal integration and the maintenance of signal specificity within the MSP.

1. Signal Integration in Multistep Phosphorelay Pathway

MSP is a backbone plant signaling pathway that allows for the integration of diverse signals. Originally discovered in relation to cytokinin signaling, MSP is now recognized for its role in regulating plant development and abiotic stress responses by coordinating multiple signaling inputs. The pathway operates through a series of protein phosphorylation events, where signal molecules trigger the activation of a phosphorelay leading to the control of signal-regulated gene expression. The integration of various signals into this system allows plants to adjust their responses according to environmental and developmental cues.

A particularly notable aspect of MSP is its ability to integrate inputs from cytokinins, light and ethylene, which were emphasized in this part.

In this section, the speaker provided a comprehensible overview of their past as well as recent (unpublished) findings on how various signal types are integrated into the MSP system to mediate plant responses.

2. Multistep Phosphorelay in Ethylene Signaling

Ethylene is a key plant hormone involved in a variety of processes, including stress responses, fruit ripening, and senescence. The lecturer detailed how ethylene signaling is integrated into the MSP pathway through an intermolecular His-Asp phosphorelay. Jan Hejatko presented the still unpublished findings, evidencing existence of a new mechanism, so far being not described in any of the biological systems exploiting the MSP signaling (bacteria, fungi and plants). The mechanism comprises intermolecular transphosphorylation of receiver domain of histidine kinase (HK) AHK5 ($AHK5_{RD}$) by the HK domain of ethylene-responsive HK ETR1 ($ETR1_{HK}$). Results of number of thoroughly performed experiments were demonstrated providing clear evidence for the $ETR1_{HK}$/$AHK5_{RD}$ intermolecular transphosphorylation and the phosphorelay down to AHP proteins both *in vitro* and *in vivo*.

3. Mechanisms Underlying Signaling Fidelity Within Multistep Phosphorelay

A critical component of the MSP pathway is its ability to integrate number of signal inputs, but at the same time to maintain signal fidelity, ensuring that the correct response is triggered by the appropriate signaling molecule. The speaker provided structural insights into the specificity of protein-protein interactions that underpin this fidelity within the MSP system.

By employing advanced structural biology techniques, the research team has identified key protein complexes that mediate the specificity of signal transmission. These findings shed light on how MSP signaling pathways are both flexible, allowing for the integration of multiple signals, and precise, ensuring that each signal elicits a distinct and appropriate response.

Conclusion of the lecture

The lecture highlighted the significant role of MSP in integrating complex signals such as ethylene, cytokinins and light, crucial for regulating plant development and stress responses. The speaker's research clearly contributes to a deeper understanding of how these signals are integrated via His-Asp phosphorelays. Furthermore, the structural insights into the protein-protein interactions that maintain signaling fidelity within MSP provide an important foundation for future studies aimed at enhancing our understanding of plant signaling mechanisms.

Through this work, Hejátko's team gained insight not only into the molecular basis of signal integration in plants but also into the ways plants maintain signal specificity, ensuring robust and coordinated responses to a variety of internal and external cues. This research has broad implications for improving crop resilience and understanding plant behavior under changing environmental conditions.

Discussion

The lecture sparked a lively discussion, in which questions arose from both the committee members and the audience (examples are listed below).

Questions by board members:

D.B.: How specific is the light signalling pathway in other plants, beyond Arabidopsis thaliana? How it contributes to adaptation to different environmental conditions and what is its variability among different plant species?

E.Z.: In most studies, BAP, the synthetic cytokinin was used – why? Do the other cytokinins, like native ones (i.e. isopentenyl adenine or *trans*-zeatin), have similar activation effect?

D.I.: Given the importance of MSP signalling, how would you apply this knowledge to improve beneficial crop traits, e.g., to increase their stress tolerance?

J.F.: In which stages of plant development can we observe major changes in cytokinin and ethylene signalling?

D.H.: Why a weaker interactor produces a stronger response? Can you speculate on a possible mechanism?

Questions by the audience (example):

Can you explain a specificity of ethylene effect on root elongation? Why we see the complementation (of ahk5-1 by a histidine kinase-deficient version of AHK5) dominantly in columella?

In his answers to all questions, Jan Hejátko showed a broad knowledge across different disciplines and excellent orientation in this field, as corresponds to his position of one of the leading experts in the field. The board members unanimously expressed deep appreciation for the multidisciplinary approach that the candidate uses in his research. Importantly, he also appropriately considers application perspectives of his research.

Concluding evaluation note:

The lecture delivered by Jan Hejátko, entitled "Mechanisms of Signal Integration and Specificity in Plants" and delivered as part of the professor appointment procedure, **demonstrated** very convincing scholarly qualifications and pedagogical capabilities expected of applicants participating in professor appointment procedure in the field of Genomics and Proteomics.

The lecture took place in a hybrid form at 2:30 p.m. The above-mentioned members of the board attended the lecture in person (J. Fajkus and D. Honys) and online (D. Inzé, D. Bartels, E. Zažímalová) and provided its evaluation. All designated evaluators are familiar with the text of the evaluation and agree with it.

Date: Dec 3rd, 2024

Jiří Fajkus

David Honys