Auxins are one of the key groups of plant hormones responsible for regulating developmental processes in plants and their adaptation to changing environmental conditions. They participate, among other, in cell elongation, tissue differentiation, organ initiation, tropic responses, and interactions with other organisms. Their action depends on cellular concentration and must be precisely controlled—temporally and spatially—to enable plants to respond properly to environmental stimuli and maintain hormonal homeostasis. One of the mechanisms regulating the level of active auxin in cells is its reversible inactivation through the binding of additional functional groups and the formation of inactive conjugates. One of the lesser-known but potentially important forms of reversibly modified auxin is meIAA, the methyl ester of indole-3-acetic acid (IAA), the main natural form of this hormone. Unlike well-known auxin conjugates, such as amino acid-bound or oxidized forms of IAA, meIAA remains relatively poorly studied. Although its presence has been confirmed in many plant species, its biological function remains unclear. It is assumed that meIAA plays the role of a "storage" of auxin in an inactive form, which in response to an internal or external signal can be hydrolyzed to active IAA. However, phenotypic observations of plants with impaired meIAA synthesis or hydrolysis, as well as results of experimental studies (including exogenous treatment of plants with meIAA), suggest that this metabolite has a more complex function. There are indications that meIAA may be a signaling molecule, acting independently of conversion to IAA, and that it may be a transport form of IAA, facilitating its local and long-distance distribution throughout the plant. The aim of the proposed project is to comprehensively investigate the biological function of meIAA in the development of the model plant species - Arabidopsis thaliana. The main research questions are whether meIAA performs only a storage function or has its own biological activity, whether it participates in the transport of auxins within the plant, and whether it undergoes hydrolysis at its target place. To this end, molecular biology, functional genetics, and microscopic imaging techniques will be applied.

The research will be conducted using Arabidopsis lines - both mutants (with impaired ability to synthesize or hydrolyze meIAA) and plants overexpressing enzymes involved in the metabolism of this compound. In addition, these lines will be crossed with transgenic plants containing fluorescent biosensors for auxin signaling, which will enable real-time monitoring of auxin activity. By using three different biosensors, it will be possible to detect the successive stages of the auxin signaling pathway - from the presence of free IAA in the cell, through the activation of hormone receptors, to the effective transcriptional response. The experiments will also include analysis of the expression of genes encoding enzymes that synthesize and hydrolyse meIAA, as well as the localization of enzymes at the tissue level at different stages of plant development. Phenotypic studies are also planned, including assessment of root and hypocotyl growth, gravitropic response, and the number and distribution of adventitious roots - with treatement of plants with exogenous meIAA and IAA. It will also be investigated how meIAA affects the auxin transporter distribution and whether it thus influences the regulation of auxin transport in the plant. In addition, to test the potential role of meIAA as a transport form of auxin, the mutant lines (mes17, iamt1) and the 35S::IAMT1 overexpression line will be grown on media with the chemical inhibitor of polar auxin transport, NPA. Finally, the interaction of meIAA with auxin signaling pathways will be investigated by analysing the effect of this conjugate on the phenotypes of plants with mutations or overexpression of known auxin receptors and components of its various signaling pathways. These experiments will help determine whether meIAA acts through the same receptors as IAA, whether it must be converted to free auxin to have biological activity, or whether it activates separate signaling pathways.

The results of the project will contribute to answering fundamental questions about hormonal regulation mechanisms in plants. Elucidating the function of meIAA will provide a better understanding of how plants manage the local availability of active auxin and how they maintain a balance between its synthesis, transport, and deactivation. This research also has potential practical applications. A better understanding of auxin metabolism and signaling pathways may enable the development of new strategies in the future to improve crop yields, especially in the context of ongoing climate changes.