Department Of
Biochemistry &
Molecular Biology

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Dissertation Defense

Emily Frankman

Ferulate cross-linking and conjugation: the role of ferulate in the grass cell wall and specialized metabolism

Grasses are one of the most economically important families of plants, and include maize, rice, wheat and sorghum. This family of crops not only accounts for about half of the human diet, they are also an attractive source of renewable bioenergy. However, the recalcitrance of the plant cell wall makes it difficult for extracting liquid biofuel and valuable bioproducts from plants. The cell wall is vital for plant strength, allows it to grow upright, and acts as protection against pests and the environment. Grasses have a unique cell wall, containing glucuronoarabinoxylan instead of xyloglucan and a large amount of hydroxycinnamic acid. Hydroxycinnamates are derived from phenylalanine in the phenylpropanoid pathway and serve many roles in the plant, including cell wall biosynthesis and specialized metabolism. One of these hydroxycinnamates is ferulate, which can modify the arabinose side-chain of xylan. These feruloylated arabinoxylan moieties are able to dimerize via radical coupling to form diferulates, which can then cross-link between xylan polymers and between xylan and lignin. Cross-linking in monocots contributes to cell wall strength, resulting in a less digestible plant. While this occurrence in grasses is known, the enzyme that makes this addition is currently unidentified. In order to better understand ferulate-mediated cross-linking in grasses, our goal has been to find the gene encoding the enzyme responsible for adding ferulate onto arabinose residues in arabinoxylan, namely the arabinoxylan ferulate acyltransferase (AraFAT). Our approaches to finding this gene were selecting candidates in a grass-specific BAHD acyltransferase clade that were highly differentially expressed in monocots compared to dicots, and then performing protein assays with arabinose-containing substrates and feruloyl-CoA. We also explored wheat seedling protein extractions as a method of determining a testable assay for AraFAT.

During our search for the AraFAT gene, we discovered that one of the genes, Bradi1g36980, in this grass-specific clade is able to add ferulate to phenylamines, creating phenylamide conjugates. These phenylamides have several functions, including defense responses to pathogens and wounding. Phenylamides also play an important role in the plant cell wall, providing crosslinks between polymers that add rigidity and strength. The Bradi1g36980 enzyme has activity with donor substrate feruloyl-CoA and the acceptors tyramine, 2-phenylethylamine, tryptamine, and serotonin. To our knowledge, this is the first time a BAHD acyl-transferase has shown to use tryptamine or serotonin as a substrate. However, its preferred substrates are unknown. Because these phenylamides are known to increase in plants exposed to jasmonate, a hormone involved in wound response pathways, we subjected Brachypodium seedlings to methyl-jasmonate and measured the relative expression level of Bradi1g36980 at various time points. There was no significant increase in Bradi1g36980 expression level, suggesting that this gene is not transcriptionally regulated by the jasmonate response pathway.

Committee Members
Dr. Curtis Wilkerson - Mentor
Dr. Dan Jones
Dr. Michael Garavito
Dr. John Ralph
Dr. Susanne Hoffmann-Benning