31. Measurement and modeling of phenylpropanoid metabolic flux in Arabidopsis

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Project Goals: We propose to develop a kinetic model for the shikimate and phenylpropanoid pathways. Kinetic models provide insights into the distribution of flux control, thus permitting more intelligent, predictive and effective design of experiments to modulate fluxes towards pathway end products. For this work, we will compare flux measurements in wild-type Arabidopsis plants to plants that are mutant or down-regulated for genes of the lignin biosynthetic pathway, and, those that have been metabolically engineered to bypass the shikimate dependent branch or direct carbon away from lignin biosynthesis to the production of 2-phenylethanol. The outcomes of our proposed kinetic modeling are to identify what remains unknown about the regulation and control of metabolic fluxes to lignin, and to allow development of strategies and predictions of what targets are the most promising candidates for alteration of metabolic flux to lignin.

Lignin is a heterogeneous phenolic polymer that constitutes about 30% of the carbon fixed by photosynthesis in terrestrial plants. Deposited with polysaccharides in the plant secondary cell wall, lignin provides strength and hydrophobicity to plant tissues, but impedes the utilization of lignocellulosic biomass for forage, paper making, and biofuel production. Lignin is derived from the phenylpropanoid pathway, the architecture of which is well understood based upon the biochemical and genetic investigations conducted to date into the enzymes and the genes encoding them. In contrast, we lack a systematic and quantitative view of the factors that determine carbon flux into and within this branched metabolic pathway in plants. Several enzymatic steps in the lignin biosynthetic pathway have been hypothesized to be critical integrators of phenylalanine and lignin biosynthetic flux. To explore the control of carbon allocation in the lignin biosynthetic pathway, we are developing a kinetic model of the pathway in Arabidopsis and performing metabolic control analysis to test the regulatory role of several key steps. We have established an experimental system for flux analysis using excised wild-type Arabidopsis stems. We have found that excised stems continue to grow and lignify in excess of 48 hours when incubated in appropriate medium, and show a distribution of PAL and 4CL activities consistent with the pattern of lignin deposition observed. In the feeding process, PAL and 4CL activities remain constant in the stem tissue. When 13C6-ring labeled phenylalanine was supplied to excised stems, corresponding isotopologues of a number of intermediates have been detected and quantified by LC/MS/MS, and incorporation of 13C6-ring labeled monolignols into lignin was demonstrated by DFRC/GC/MS. We are analyzing the isotope abundance and metabolite concentrations in the pathway in the established system, and using these data for kinetic modeling and metabolic control analysis. Our initial modeling of the first steps of pathway shows a good fit for Phe and p-coumarate. Following the generation of kinetic model in wild-type Arabidopsis, we will take advantage of monolignol biosynthetic mutants in hand to evaluate and refine the model.

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