Lignin breakthroughs serve as GPS for plant research

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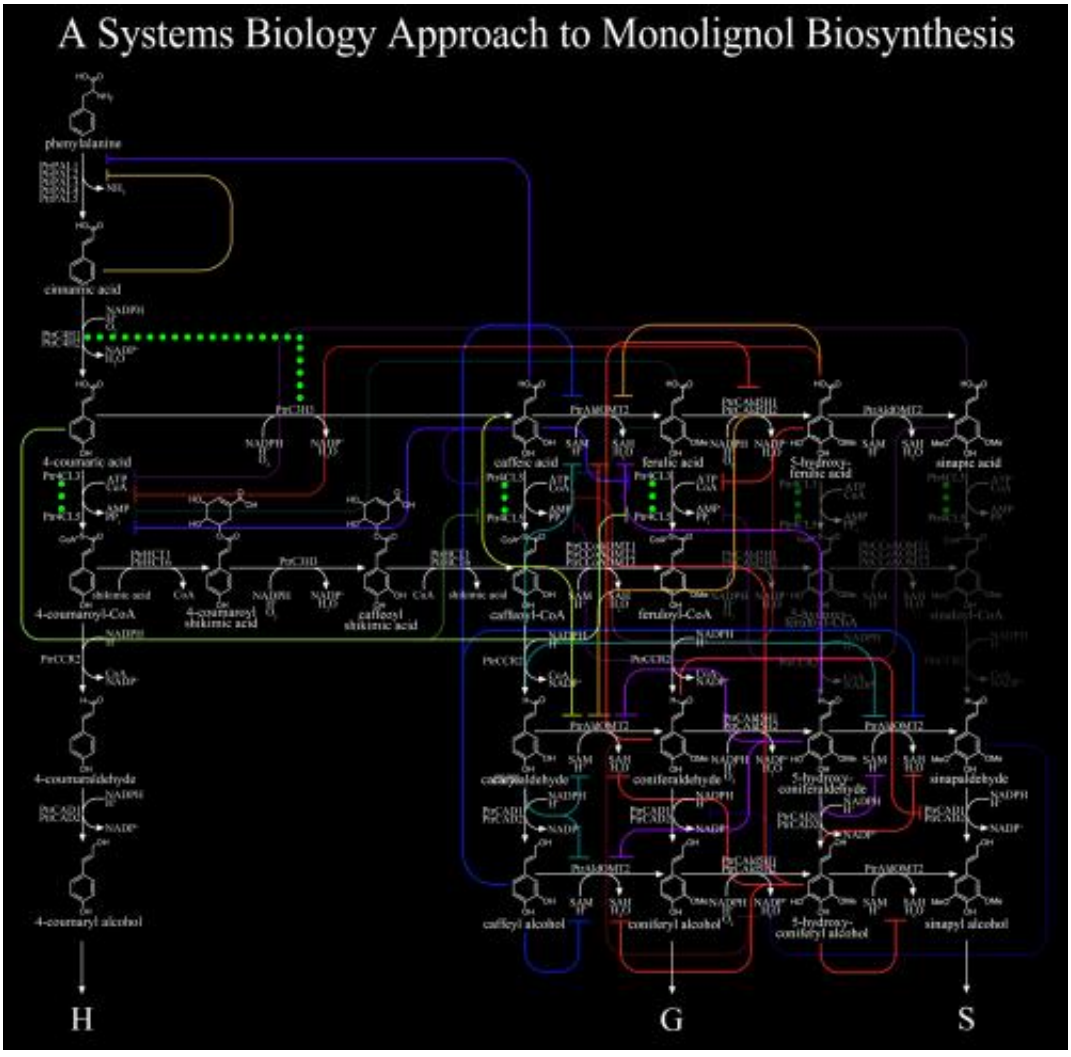

This illustration shows detailed work on lignin biosynthesis from North Carolina State University that offers step-by-step directions for future plant research. Credit: Jack P. Wang

Researchers at North Carolina State University have developed the equivalent of GPS directions for future plant scientists to understand how plants adapt to the environment and to improve plants' productivity and biofuel potential.

Two articles published March 11 in *The Plant Cell* offer a step-by-step approach for studying plant traits, drawing on comprehensive, quantitative research on lignin formation in black cottonwood. Lignin, an important and complex polymer responsible for plant growth and development, provides mechanical strength and water transport that enables some trees to grow 100 meters tall. However, lignin must be removed for biofuel, pulp and paper production–a process that involves harsh chemicals and expensive treatments.

The interdisciplinary research provides a new approach integrating knowledge of genes, proteins, plant chemical compounds and engineering modeling to understand how plants make products and structures needed for growth and development. This work in the new area of plant systems biology, integrating biology, chemistry and engineering, sets a new standard for understanding any complex biological feature in the future.

"I describe these findings as MapQuest for plant scientists," says Vincent Chiang, co-director of NC State's Forest Biotechnology Group, the lead team for the project, which involved scientists in the College of Natural Resources, College of Engineering and College of Sciences. "For example, the systems biology approach could be applied in research to develop sweeter citrus fruit, disease-resistant rice or drought-resistant trees."

Over many years of intensive research, the interdisciplinary team led by Chiang purified 21 pathway enzymes and analyzed 189 different parameters related to lignin formation. With help from engineering

colleagues Cranos Williams and Joel Ducoste, the team developed models that predict how pathway enzymes affect lignin content and composition. One of the enzymes forms a novel four-part structure, which was discovered as part of this work, including quantitation of all 21 enzymes carried out by chemist David Muddiman.

"The model, based on a comprehensive set of equations for each step in the process, can now predict changes in the amount and composition of lignin, as well as why it's often difficult to modify lignin in plants" says Ronald Sederoff, co-director of Forest Biotechnology Group.

The GPS-like findings could reduce years of research time required to make future advances, Chiang says. "We hope that this research will stimulate similar work by young scientists. Don't be discouraged by complex biological processes. Our work shows a successful approach for such studies."

More information: Paper abstracts:

"Complete Proteomic-Based Enzyme Reaction and Inhibition Kinetics Reveal How Monolignol Biosynthetic Enzyme Families Affect Metabolic Flux and Lignin in Populus trichocarpa" Published: Online on March 11, 2014 in The *Plant Cell*. Authors: Jack P. Wang, Punith P. Naik, Hsi-Chuan Chen, Rui Shi, Chien-Yuan Lin, Christopher M. Shuford, Quanzi Li, Ying-Hsuan Sun, Sermsawat Tunlaya-Anukit, Cranos M. Williams, David C. Muddiman, Joel J. Ducoste, Ronald R. Sederoff, and Vincent L. Chiang

Abstract: We established a predictive kinetic metabolic-flux model for the 21 enzymes and 24 metabolites of the monolignol biosynthetic pathway using Populus trichocarpa secondary differentiating xylem. To establish this model, a comprehensive study was performed to obtain the reaction and inhibition kinetic parameters of all 21 enzymes based on

functional recombinant proteins. A total of 104 Michaelis-Menten kinetic parameters and 85 inhibition kinetic parameters were derived from these enzymes. Through mass spectrometry, we obtained the absolute quantities of all 21 pathway enzymes in the secondary differentiating xylem. This extensive experimental data set, generated from a single tissue specialized in wood formation, was used to construct the predictive kinetic metabolic-flux model to provide a comprehensive mathematical description of the monolignol biosynthetic pathway. The model was validated using experimental data from transgenic P. trichocarpa plants. The model predicts how pathway enzymes affect lignin content and composition, explains a long-standing paradox regarding the regulation of monolignol subunit rations in lignin, and reveals novel mechanisms involved in the regulation of lignin biosynthesis. This model provides an explanation of the effects of genetic and transgenic perturbations of the monolignol biosynthetic pathway in flowering plants.

"Systems Biology of Lignin Biosynthesis in Populus trichocarpa: Heteromeric 4-Coumaric Acid:Coenzyme A Ligase Protein Complex Formation , Regulation, and Numerical Modeling" Published: Online on March 11, 2014 in The *Plant Cell*. Authors: Hsi-Chuan Chen, Jina Song, Jack P. Wang, Ying-Chung Lin, Joel Ducoste, Christopher M. Shuford, Jie Liu, Quanzi Li, Rui Shi, Angelito Nepomuceno, Fikret Isik, David C. Muddiman, Cranos Williams, Ronald R. Sederoff, and Vincent L. Chiang

Abstract: As a step toward predictive modeling of flux through the pathway of monolignol biosynthesis in stem differentiating xylem of Populus trichocarpa, we discovered that the two 4-coumaric acid:CoA ligase (4CL) isoforms, 4CL3 and 4CL5, interact in vivo and in vitro to form a heterotetrameric protein complex. This conclusion is based on laser microdissection, coimmunoprecipitation, chemical cross-linking, bimolecular fluorescence complementation, and mass spectrometry. The

tetramer is composed of three subunits of 4CL3 and one of 4CL5. 4CL5 appears to have a regulatory role. This protein-protein interaction affects the direction and rate of metabolic flux for monolignol biosynthesis in P. trichocarpa. A mathematical model was developed for the behavior of 4CL3 and 4CL5 individually and in mixtures that form the enzyme complex. The model incorporates effects of mixtures of multiple hydroxycinnamic acid substrates, competitive inhibition, uncompetitive inhibition, and self-inhibition, along with characteristic of the substrates, the enzyme isoforms, and the tetrameric complex. Kinetic analysis of different ratios of the enzyme isoforms shows both inhibition and activation components, which are explained by the mathematical model and provide insight into the regulation of metabolic flux for monolignol biosynthesis by protein complex formation.

Provided by North Carolina State University