Developing a molecular-level model of cofactor-trafficking in chloroplasts

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Project Goals: Bioenergy crops that thrive in marginal soils and maintain performance in diverse and fluctuating environments are an essential component of a sustainable energy and carbon portfolio. However, understanding and predicting productivity in these environments is challenging, in part, because of the general lack of sequence-to-function information in the plant lineage. The Quantitative Plant Science Initiative (QPSI) is a versatile and scalable capability that aims to bridge the knowledge gap between genes and their functions. A central aspect of our strategy is combining genome-wide experimentation and comparative genomics with gene-, protein-, and molecular-level experimentation. In this way, we leverage the scalability of 'omics data and bioinformatic approaches to capture system-level information for DOE-relevant crops, while generating experimentally determined sequence-specific understanding of gene and protein function. By incorporating molecular-level experimentation into our workflow, we can address the question of how a protein functions and establish mechanistic insight into how sequence variation impacts phenotype. This knowledge also serves as a touchstone for accurate genome-based computational propagation across sequenced genomes and forms the foundation for robust predictive modeling of plant productivity in diverse environments.

To ensure that sustainable, low-impact bioenergy can be developed, a fundamental understanding of how plants can acclimate to poor nutrient quality is needed. Micronutrients are of growing importance in maximizing bioenergy/bioproduction crop yield, and the trace metal nutrients present unique challenges. Bioavailability of these nutrients in the soil is dynamic and variable, and yield-impacting deficiency can suddenly appear as more intensive cropping, higher yielding crops, insufficient nutrient management, and nutrient imbalances are becoming more widespread. Additionally, because these elements are essential for the proper assimilation and metabolism of macronutrients, such as nitrogen, poor macronutrient availability is exacerbated by metal deficiency. To support the development of bioenergy crops with improved micronutrient stress resilience, our goal is to develop a genome-based, molecular-level and system-level understanding for the two most abundant trace metal nutrients in plants: zinc and iron.

In addition to the thylakoid membrane, many metal-dependent proteins localize to the chloroplast. They do not necessarily participate in photosynthetic electron transfer directly but serve a support role ensuring the proper functioning of photosynthesis. The chloroplast is also the site of multiple metabolic pathways that are dependent on a transition metal at one or more steps. To ensure metalloprotein biogenesis in the chloroplast, plants have unique challenges to overcome. Cofactors must be transported across multiple membranes, while avoiding potentially cytotoxic interactions.

While permeases involved in chloroplast metal transport are relatively well characterized, how metal ions are loaded into most metalloproteins is unknown. Since unchelated metal ions can be toxic, mechanisms that mediate trafficking from the site of transport to each metalloprotein should exist. Here, we present the discovery of (1) a chloroplast metal transferase, which we propose is a zinc-chaperone essential for proper translation and protein modification during zinc scarcity, and (2) an unprecedented trinuclear zinc-heme-zinc protein, which we propose may function as a heme chaperone in cyanobacteria, but with the addition of an oxidase domain, has evolved a function in heme degradation in plants. We also present our work toward developing a plant subsystem editor. This public platform will enable the *in silico* integration of functional annotations, comparative plant genomics and metabolic reconstruction, which we are using to build process-level models of how plants mediate cofactor trafficking and metabolism-centric adjustments to cofactor use in the chloroplast.

This research was supported by the DOE Office of Science, Office of Biological and Environmental Research (BER).