Transforming our understanding of chloroplast-associated genes through comprehensive characterization of protein localizations and protein-protein interactions

Lianyong Wang*[1](lianyong@princeton.edu), Bibin Paulose[2], **Danny J. Schnell[2]**, and **Martin C. Jonikas[1]**

[1]Princeton University, Princeton, NJ; [2]Michigan State University, East Lansing, MI

Project Goals: Our project aims to generate a map of protein localizations and protein-protein interactions for 5,906 genes associated with the chloroplast. We use two synergistic organisms, the unicellular model green alga *Chlamydomonas reinhardtii* and the dedicated biofuels oilseed crop *Camelina sativa*. Objectives 1 and 2 are to generate a searchable online resource of protein localizations and protein- protein interactions for nearly all chloroplast-associated proteins. We seek to achieve these objectives by leveraging high-throughput protein tagging, microscopy and affinity purification-mass spectrometry in *Chlamydomonas*. Objective 3 is to illustrate the value of this resource to biofuel crops by validating high-priority localizations and protein-protein interactions in *Camelina* and by building on the newly generated knowledge to advance our understanding of protein interaction networks that impact yield and stress resistance.

Our efforts are focused around the chloroplast because of the organelle's central roles in photosynthesis, metabolism and intracellular signaling, all of which are targets of ongoing biofuels crop engineering efforts. Furthermore, chloroplast-associated genes are particularly underrepresented in existing systems-level datasets because most high-throughput studies to date were performed in model systems that lack chloroplasts. As demonstrated in yeast, protein localization and protein-protein interaction data transform our understanding of the genes under study by immediately generating specific hypotheses about the mechanism of action of their protein products.

So far, we have determined the localization of 1,056 proteins. Of these proteins, 585 localized to the chloroplast. Intriguingly, 305 of the proteins that localized to the chloroplast were also observed in other subcellular compartments, suggesting proteins with possible signaling roles, dual functions, and possible alternative targeting routes. We are currently analyzing the localization data and identifying protein-protein interactions by affinity purification-mass spectrometry of tagged proteins. We anticipate that the localization and protein-protein interaction data will provide key information on the functions of thousands of uncharacterized proteins, many of which have no recognizable protein motifs. The project will also have a long-term impact as the scientific community utilizes the resource of strains, constructs and data.

This research is supported by the U. S. Department of Energy, Office of Science, through the Genomic Science Program, Office of Biological and Environmental Research, grant no. DE-FOA-0002060.