## Characterization of a Novel Zinc Chaperone in Arabidopsis

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## Project Goals: Short statement of goals. (Limit to 1000 characters)

The Quantitative Plant Science Initiative (QPSI) is a 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 molecular-level experimentation. In this way, we leverage the scalability of 'omics data and bioinformatic approaches to capture system-level information, while generating sequence-specific understanding of gene and protein function. By incorporating molecular-level experimentation in our workflow, we are addressing the question of how a protein functions and establishing mechanistic insight into how sequence variation impacts phenotype. This knowledge 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.

Transition metals occupy numerous and often essential positions within the biochemical framework of plant metabolism. As protein cofactors, these elements have expanded the breadth of protein-catalyzed reactions and enabled pivotal energy-intensive reactions. To use metal ions as catalysts, the cell must balance a fundamental dichotomy: nutrient and toxin. Metal homeostasis has evolved to tightly modulate the availability of metals within the cell, avoiding cytotoxic interactions due to excess and protein inactivity due to deficiency. Even in the presence of homeostasis processes, however, low bioavailability of these essential metal nutrients in soils can negatively impact crop health and yield. While research has largely focused on how plants assimilate metals, acclimation to metal-limited environments, such as marginal soils, requires a suite of strategies that are not necessarily involved in metal transport. The identification of these assimilation-independent mechanisms provides an opportunity to improve metal-use efficiency and optimize feedstock yield in low nutrient soils without supplementing with expensive and environmentally damaging fertilizers.

Zinc is a vital micronutrient for plants. It is a constituent of approximately 2400 Arabidopsis proteins and an essential cofactor of enzymes and many regulatory proteins [1]. However, how zinc-dependent proteins are loaded with zinc was previously unknown. By leveraging phylogenomic and data-mining analyses combined with an inter-disciplinary experimental approach, we have discovered a novel metal chaperone that delivers zinc to an essential zinc-dependent enzyme during zinc deficiency. We provide evidence that this function is universally conserved from fungi to plants. In plants, we propose that duplication has resulted in analogous zinc-trafficking pathways in the cytosol and chloroplast. In Arabidopsis, there is one zinc chaperone (ZNG1) in the cytoplasm, and two paralogous chaperones (GMC2A1 and GMC2A2) localized to chloroplast. We have obtained and characterized corresponding mutants for ZNG1, GMC2A1, GMC2A2, MAPs (the target zinc-dependent proteins) and made several

crosses among these mutants. Here we present preliminary data of the characterization of plant ZNG1. Using Yeast two-hybrid (Y2H) and bimolecular fluorescence complementation (BiFc) studies, we show that ZNG1 can interact with the cytosolic target, methionine aminopeptidase, MAP1A, as we have found in yeast. Our hypothesis is that Plant ZNG1 is an activator of cytosolic MAP1, and cytosolic MAP2 can function as a back-up enzyme when cytosolic MAP1 activity is compromised.

## References

1. Clemens S, 2019. Zn-a versatile player in plant cell biology. In: Cell Biology of Metals and Nutrients. Springer, Berlin, Heidelberg, pp.281-298.

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