A New Structural Paradigm In Heme Binding – A Novel Family Of Plant Heme Oxidases.

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https://genomicscience.energy.gov/research/sfas/bnlqpsi.shtml

Project Goals: The Quantitative Plant Science Initiative (QPSI) is a capability that aims to bridge the knowledge gap between genes and their encoded function. A central aspect of our strategy is combining genome-wide experimentation and comparative genomics with molecular-level methodologies. Our program leverages the scale 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 nutrient-limited environments.

Up to 90% of the iron found in leaves is located in the chloroplasts, where every membrane-spanning photosynthetic complex has an absolute requirement for iron cofactors, such as heme. Specialized biogenesis pathways involved in heme trafficking and insertion have been described specifically for cytochrome *b*- and *c*-type hemoproteins. These pathways exist to ensure fidelity of cofactor synthesis and limit potential oxidative stress caused by free heme. However, the existence of a generalized heme chaperone that can interact with the labile heme pool in plant cells to protect and deliver heme has yet to be identified.

Using a phylogenomic approach, we identified a large protein family consisting of uncharacterized or putative heme-binding proteins. Our analysis suggested that distinct members of this family have evolved discrete functions as heme-sensing regulators, heme oxidases, and heme chaperones. Specifically, we identified three distinct, but related, subfamilies of phototroph-specific homologs. The first subfamily includes the previously characterized AtGluTRBP that binds to GluTR and plays a pivotal role in heme biosynthesis regulation. The second subfamily, which we predict contains novel heme oxidases, is composed of uncharacterized plant and algal proteins, and the third subfamily, which we predict contains novel heme chaperones, is composed of uncharacterized cyanobacterial proteins.

To test these computationally derived hypotheses, we purified protein homologs from the green alga, *Chlamydomonas reinhardtii*, the bioenergy feedstock *Populus trichocarpa*, and the cyanobacterium, *Synechocystis* sp. PCC 6803. We demonstrated that the algal and land plant proteins can bind and degrade heme *in vitro*, suggesting that these proteins, which are localized in the chloroplast, present a new family of plant heme oxidases. In contrast, the cyanobacterial homolog can bind but not degrade heme in the presence of an exogenous electron donor, suggesting that this subfamily may function as either heme storage or heme chaperones.

Determination of crystal structures of the cyanobacterial homolog in the presence and absence of heme revealed unprecedented features. In the presence of heme, the protein forms a dimer where the heme is saddled by two zinc ions. By analogy with the protective axial histidines found in a bacterial heme transporter, we propose that the zinc saddle found here protects heme from oxidation. By homology modelling, this structure also helps to understand the structure-function relationship of plant homologs where this protective function is not conserved. The discovery of this family of novel heme oxidases and putative heme chaperones provides new molecular and genomic insights into the evolution of heme regulation in photosynthetic organisms.

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