

## **Functional analysis of copper and silver storage sites and their role in metal homeostasis in *Chlamydomonas***

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**Project Goals: Processes regulating metal bioavailability and homeostasis impact cellular functions in all forms of life and their dysfunction contributes to reduced primary productivity as well as disease in livestock and humans. This project seeks to understand the mechanisms of Cu homeostasis and how its toxic analog Ag influences these processes in the model alga *Chlamydomonas reinhardtii*. We will investigate factors that control Cu storage and bioavailability. In so doing, this project will contribute broadly to the understanding of metal metabolism in the context of photosynthesis as well as the environmental impact of heavy metals.**

Copper (Cu<sup>+</sup>) is an essential cofactor required for protein function in various cellular pathways, ranging from DNA synthesis to respiration. However, Cu<sup>+</sup> uptake and distribution is highly regulated because (1) the reactivity of Cu<sup>+</sup>, which makes it useful in biology, can also lead to cytotoxicity, (2) the interplay between pathways for metabolizing Cu<sup>+</sup> and other essential metals has the potential for broad disruption of metabolism if even one metal is present at too low or high a level and (3) pathways required for the uptake of Cu<sup>+</sup> can also lead to cellular accumulation of toxic metals in the environment. The advent of analytical techniques has revealed considerable detail concerning Cu metabolism, but mechanistic and regulatory details of intracellular distribution and storage of Cu<sup>+</sup> are under-investigated. The Merchant laboratory has developed the green alga *Chlamydomonas reinhardtii* as a powerful eukaryotic reference organism for fundamental discovery in the field of trace metal homeostasis, including mechanisms of elemental sparing (in face of deficiency or other stress), pathways of metal assimilation and distribution (especially for Cu and Fe), and most recently, the visualization of intracellular compartmentalization sites for Cu, Fe and Mn. These storage sites are dynamic: they sequester excess metals to prevent cytotoxicity but the essential elements are bio-available upon transfer of the organism to a situation of deficiency. During Zn or Cu limitation, *Chlamydomonas* accumulates Ag, a process that is influenced by expression of a Cu transporter, CTR2. The goal of this project is to understand how nutritionally essential Cu<sup>+</sup> and its toxic analog Ag<sup>+</sup> are sequestered and stabilized and how the metals are trafficked into and out of intracellular compartments.

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