Trace Metal Storage in Metal-Specific, Lysosome-Related Vacuolar Compartments

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Project goals: Transition metals are of crucial importance for primary productivity; their scarcity limits crop growth and carbon sequestration on a global scale. The most important elements are copper (Cu), iron (Fe) and manganese (Mn), which all serve as cofactors to enable redox biochemistry, especially for oxygenic photosynthesis, the process that transformed earth's primitive anoxic environment and is largely responsible for primary production today. The single-celled, eukaryotic green alga *Chlamydomonas reinhardtii* is an excellent model system to study trace metal biology in phototrophic organisms, with all the advantages of a microbial system, well-characterized photosynthetic and trace metal metabolic machinery. The goal of this project is to identify, differentiate and characterize the different trace metal storage compartments in the Chlamydomonas cell, and uncover the dynamics of trace metal storage and mobilization in situations of excess and limitation in single cells.

Abstract: *Chlamydomonas reinhardtii* is a unicellular green alga that has been widely used as a plant reference system for six decades, it has a quick generation time (~ 6h), can be synchronized and grown to high densities and its three genomes are sequenced and well-annotated [1]. We have utilized Chlamydomonas as a reference organism for decades to understand the principles underlying trace metal utilization and economy in a photosynthetic cell, and have identified a repertoire of assimilatory and distributive transporters, discovered mechanisms for reducing the metal quota and recycling metal cofactors from non-essential to essential proteins in situations of sustained elemental deficiency [2].

Chlamydomonas requires a broad spectrum of metal cofactors to sustain its photosynthetic, respiratory and metabolic capabilities, and iron (Fe), copper (Cu) and manganese (Mn) are the major transition metals involved in these processes. The metal catalysts in the photosynthetic electron transfer chain span a potential of about 2V, with a strong Mn-containing oxidant in photosystem II, enabling the oxidation of water and a strong, Fe-containing reductant in PS I enabling the reduction of $NADP^+$ for the synthesis of reduced carbon-containing compounds from CO_2. While indispensable and often growth-limiting when absent, redox active metals are

toxic for cells, and are therefore tightly bound or sequestered upon uptake. They are either handled by a set of intracellular ligands (protein chaperones, metallothioneins, phytochelatins or glutathione, for example) or sequestered into specific compartments, like vacuoles.

We identified and characterized the acidocalcisome as a major storage site for Cu (in Zn deficiency and Cd toxicity), Fe (in Zn deficiency and in Fe excess conditions) and Mn (in Mn excess conditions). The acidocalcisome is an acidic vacuole in the cytosol, defined by the presence of pyrophosphate and polyphosphate complexed with calcium [3,4]. It can be identified as an electron-dense granule by transmission electron microscopy, or more precisely with multimodal X-ray fluorescence microscopy (XFM) analysis according to its characteristic high calcium and phosphorus content. XFM allows to absolutely quantify cellular trace metal contents, since no sectioning is required for one Chlamydomonas cells and metabolic states can be conserved rapidly using either vitrification or chemical fixation. We used XFM on the bionanoprobe (beamline 9-ID-C) at the Advanced Photon Source at the Argonne National Laboratory to determine the spatial distribution of trace metals within algae cells, and quantified the contents of the acidocalcisomes in situations of various trace metal hyper-accumulation (Fe, Cu, Mn). We utilized a set of different mutants, including the vacuolar transporter chaperone (*vtc1*) mutant strains, that are defective in polyphosphate synthesis and where acidocalcisomes are highly diminished [5], and the copper transporter (*ctr2*) mutant strain to distinguish two distinct vacuolar sub-types, depending or their elemental composition. Additionally, quantification of the trace metal content of individual cells via XFM and comparisons to data for cell cultures acquired with inductively-coupled plasma mass spectrometry (ICP-MS/MS) allowed us to distinguish the nutritional state for Cu and Fe in single cells.

References:

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