165. Intracellular Iron Distribution in Chlamydomonas in Response to Fe Nutrition Status

Marcus Miethke1* (miethke@chem.ucla.edu), Jefferson Chan,2 Peter K. Weber,3 Christopher J. Chang,2 Joseph A. Loo,1,4 Jennifer Pett-Ridge,3 and Sabeeha S. Merchant1,4

1Department of Chemistry and Biochemistry, University of California, Los Angeles, CA; 2Department of Chemistry and Howard Hughes Medical Institute, University of California, Berkeley, CA; 3Lawrence Livermore National Laboratory (LLNL), Livermore, CA; 4Institute of Genomics and Proteomics, University of California, Los Angeles, CA

http://www.chem.ucla.edu/dept/Faculty/merchant/#research

Project Goals: The LLNL Biofuels SFA seeks to support robust and sustainable microalgae fuel production through a systems biology understanding of algal-bacterial interactions. We hypothesize that by understanding the factors that control cellular physiology and biogeochemical fluxes in and out of algal cells, particularly through the phycosphere, we can advance the efficiency and reliability of algal biofuel production. Our research includes studies of probiotic traits of phycosphere-associated bacteria, systems biology studies of model algae, and genome-enabled metabolic modeling to predict the interspecies exchanges that promote algal growth, lipid production and healthy co-cultures. Our overall goal is to develop the comprehensive understanding of complex microbial communities needed to advance the use of biological properties for practical energy production.

Abstract: The green alga Chlamydomonas requires metal cofactors to sustain its photosynthetic and respiratory capacities, and of these Fe is the most dominant. The demand for Fe is reflected by the intracellular quota (~2 x 10^8 atoms/cell), which in Chlamydomonas is strongly dependent on the available external concentration. Fe can accumulate beyond the quota when excess Fe is supplied in the external growth milieu. To better understand acclimations resulting in Fe homeostasis in a phototrophic organism while it is facing changes in iron bioavailability, we have used a suite of complementary methods including (LC)-ICP-MS, confocal microscopy (Fe(II)-specific fluorescent sensors), NanoSIMS isotopic imaging and quantitative proteomics. Our results identify the pathways and compartments involved in Fe metabolism in the algal reference organism, Chlamydomonas. We grew Chlamydomonas in media where Fe concentrations ranged from 0.1 µM Fe (severe limitation) to 200 µM Fe (excess), and found the intracellular Fe content increases more than 20-fold. Strikingly, Chlamydomonas expresses one of its major iron storage proteins (ferritin 1, Fer1) under low Fe conditions, while its expression under high Fe is rather low. We separated the soluble Fer1 fraction from cultures grown under different Fe concentrations by size exclusion chromatography and quantified both protein and Fe contents by LC-MSE and ICP-MS analysis, respectively (Fig. 1). Between low and high Fe conditions, the total Fer1 pool decreases ~12-fold, however, the average iron content per Fer1 oligomer increases ~150-fold. Further, the total cellular Fe content during growth under high iron is ~5-fold higher than the Fer1-associated Fe pool.

To understand how accumulated Fe is stored at the individual cell level, we stained cells with a Fe(II)-specific fluorescent dye, and in the high iron treatment, found it appears to be sequestered in distinct subcellular compartments. Complementary NanoSIMS studies revealed a partial co-localization of Fe, Ca and P in these sites, suggesting that excess intracellular Fe is stored in lysosome-associated organelles known as acidocalcisomes1. Chlamydomonas mutant strains that are not capable of acidocalcisome formation did not show bulk scale Fe accumulation under high Fe conditions, nor did they form obvious sites of Fe sequestration at the subcellular scale. Using 57Fe stable isotope tracing followed by LC-ICP-MS and NanoSIMS isotopic imaging, we studied how the kinetics of Fe enrichment shifted from low to high Fe conditions. When cells were shifted from a 56Fe to a 57Fe enriched media, we found the isotope
label accumulated in the ferritin compartment during the first 12 hours of incubation. While the ferritin-associated Fe pool subsequently decreased; the $^{57}$Fe enrichment of lysosome compartments (acidocalcisomes) continued to increase up until 24 h (Fig. 2). These studies are allowing us to dissect the intracellular Fe distribution pathways both kinetically and spatially during the initial stages of cell growth under Fe excess; in our upcoming work we plan a step-by-step characterization of the molecular components that constitute these pathways.

**Figure 1**

**Figure 2**

**Publications**


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