

**Iron storage in ferritin versus a lysosome-related compartment in the green alga
*Chlamydomonas reinhardtii***

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Project goals: Iron is an element of crucial importance, iron-deficiency limits primary productivity on a global scale, in croplands as well as in the oceans. The photosynthetic apparatus is thereby a key link, because of its central role in light-driven carbon assimilation, its abundance and iron-dependence. The single-celled, eukaryotic green alga *Chlamydomonas reinhardtii* is an excellent model to study iron deficiency, with all the advantages of a microbial system and the components of the iron assimilation machinery identified. In eukaryotes, the predominant site for iron storage is either ferritin or a vacuole. While ferritin is more and more viewed as a dynamic reservoir or iron buffer, the vacuole recently gained more attention as an iron storage site. The goal of this project is to provide a cellular view of iron metabolism, by understanding the pathways and dynamics of iron distribution between different iron storage compartments, especially during metabolic transitions.

Abstract: *Chlamydomonas* requires a broad spectrum of metal cofactors to sustain its photosynthetic and respiratory capacities, and iron (Fe) is one of the major transition metals involved in these processes [1]. The demand for Fe is reflected by its intracellular quota, which in *Chlamydomonas* strongly depends on the lifestyle (phototrophic, heterotrophic or photoheterotrophic) and the available external Fe concentrations. Four stages of iron nutrition can be distinguished in *Chlamydomonas*: iron-limited (below 0.5 μM in the growth media), iron-deficient (between 1-3 μM), iron-replete (5-30 μM) and iron excess (above 50 μM). While iron-limited cells are chlorotic and growth-inhibited, iron-deficient cells have no obvious phenotype, but already activated the full transcriptional response towards iron deficiency. Iron-replete and iron-excess cells have not activated this response, but can be distinguished by their intracellular iron content. Especially interesting regarding iron storing, is that the more abundant of the two ferritin proteins that are encoded in the *Chlamydomonas* genome, the chloroplast-targeted Fer1, shows an unusual accumulation pattern upon different external iron concentrations [2]. Fer1 preferentially accumulates in iron deficient and iron-limited cells, while it is less abundant in iron-replete and -excess conditions, when iron is abundantly present, suggesting a different role than Fe storage.

To better understand Fe homeostasis in this reference organism during acclimation to changing Fe bioavailability, we used a suite of complementary techniques including (HPLC)-ICP-MS, confocal microscopy with Fe(II)-specific fluorescent sensors, NanoSIMS isotopic imaging and XAS to visualize individual compartments of iron storage, while providing different concentrations of extracellular Fe, ranging from 0.1 μM Fe (iron-limited) to 200 μM Fe (iron-excess). We found that intracellular iron concentrations observed in these conditions vary from 3×10^7 atoms/cell at the lowest concentrations up to 6×10^8 atoms/cell in excess iron conditions. We isolated the soluble Fer1 fraction grown under different Fe concentrations by size exclusion chromatography and quantified both protein and Fe contents. Fe content / ferritin varies dramatically between the different conditions, and is especially increased in iron-excess conditions, where ferritin abundance is reduced but cellular Fe content is at its peak. In iron-limited and iron-deficient cells the major iron storage compartment is indeed ferritin, where most of the intracellular iron is found. To understand how accumulated Fe in excess conditions 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, suggesting that at higher external iron concentrations an additional iron storage compartment was identified, linked to a lysosome-related structure known as acidocalcisome [3]. Mutants lacking the acidocalcisome compartment were not capable of accumulating additional Fe in iron-excess conditions and didn't display the additional Fe sequestration sites. Using stable-isotope labelling (^{57}Fe), we were able to follow the kinetics of Fe enrichment in the different storage compartments during a shift from iron-limited conditions (^{56}Fe) to iron-excess conditions (^{57}Fe). During the initial hours of the shift, the ^{57}Fe label was strongly enriched in the Fer1 pool before it accumulated in the lysosome-related compartment and subsequently was depleted from the Fer1 pool. Thus, these studies allowed us to dissect the intracellular Fe distribution pathways both kinetically and spatially in different Fe regimes.

References

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