

Orchestrated Response of *Dunaliella* for Coping with Iron Limitation

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Project goals: The specific goal of this project is to provide a broader view of iron assimilation, sub-cellular compartmentation and metabolism, by discovering pathways and mechanisms of intracellular iron trafficking and storage for iron re-use and re-cycling in the green microalgae *Dunaliella bardawil*.

Algae are responsible for half of global primary productivity and have an important role in biogeochemical cycling. Algae can thrive in nutrient-rich and nutrient-depleted environments, indicating their wide plasticity. Iron, which is one of the key elements for life on Earth, limits all forms of life due to its limited bioavailability. Photosynthetic organisms which have greater iron demand due to its use in photosynthesis in addition to respiration, can be in a chronic state of iron deficiency; Consequentially, have adapted iron economy mechanisms involving sparing and recycling. The goal of this project is to provide a cellular view of iron metabolism in *Dunaliella bardawil* by discovering and understanding the pathways and mechanisms for visualizing the dynamics of iron distribution during metabolic transitions, from iron luxury to iron economy conditions.

The genus *Dunaliella* represents globally abundant, broadly distributed unicellular green microalgae that are well known for their high tolerance to extreme abiotic stress conditions of salinity, light, temperature and pH, but are otherwise not well studied at the molecular level. One species, *D. bardawil*, is famous for its ability to accumulate β -carotene. Most importantly, a draft genome sequence of the closely related *D. salina*, has been published recently, enabling genome-level studies. Biochemical and physiological experiments indicate that *D. bardawil* can reduce its iron quota with only moderate chlorosis. Combination of transcriptome and proteome experiments on iron limited vs. replete cells, identified sentinel genes and proteins for monitoring iron status, with the most dramatic increases in the transcriptome being recapitulated in the proteome, such as transferrin and FOX1 in iron assimilation and Tidi and flavodoxin in the photosynthetic apparatus. The iron assimilation components include activities and proteins related to those known in *Chlamydomonas*, like FOX1, proteins known in diatoms, like p130b/ ISIP1 and CREG1, and novel proteins like transferrin, supporting the model that algal Fe assimilation pathways are a heterogenous mix and match of individual Fe uptake modules. Choosing *D. bardawil* as an organism of interest with its importance in industrial applications and its evolutionary relationship to *C. reinhardtii* allows us to both build on the knowledge that has been acquired already for *C. reinhardtii* and also to discover diverse strategies for acclimation to iron limitation. A systematic understanding of diverse strategies for optimizing iron utilization is a pre-requisite for exploiting iron-poor environments for food and fuel production.

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