The Algal Ferredoxin Interactome

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Project Goals: Our goal is to unravel the specific roles of the different ferredoxins in the green alga, *Chlamydomonas reinhardtii* and their contribution, either singly or in combination, towards overcoming different physiological and environmental stresses.

Abstract: Ferredoxins (FDX) are small, iron-sulfur cluster containing proteins with strong negative redox potentials (-350 to -450 mV) that transfer electrons to reductive steps in various metabolic pathways. There are 13 FDX isoforms predicted in the *Chlamydomonas* genome that are differentially expressed in response to varying environmental conditions such as availability of copper, oxygen, iron and ammonium. Previous research in our lab has established a global FDX interaction network along with demonstrating that they may have redundant functions (Peden et al. 2013, Boehm et al. 2015). That work revealed that (1) FDX2 may have an overlapping role with FDX1 in donating electrons for H_2 production and $NADP^+$ reduction and, possibly, in mediating state transitions and/or cyclic electron transport; (2) FDX3, together with FDX1 and FDX2 is involved in nitrogen assimilation; (3) FDX4 interacts with glycolytic enzymes and enzymes involved in protection against ROS; and (4) FDX5 may be required for hydrogenase maturation, together with FDX2 and FDX4, and has been shown to be involved in fatty acid synthesis in the dark (Yang et al. 2015). It has also been shown in the literature that overexpression of specific FDXs (FDX1 and FDX5) results in an increase in starch and oil content as well as increased tolerance to heat-, salt-, and oxidative- stresses in this alga.

Towards understanding the specific role of the FDXs, we have analyzed the response of the *FDX2* gene knock-out (Δ*fdx2*) towards H_2 production, starch content, oxidative stress, and triacylglycerol (TAG) accumulation, under sulfur deprived (-S) conditions. Based on our observation, there are no structural phenotypic differences between the wild-type and the Δ*fdx2* strain. Both the wild-type and the Δ*fdx2* strain produce ~ 18 mmol H_2 over a period of 72 h under -S conditions. They have similar starch accumulation profiles, which increases up to 24 h after sulfur deprivation, followed by a gradual reduction in starch levels, which is accompanied by a consistent increase in H_2 production. Although starch accumulation was found to be similar in both the strains, chlorophyll synthesis was found to be slightly higher in the wild-type strain (27 µg/ml) in comparison to the Δ*fdx2* (22 µg/ml) during this time period. Metabolite analysis revealed that both strains accumulated almost similar levels of ethanol and formate, measured at 72 h post sulfur-deprivation. The levels of acetate decreased in the culture at 24 h post sulfur-deprivation and then remained the same till 72 h. This was consistent with consumption of

acetate during the oxic phase of the culture. On the other hand, our plate-based assay to determine tolerance to oxidative stress revealed that Δ*fdx2* strain was more sensitive to H_2O_2 under –S conditions. This effect was more pronounced under high light intensities, as expected. Based on microscopy, there was no apparent change in the neutral lipid content of Δ*fdx2* strain. Overall, deletion of *FDX2* gene seems to affect the strain's ability to combat reactive oxygen (ROS) species in this organism, especially under high light conditions when ROS levels are high. On the other hand, preliminary analysis reveals that Δ*fdx2* shows lower H_2 photoproduction activity (~ 30%) during a 24 h dark, anaerobic induction period, as well as slightly lower (~ 10%) dark fermentative H_2 production.

References and publications generated by this project:

1. **Boehm M**, Alahuhta M, Mulder DW, **Peden EA**, Long H, Brunecky R, Lunin VV, King PW, **Ghirardi ML**, **Dubini A**. (2015). Crystal structure and biochemical characterization of Chlamydomonas FDX2 reveal two residues that, when mutated, partially confer FDX2 the redox potential and catalytic properties of FDX1. Photosynth. Res. DOI 10.1007/s11120-015-0198-6.

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