

The Algal Ferredoxin Interactome

Venkataramanan Subramanian^{1*} (venkat.subramanian@nrel.gov), Matt Wecker², Nitya Subrahmanian⁴, Wei Xiong¹, Alexandra Dubini³, David Gonzalez-Ballester³, Patrice Hamel⁴, **Maria Ghirardi¹**

¹National Renewable Energy Laboratory, Golden, CO; ²GeneBiologics LLC, ³Universidad de Córdoba, Córdoba, Spain; ⁴The Ohio State University, Columbus; OH.

Project Goals: To unravel the specific roles of the different ferredoxin homologs in the green alga, *Chlamydomonas reinhardtii* and their contribution, either singly or in combination, in mediating electron transfer within specific metabolic pathways and under different stress conditions.

Abstract: Ferredoxins (FDX) are small, iron-sulfur cluster-containing proteins with strong negative redox potentials (-320 to -450 mV) that mediate redox transfer in various metabolic pathways. There are 13 FDX isoforms predicted in the *Chlamydomonas* genome that are differentially expressed in response to varying environmental conditions. Previous research in our lab has established a global FDX interaction network that suggests that they may have both specific and redundant functions (Peden et al. 2013).

This year, we focused our efforts on finishing our characterization of a *fdx5* mutant provided by the Grossman's group (Yang et al., 2016), and generation of a new *fdx2* strain, due to the loss of phenotype of the previously generated *fdx2* mutant. The *fdx5* strain was characterized under H₂ producing sulfur deprived conditions. When compared to its WT, CC-124, we observed (a) decreased O₂ evolution capacity under saturating light (in contrast with Yang et al., 2016) but similar respiratory capacity; (b) similar growth pattern and chlorophyll concentration under S-deprivation but a 24-h delay in starch accumulation and degradation, start of anaerobiosis, and H₂ and fermentative metabolites production; (c) lower initial level of C16:0 and C18:0 saturated fatty acids in *fdx5*, followed by decrease in C16:4 and C18:3n3 after 120 h S-deprivation in both strains (in contrast with the response of *fdx5* under dark incubation, Yang et al., 2016), resulting in similar ratio of saturated/unsaturated fatty acids for both strains at the end of the process; and (d) similar neutral lipid content, as measured by Bodipy staining, but better cell integrity in *fdx5* after 120 h S-deprivation. These results highlight the different effects of dark incubation vs. S-induced anaerobiosis on the *fdx5* strain and potentially support the observed interactions between FDX5 and the cell wall glycoprotein GP2; the Squamosa promoter binding protein, a central regulator of gene transcription; the programmed cell death protein; starch branching enzyme SB3; and the HYDEF hydrogenase maturation proteins. Clearly these interactions must be further verified by additional in vitro assays whenever possible.

An additional property of the mutant, based on potential interaction of FDX5 with peroxiredoxin 1 (PRX1), as suggested by previous yeast two-hybrid (Peden et al., 2015) and pull-down assays, was similarly examined under S-deprivation. We showed that *fdx5* was significantly more resistant to H₂O₂ following prolonged exposure to it but only at and after 72 h

of S-deprivation, suggesting changes in the cell wall protein composition under S-deprivation that are favorable to *fdx5*, which is also consistent with the well-known major membrane restructuring following S-deprivation.

To identify a potential compensatory response to explain some of the above results by increase in the levels of other FDXs, we compared their transcript and protein levels at different points after S-deprivation. Our results show a major increase in FDX1 and FDX2 protein levels at $t=0$ in *fdx5* but no corresponding difference in their transcript levels. During S-deprivation, the transcript and protein levels of FDX1 are higher in *fdx5* while those of the other FDXs do not undergo significant changes with respect to the WT strain; the levels of the FDX2 protein decrease to undetectable levels soon after S-deprivation is initiated. Interestingly, the transcript levels one of the desaturases reported by Yang et al., 2016 as being dependent on FDX5 activity decreases substantially in *fdx5* during the later times of the S-deprivation process. We propose that the observed changes in FDX1 and FDX2 levels may represent a compensatory response of the mutant strain to the loss of FDX5 activity, and down-regulation of the desaturase FAD4 may be related to decreased need for its activity in *fdx5* under S-deprivation.

All the above observations are being further verified by transcriptomic analyses done with WT and *fdx5* under S-deprivation, and flux analysis under low light intensity. Moreover, since the *FDX5* mutation is present in a *nit-* background, we have generated a *fdx5* mutant in a *NIT+* strain. This process has led to the discovery of a possible genetic suppressor of the *FDX5* mutation that can compensate for the dark no-growth phenotype of the *fdx5* mutant. Genomic identification of this suppressor is in progress.

Finally, initial characterization studies of a new FDX2 down-regulated strain in a *nit-* background, is being carried out; initial results show that *fdx2*'s sensitivity to H₂O₂ is higher than WT under high light intensity but decreases to WT levels in the presence of potassium nitrate. These preliminary results strongly link FDX2 directly or indirectly to ROS detoxification or protection via the presence of nitrate. Further studies addressing this phenotype will be presented

References and publications generated by this project and cited above:

1. **Peden EA, Boehm M**, Mulder DW, Davis R, Old WM, King PW, **Ghirardi ML, Dubini A**. (2013). J. Biol. Chem. 288(49): 35192-209.
2. Yang W, Wittkopp TM, Li X, Warakanont J, **Dubini A**, Catalanotti C, Kim RG, et al. (2015). Proc Natl Acad Sci U S A. 112(48):14978-83.
3. **Subramanian, V, Dubini, A and Ghirardi, ML** (submitted review) The Role of Chlamydomonas Ferredoxins in Hydrogen Production and Other Related Metabolic Functions. In "Microbial Hydrogen Production: Achievements and Perspectives" (Eds. Seibert M. and Torzillo, G.) Springer; *submitted*.

Two additional manuscripts are in preparation, addressing (i) the physiological characterization of *fdx5* under S-deprivation, and (ii) the preliminary flux analysis of *fdx5* strain under very low light intensity.

This research is being supported by DOE's Office of Science, Biological and Environmental Research Office under a Science-Focused Area (SFA) project to NREL.