The Algal Ferredoxin Interactome

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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). Among other results, that work revealed that (1) FDX2 may have an overlapping role with FDX1 in donating electrons for H_2 production and $NADP^+$ reduction, which we confirmed in vitro (Boehm et al., 2015); (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 (Yang et al. 2015).

This year, we focused our efforts on the roles of FDX1, 2 and 5. The *FDX5* knock-out mutant strain (*fdx5*) was characterized under different growth conditions. When compared to its wild-type (WT), CC-124, we observed decreased H_2 production following incubation in the dark, low light and low light but with an open reactor (the two light conditions require both photosynthesis and fermentation to occur concomitantly). The *fdx5* strain was also more sensitive to H_2O_2 than the WT as detected by inhibition of growth on H_2O_2-containing plates exposed to HL. Finally, in addition to its lack of growth in the dark (Yang et al. 2015), no differences were observed between WT and mutant with respect to starch accumulation, lipid and organic acid production in any of the above studied conditions. On the other hand, *fdx5* showed less starch and low fermentative metabolite accumulation (including H_2) levels under sulfur (S) deprived conditions. Studies comparing the levels of the various FDXs in this mutant are being conducted.

FDX1 and FDX2 knock-down mutants were generated in the *Chlamydomonas* 704 (nit^+) strain using micro-RNA-mediated silencing techniques. Analyses of the *fdx1* mutants revealed a strain with a 55% drop in *fdx1* transcript levels, which was accompanied by an *increase* in growth when cultivated photoautotrophically or photoheterotrophically in liquid medium at non-saturating light intensity. The photosynthetic capacity of the mutant (under saturating light) was

12% lower than that of WT, and its respiratory capacity 80% of WT. However, the mutant's initial rates of H_2 photoproduction measured under saturating light were over 2X higher than its WT counterpart. Maximum production rates were achieved after 2 hours of anaerobic induction for both strains. Inhibitor experiments and starch level evaluations are underway to examine the source of reductant to the hydrogenase under various illumination conditions.

Under S-deprivation, various changes between WT and *fdx1* were observed: the O_2 concentration in the gas phase was still high after 48 hours of S-deprivation in both strains (although even higher in the *fdx1* mutant vs WT) and did not decrease substantially during the remainder of the treatment; H_2 detection was delayed by 24 h in the *fdx1* mutant, but the levels of H_2 accumulated were 3.5-fold higher after 96 hours of –S treatment when compared to the WT. Among the fermentative products, *fdx1* showed large amounts of lactate accumulation in the extracellular medium, while formate secretion was lowed compared to the WT. Clearly, there is a shift in the nature of the fermentation products as well as on the time-point at which each one is detected in the mutant vs. WT strain. We are currently determining starch levels and changes in the expression of all ferredoxins to verify if there is a complementary up-regulation of any of the minor ferredoxins (particularly FDX2) in *fdx1*.

The FDX2 knock-down mutant, which expresses only about 20% of the *FDX2* gene, shows lower nitrite assimilation activity, as expected, with nitrite secretion observed when the culture is grown in the presence of nitrate.. The mutant produces less H_2 than WT when grown under TAP-NH_4, S-deprivation, and in either low light or dark conditions. These results confirm the involvement of FDX2 in nitrogen assimilation and suggest its role in H_2 production (fermentative and photoproduction) under stress conditions. As in the case of the *fdx1* mutant, transcript and protein levels of all the FDX isoforms will be determined in this mutant.

Finally, double *fdx2/fdx5* mutants, as well as overexpressing strains of FDX2, FDX5 and both (in a WT background and subsequently in in the *fdx1* mutant) are being constructed.

References and publications generated by this project and reference above:

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