Integration of the iron-sulfur transfer protein NFU1 of the microalga *C. reinhardtii* in chloroplast metabolism

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Iron-sulfur (Fe-S) proteins are required for several chloroplastic processes such as photosynthesis, chlorophyll, and amino acid metabolisms [1]. NFU1 (Cre17.g710800) is one of the two chloroplastic NFU proteins of *Chlamydomonas* required for the insertion of Fe-S clusters in client proteins [2]. Insertional mutants of the *NFU1* gene were analyzed in order to decipher the network of client proteins of this late maturation factor.

Mutant growth was slightly affected in the light. This defect was not linked to photosynthesis since photosystem I and II activities were not impacted and no major decrease of core PSI or PSII subunits was observed. Pigment analysis showed no major changes of the composition or amount.

To find putative targets of NFU1, a semi-quantitative proteomic analysis of the *nfu1* mutants and the corresponding wild-type strain was performed. Four putative (4Fe-4S) client proteins were found in reduced amounts in the *nfu1* mutants: the large subunit of the 3-isopropylmalate dehydratase LEU1L, the hybrid cluster proteins HCP2 and HCP3, the hydrogenase HYDA1. These results pointed out towards a defect in anaerobiosis and prompted us to investigate the dark fermentative metabolism of the microalga. In addition to HYDA1, another (4Fe-4S)-containing enzyme, the pyruvate-ferredoxin oxidoreductase (PFL1), was found in reduced amounts in dark anoxic conditions by western blotting experiments. A decreased production of fermentative products such as acetate and a decreased activity of the hydrogenase were also measured.

The *nfu1* mutants also showed a yellow phenotype in the dark and accumulated protochlorophyllide *a*. This result suggests a defect in the dark-operative protochlorophyllide *a* oxidoreductase (DPOR), a (4Fe-4S) enzyme responsible for chlorophyll synthesis in the dark.

A diploid strain *nfu1*/NFU1 and a complementated strain containing a wild-type version of the NFU1 gene were isolated. The strains presented a restoration of the wild-type phenotype both in dark oxic and anoxic conditions. This result demonstrates that NFU1 is indeed responsible for the observed defects and thus plays a major role in the transfer of (4Fe-4S) clusters to client proteins in these two specific growth conditions.

At last, binary yeast two-hybrid experiments confirmed the interaction between NFU1 and HCP3. Co-immunoprecipitation experiments are currently undertaken on chloroplasts isolated from cells grown in different conditions to validate additional direct interactions.

In conclusion, our results suggest that NFU1 is involved in the maturation of several key algal [4Fe-4S] of the chloroplast metabolism.

[1] Przybyla-Toscano J et al (2018) *J Biol Inorg Chem* **23**, 545–566

[2] Przybyla-Toscano J et al (2021) *J Int Mol Sci* **22**, 3175