Integration of the iron-sulfur transfer protein NFU1 of the microalga *C. reinhardtii* in chloroplast metabolism in the light, in the dark and in anaerobiosis

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

In the light, growth of the mutants is slightly affected. However, this defect is not linked to photosynthesis since photosystem I (PSI) and II (PSII) activities are not impacted and no major decrease of proteins constituting the core of PSI or PSII is observed. Pigment analysis shows no major changes in terms of composition or amounts, except for the accumulation of 7 hydroxy-methyl chlorophyll *a*, a degradation product of chlorophyll *a* accumulated during nitrogen starvation. This latter result could indicate that the hydroxy-methyl chlorophyll *a* reductase, a [4Fe-4S] cluster-containing enzyme is affected in the mutants.

In the dark, the *nfu1* mutants are yellow and accumulate protochlorophyllide *a*. This result suggests a defect in the dark-operative protochlorophyllide *a* oxidoreductase (DPOR), a [4Fe-4S] cluster-containing enzyme that uses this metabolite as substrate and is responsible for chlorophyll synthesis in these specific growth conditions. Complementation of the mutants with a synthetic version of the *NFU1* gene restores a green-in-the-dark phenotype.

Under dark anaerobiosis, western blotting experiments show that several [4Fe-4S] cluster-containing enzymes are present in reduced amounts in the *nfu1* mutants such as the hydrogenases (HYDA1 and HYDA2), the pyruvate ferredoxin oxidoreductase (PFO) or the hybrid cluster proteins (HCPs). In addition, the hydrogenase activity is significantly reduced. Binary yeast two-hybrid experiments validate the interactions of NFU1 with one of the HCPs (HCP3).

At last, in an attempt to find additional targets, a semi-quantitative proteomic analysis has been undertaken. It confirms the diminution of several of the client proteins cited above in the *nfu1* mutants (HCP3, HYDA1, HYDA2, ChlL encoding one of the DPOR subunits). Additional [4Fe-4S] chloroplastic targets of NFU1 could also be proposed based on their statistically significant reduction in the mutants such as HYDG, one of the specific radical SAM maturation factors of hydrogenases, and 3-isopropylmalate dehydratase involved in leucine biosynthesis. In agreement with this last result, we found a decrease amount of branched chain amino acids (leucine, valine and isoleucine) in the mutants.

In conclusion, our results suggest that NFU1 is involved in the maturation of several key algal [4Fe-4S] chloroplastic enzymes expressed in both oxic and anoxic conditions, as well as in the light and in the dark.

[1] Przybyla-Toscano, J., Roland, M., Gaymard, F., Couturier, J., Rouhier, N. (2018) Roles and maturation of iron-sulfur proteins in plastids. *J. Biol. Inorg. Chem.* **23**, 545–566.
[2] Przybyla-Toscano, J., Couturier, J., Remacle, C., Rouhier, N. (2021) Occurrence, evolution and specificities of iron-sulfur proteins and maturation factors in chloroplasts from algae. *Int. J. Mol. Sci.*, **22**, 3175.