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

Claire Remacle[1], Jonathan Przybyla-Toscano[1,2], Anna Caccamo[1], Sébastien Pyr dit Ruys[3], Gwenaelle Gain[1], Zouheir Alsafra[4], Damien Godaux[1], Gauthier Eppe[4], Didier Vertommen[3], and Nicolas Rouhier[2]

[1]University of Liege, Genetics and physiology of microalgae, InBios/Phytosystems RU, Liege, Belgium
[2]Université de Lorraine, INRAE, IAM, Nancy, France
[3]UCLouvain and de Duve Institute, Université Catholique de Louvain, Brussels, Belgium
[4]University of Liege, LSM/Inorganic Analytical Chemistry, MolSys RU, Liege, Belgium
c.remacle@uliege.be

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]. Mutants of the *NFU1* gene are analyzed in order to decipher the network of client proteins of this maturation factor.

In the light, mutant growth is slightly affected. This defect is not linked to photosynthesis since photosystem I (PSI) and II (PSII) activities are not impacted and no major decrease of core PSI or PSII subunits is observed. Pigment analysis shows no major changes of the composition or amount.

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] enzyme responsible for chlorophyll synthesis in the dark. Complementation with the *NFU1* gene restores a green-in-the-dark phenotype.

Under dark anaerobiosis, western blotting experiments show that [4Fe-4S] enzymes such as the hydrogenases (HYDA1 and HYDA2), the pyruvate ferredoxin oxidoreductase and the hybrid cluster proteins (HCPs) are in reduced amounts. In addition, the hydrogenase activity is decreased. Binary yeast two-hybrid experiments validate the interaction between NFU1 and HCP3.

At last, in an attempt to find additional targets, a semi-quantitative proteomic analysis has been undertaken. It confirms the diminution of several client proteins cited above in the *nfu1* mutants (HCP3, HYDA1, HYDA2, ChlL encoding a DPOR subunit). Additional [4Fe-4S] chloroplastic targets of NFU1 could also be proposed based on their 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 in the mutants.

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

Funded by FNRS-FWO EOS Project 30829584 and CDR J.0175.20

[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