INTEGRATION OF THE IRON-SULFUR CLUSTER TRANSFER PROTEIN NFU1 OF THE GREEN MICROALGA *CHLAMYDOMONAS* IN CHLOROPLAST METABOLISM

Claire Remacle[1], Jonathan Przybyla-Toscano[1,*], Antoine Kairis[1,2], Anna Caccamo[1], Sébastien Pyr dit Ruys[3,**], Didier Vertommen[3] and Nicolas Rouhier[2]

*[1]University of Liege, Genetics and physiology of microalgae, InBios/Phytosystems RU, Liege, Belgium; [2]University of Lorraine, INRAE, IAM, Nancy, France; [3]UCLouvain and de Duve Institute, Brussels, Belgium present addresses: *University of Grenoble CEA, INRAE, CNRS, IRIG, LPCV, Grenoble, France, ** UCLouvain, Faculty of Pharmacy and Louvain Drug Research Institute, Brussels, Belgium*

c.remacle@uliege.be

Iron-sulfur (Fe-S) proteins are required for various chloroplastic processes such as photosynthesis, pigment, and amino acid biosynthesis [1]. NFU1 is one of the two chloroplastic NFU proteins of *Chlamydomonas* required for the insertion of Fe-S clusters into client proteins [2]. Chlamydomonas NFU1 has an atypical architecture since an additional N-terminal domain with putative endonuclease activity is fused to the usual NFU domain serving for Fe-S cluster binding. Two insertional mutants of the *NFU1* gene (*nfu1-1* and *nfu1-2*) have been analyzed to investigate the role of this late maturation factor.

Growth of the mutants in the light was unaffected. Photosynthesis was not impacted, and pigment analysis showed no major changes in composition or amount. A semi-quantitative proteomic analysis of *nfu1-1*, *nfu1-2* and a complemented strain confirmed no changes in the abundance of the major subunits of the photosynthetic chain and the enzymes of the carotenoid synthesis. In contrast, the abundance of one of the subunits (chlL) of the dark-operative protochlorophyllide *a* oxidoreductase (DPOR), a [4Fe-4S] enzyme responsible for chlorophyll synthesis in the dark, and of the hydrogenase enzyme (HYDA1), another [4Fe-4S]-containing enzyme, typical of the fermentative metabolism, was reduced.

These results led us to analyze the *nfu1* mutants when they are cultivated in the dark or subjected to anoxia. When grown in the dark, the *nfu1* mutants were yellow and accumulated protochlorophyllide *a*, the substrate of the DPOR enzyme, in contrast to the wild-type and complemented strains which remained green. When *nfu1* cells were subjected to dark anoxia after growth in the light, the amounts of HYDA1, and of another [4Fe-4S]-containing enzyme, the pyruvate-ferredoxin oxidoreductase (PFOR) were strongly decreased as shown by immunoblot experiments. This was accompanied by a reduced accumulation of fermentative products and a reduced hydrogenase activity. These results demonstrated that NFU1 plays an important role in the transfer of [4Fe-4S] clusters to client proteins under these conditions. To combine these two stresses, cells were then cultivated in the dark followed by dark anoxia. Semi-quantitative proteomic analysis revealed that in this particular growth condition, the amount of HYDA1 was increased by more than 2-fold in the *nfu1-1* mutant. This increase was accompanied by a 2-fold increase of another maturation factor, HCF101. These results suggest a possible redundancy between NFU1 and HCF101, which only occurs when cells are exposed to prolonged stress conditions and highlight the flexibility of the Fe-S cluster assembly machinery of Chlamydomonas.

Finally, strains complemented with the single catalytic NFU domain were isolated. These strains showed a very partial complementation, presenting a very low amount of chlorophyll per cell when grown in the dark and no detectable HYDA1 when anoxia was induced after growth in the light. These results indicate that the catalytic domain is not sufficient to restore the function of NFU1 and that the N-terminal extension is required for the proper functioning of NFU1.

[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