Title:
Characterization of NFU1 and HCF101, two [4Fe-4S] cluster transfer proteins of the sulfur mobilization (SUF) machinery in Chlamydomonas chloroplast

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Abstract:
Iron-sulfur (Fe-S) proteins are required for several chloroplastic processes such as photosynthesis, and chlorophyll or amino acid metabolisms. The sulfur mobilization (SUF) machinery is responsible for the synthesis of the required Fe-S clusters. It involves around 15-20 proteins and allows the synthesis, transfer and insertion of Fe-S clusters into apo-targets. The final Fe-S cluster transfer step relies on several Fe-S transfer proteins, including HCF101 and NFUs. The role(s) of these maturation factors has been investigated in the model plant *Arabidopsis thaliana* [1]; however, a detailed study of their algal counterparts is currently missing. NFU1 is one of the two predicted chloroplastic NFU proteins present in Chlamydomonas [2]. Two insertional *nfu1* mutants of the CLiP library and complemented lines were analyzed. The physiological and molecular analyses revealed that the growth in control light conditions and photosynthesis are not impacted. However, further analyses under specific growth conditions pointed to a role of NFU1 in the maturation of [4Fe-4S] clusters present notably (i) in the dark-operative protochlorophyllide *a* oxidoreductase (DPOR) responsible for chlorophyll synthesis in the dark and (ii) in several Fe-S enzymes involved in the fermentative metabolism. In contrast, an *hcf101* mutant of the CLiP library showed reduced growth in control light growth conditions. Analyses of photosynthesis showed that the maximum quantum yield of photosynthesis (Fv/Fm), the relative electron transport rate (ϕPSII), state transitions and the PSI/PSII ratio are affected in the *hcf101* mutant, indicating that photosynthesis is impaired in these cultivation conditions. These analyses therefore revealed that NFU1 plays a role in specialized pathways not conserved in land plants while HCF101 is likely involved in the biogenesis of PSI subunits by mediating the delivery of [4Fe-4S] clusters to PsaA, PsaB and/or PsaC as observed in land plants [1]. Considering that around 50 proteins are bearing [4Fe-4S] clusters in the chloroplast [1, 2], additional experiments (co-IP, comparative proteomics) aiming at defining the whole network of client proteins of these 2 transfer proteins are currently performed to identify new partners.

[1] Przybyla-Toscano J et al., (2018) *J Biol Inorg Chem* **23**, 545–566
[2] Przybyla-Toscano J et al., (2021) *Int J Mol Sci* **22**, 3175