

# Role of the chloroplastic NFU3 iron-sulfur transfer protein in the microalga *Chlamydomonas reinhardtii*

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NFU3 is one of the two NFU proteins present in the chloroplast of *Chlamydomonas* involved in the insertion of iron-sulfur (Fe-S) clusters in client proteins. Fe-S proteins are found in several chloroplastic processes such as photosynthesis, chlorophyll, amino acid and sulfur metabolisms (see abstract Przybyla-Toscano et al. for more information). Three insertional mutants of the CLIP library [1] bearing a disruption of the *NFU3* gene are analyzed. Growth of the mutants is affected in the light and to a lesser extent in the dark when cells are grown on acetate. The maximum photosynthesis efficiency and  $\phi$ PSII are slightly reduced. No major decrease of proteins constituting the core of Photosystem I is observed by Western blotting. Chlorophyll metabolism is investigated. Two Fe-S enzymes are affected in the *nfu3* mutants: the dark-operative protochlorophyllide oxidoreductase (DPOR) and the 7 hydroxy-methyl chlorophyll *a* reductase (HCAR). DPOR is an enzyme catalyzing the synthesis of chlorophyll in the dark, which is present in algae, but lost in angiosperms. HCAR plays a critical role in chlorophyll degradation, when cells are starved of nitrogen [2]. To find additional targets, the promoter sequence of *NFU3* was scanned for putative *cis*-regulatory elements. Motifs for anaerobiosis and sulfur-deprivation are present. Fe-S enzymes of the fermentative metabolism are currently investigated. First results show that the pyruvate ferredoxin oxidoreductase and H<sub>2</sub> metabolism would be impacted. Binary yeast two-hybrid experiments aiming at validating the interactions of NFU3 with the candidate client Fe-S proteins are underway (see abstract Przybyla-Toscano et al.). Additional targets present in the sulfur or amino acid metabolism will be investigated. Overall, our results suggest that NFU3 is involved in maturation of both classical and algal-specific Fe-S enzymes, which could be relevant biotechnological applications.

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

[1] X. Li et al. *Nature Genetics* 51, 627 (2019).

[2] S. Schmollinger et al. *Plant Cell* 26, 410 (2014).