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Conserved and plant-specific roles of NDUFAF3 in the assembly of the Q/P module of respiratory complex I in the green microalga *Chlamydomonas reinhardtii*

Claire Remacle, Mitchell Ticoras, Thalia Salinas-Giegé, Florent Waltz, Nadine Coosemans, Steven Fanara, Gaetan Herinckx, Didier Vertommen, Johana Chicher, Philippe Hammann, Patrice P Hamel

The NADH: ubiquinone oxidoreductase, or complex I, is the principal entry point for electrons into the respiratory chain. The modular assembly of complex I is well characterized in humans. However, this process remains relatively poorly described in plants where the holoenzyme exhibits plant-specific features, like the recently described ferredoxin bridge connecting the matrix and the membrane arms of complex I at the level of the γ carbonic anhydrase domain. Amongst the assembly factors of the matrix arm, NDUFAF3 is known to function in the stability and assembly of the Q/P_d modules in humans. To investigate the role of NDUFAF3 in plant complex I, we used the plant model system *Chlamydomonas reinhardtii* and generated a strain producing a tagged NDUFAF3-3xFLAG. In this study, we document that NDUFAF3 is also recruited for the assembly of the Q/Pd modules level similar to its human counterpart. Additional plant-only complex I subunits are also identified as NDUFAF3 interactors, including the three proteins of the γ carbonic anhydrase domain and the C1-FDX protein of the ferredoxin bridge. The structural predictions suggest an additional role for NDUFAF3, at a stage preceding the consolidation of the plant-specific ferredoxin bridge in complex I. This placement in the sequential assembly of complex I implies that NDUFAF3 plays a crucial role in the connection between the two arms of the algal complex I. These results highlight the conserved and specific roles of this assembly factor in plants.