

NDUFAF3 is essential for the assembly of the Q/P modules of respiratory complex I in *Chlamydomonas*

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Abstract

The mitochondrial respiratory complex I (CI) is composed of a hydrophobic arm comprising the P module and a hydrophilic arm comprising the N and Q modules. CI assembly is well characterized in humans and catalyzed by a series of assembly factors that join the 3 modules. The CI of protists and plants contain additional ancestral features whose mechanisms of assembly are unknown, namely a ferredoxin bridge that connects the matrix and the membrane arms and a gamma carbonic anhydrase domain. We previously showed that the absence of the assembly factor NDUFAF3 results in a lack of fully-formed CI in *Chlamydomonas* and a decreased abundance of subunits of the Q module (1). In this work, a strain where NDUFAF3 has been tagged with a 3xFLAG is investigated. Like its human homolog, NDUFAF3 interacts strongly with the classical subunits of the Q and P modules, but also with the gamma carbonic anhydrase domain and a subunit of the ferredoxin bridge. The predicted structural positioning of NDUFAF3 within the Q module suggests a role in the formation of this bridge. In contrast, subunits of the N module are loosely associated with NDUFAF3. We further demonstrate that the N module is attached at a later stage of assembly, suggesting that *Chlamydomonas* CI assembles in a human-like sequence. This contrasts with the situation in *Arabidopsis* where the N and the Q modules are attached together before anchoring to the P module.

Altogether, these results highlight a conserved and ancestral role of NDUFAF3 in CI manufacture.

Reference

1. Massoz, S., Hanikenne, M., Bailleul, B., and Coosemans, N., Radoux, M., Miranda-Astudillo, H., Cardol, P., Larosa, V., Remacle, C. (2017) *In vivo* chlorophyll fluorescence screening allows the isolation of a *Chlamydomonas* mutant defective for NDUFAF3, an assembly factor involved in mitochondrial complex I assembly. *Plant J.* **92**, 584-595