F_1F_O ATP synthase mutants in Chlamydomonas : stability and oligomycin resistance mediated by atypical Asa7 protein; interaction between chloroplastic and mitochondrial bioenergetics

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In this work, we first show that **mitochondrial F1FO ATP synthase from chlorophycean green algae has an atypical subunit composition** of its peripheral stator and dimerization module, with 9 subunits of unknown evolutionary origin (Asa subunits). In contrast, other classes of green algae possess a canonical subunit composition. In addition, respiration, ATP levels and **growth of Chlorophyceae are also barely affected by oligomycin concentrations** that affect representatives of the other classes of Chlorophytes.

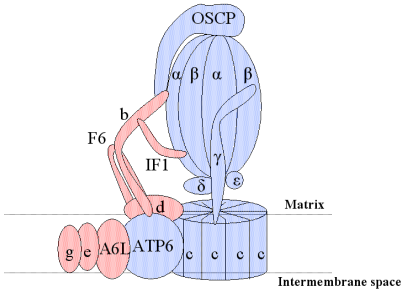


Figure 1. Schematic structure of the bovine ATP synthase. Subunits absent from the Chlorophycean enzyme are shown in red.

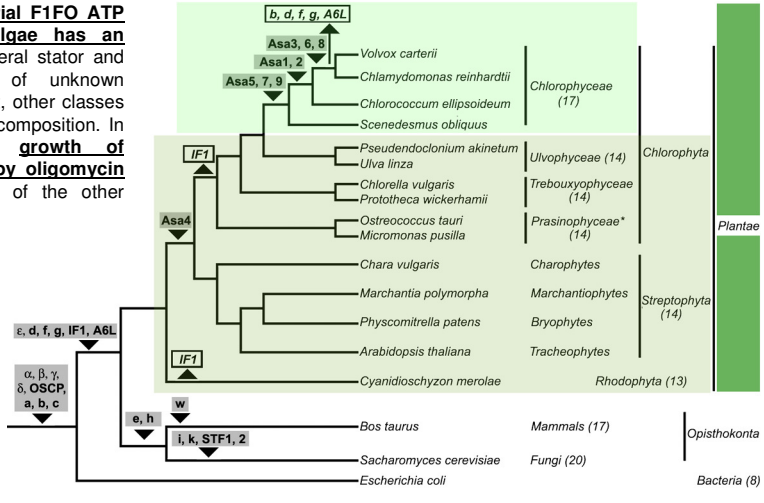


Figure 2. Phylogenetic distribution of mitochondrial ATP synthase gene gains and losses in the green lineage. Clades and species for which the subunit composition has been (at least partially) determined are indicated. Gains and losses were mapped using unweighted Dollo parsimony onto a consensus tree from other studies

Figure 3. Oligomycin sensitivity. in representative of the four classes of Chlorophytes was tested after 5 days of growth in the light

The ***Chlamydomonas reinhardtii* mutant defective in β subunit (Atp2 mutant) is an obligate phototroph** lacking ATP synthase assembly and activity coupled to the respiration. In addition, **Atp2-deficient mitochondria are deprived of cristae, and rearrangements of the photosynthetic apparatus and thylakoid organization are observed.**

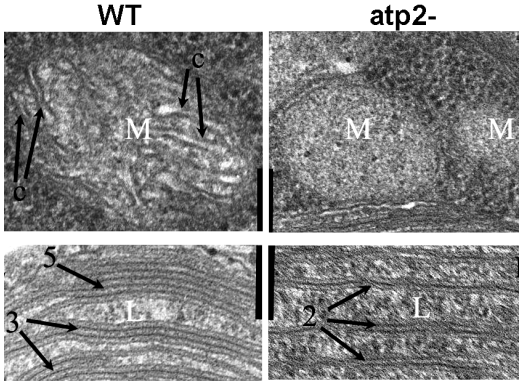


Figure 5. Ultrastructure of wild-type and atp2- mutant cells grown in the light in the presence of 5mM acetate. M, mitochondria; C, cristae; L, lumen; numbers indicate the number of thylakoid lamellae in stacks. Black bars = 0.68 µm (top) or 0.2 µm (bottom).

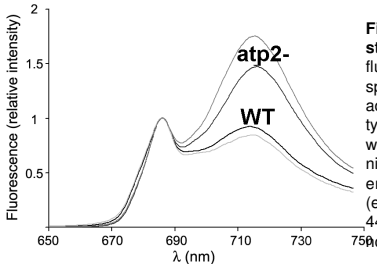
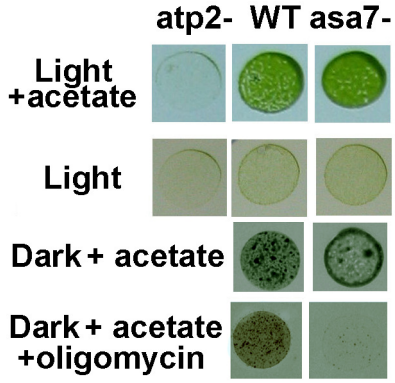


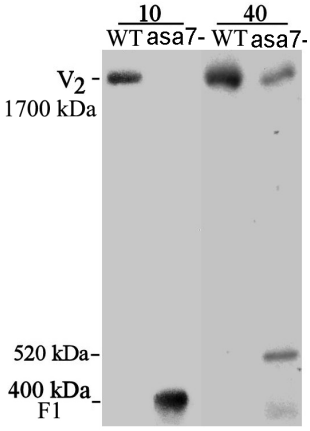
Figure 6. Analysis of state transitions by 77 K fluorescence spectroscopy. Dark-adapted cells from wild - type and atp2- mutant were freezed in liquid nitrogen. Fluorescence emission spectra (excitation wavelength = 440 nm) were recorded, normalized at 685

Figure 4. Growth of WT, atp2- and *asa7*- cells. 5 mM acetate; oligomycin 20 µM.



In contrast, **the loss of Asa7 subunit has no impact on cell bioenergetics or organelles structures, but it destabilizes the enzyme dimeric form *in vitro* and renders growth, respiration and ATP level sensitive to oligomycin.**

Figure 7. ATP synthase dimer stability in wild type and *asa7*- murant. BN-PAGE analysis. Purified mitochondria (50 µg of protein) solubilised with 10 or 40% (w/w) of n-dodecyl-β-D-maltoside. After electrophoresis, the gel was stained for ATPase activity.



Conclusions : Altogether, our results suggest that **the loss of canonical components of the stator happened at the root of chlorophycean lineage** and **was accompanied by the recruitment of novel polypeptides.** Such a massive modification of stator features might have conferred novel properties, including the stabilization of the enzyme dimeric form and the shielding of the proton channel. Our study also contributes to the understanding of the yet poorly-studied bioenergetic interactions between organelles in photosynthetic organisms.

Lapaille et al. (2010) Atypical subunit composition of the chlorophycean mitochondrial F1FO-ATP synthase and role of Asa7 protein in stability and oligomycin resistance of the enzyme, Mol Biol Evol, 27 1630-44.
Lapaille et al. (2010) Loss of mitochondrial ATP synthase subunit beta (Atp2) alters mitochondrial and chloroplastic function and morphology in Chlamydomonas, Biochim Biophys Acta, 1797:1533-9

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