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.

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

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

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 freeze thawed and measured in liquid nitrogen. Fluorescence emission spectra (excitation wavelength = 440 nm) were recorded.

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- mutant. BN-PAGE analysis. Purified mitochondria (50 μg of protein) solubilized 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.
