2P.7 F1F0 ATP synthase mutants in *Chlamydomonas*: Stability and oligomycin resistance mediated by atypical Asa7 protein; interaction between chloroplastic and mitochondrially bioenergetics

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In yeast, mammals, and land plants, F1F0 ATP synthase (complex V) comprises about 15 conserved subunits. In this work, we show that complex V 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), while complex V has a canonical subunit composition in other classes of green algae. In addition, growth, respiration and ATP levels in Chlorophyceae are also barely affected by oligomycin concentrations that affect representatives of the other classes of Chlorophyta. We then isolated *Chlamydomonas* mutants lacking ether beta subunit (Atp2) or an atypical subunit (Asa7). The Atp2 mutant is an obligate phototroph lacking complex V assembly and ATP synthesis coupled to the respiration. In addition, Atp2-deficient mitochondria are deprived of cristae, and rearrangements of the photosynthetic apparatus and thylakoid organization are observed. 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. 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.


2P.8 Purification and characterisation of F-ATPase from three species of fungi

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F-ATPase has been purified from mitochondria from *Pichia angusta*, *Pichia pastoris* and *Yarrowia lipolytica* by affinity chromatography with a monomeric pH insensitive form of the bovine yeast inhibitor protein, IF1. The subunit compositions of the complexes have been characterized by SDS-PAGE, by mass spectrometric analysis of tryptic peptides, and by measurement of the masses of the subunits by LC-MS [1]. The impact of phospholipids on specific activities and the sensitivity to oligomycin of the complexes has been studied. The enzymes have been reconstituted in phospholipid vesicles and proton pumping was measured with the pH sensitive probe amino-6-chloro-2-methoxy-acridine (ACMA). ACMA quenching was observed after addition of ATP, halted by addition of oligomycin and reversed by addition of the ionophore gramicidin. The thermostability of the purified complexes was investigated by following the ATP hydrolysis activity from 25 °C to 70 °C. As expected, the *P. angusta* enzyme was the most thermostable. It has been selected for crystallisation trials.

References
