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Divergence of the mitochondrial electron transport chains from the green alga *Chlamydomonas reinhardtii* and its colorless close relative *Polytomella* sp.

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Abstract

Compelling evidence exists that the colorless algae of the genus *Polytomella* arose from a green *Chlamydomonas*-like ancestor by losing its functional photosynthetic apparatus. Due to the close relationship between the colorless and the green chlorophyte, *Polytomella* sp. appeared as a useful indicative framework for structural studies of *Chlamydomonas reinhardtii* mitochondria. However, comparative studies reported here unexpectedly revealed significant differences between the mitochondrial respiratory systems of the two algae. Two-dimensional blue native/SDS-PAGE of isolated mitochondria indicated that cytochrome-containing respiratory complexes III and IV in the two chlorophytes contrast in size, subunit composition and relative abundance. Complex IV in *Polytomella* is smaller than its counterpart in *C. reinhardtii* and occurs in two forms that differ presumably in the presence of subunit COXIII. The cytochrome *c* and the iron–sulfur Rieske protein of both chlorophytes revealed structural differences on the amino acid sequence level. Under comparable culture conditions, the colorless alga exhibits lower levels of cytochrome *c* and complex IV but a higher respiratory activity than the green alga. Cytochrome *c* levels were also found to be differently regulated by the growth conditions in both algae. The divergence between the respiratory systems in the two related chlorophytes can be viewed as a consequence of the loss of photosynthetic activity and/or of the adaptation to the environment via the acquisition of a more flexible, heterotrophic metabolism. Our understanding of mitochondrial function and evolution is expected to be greatly enhanced via further parallel studies of photosynthetic/non-photosynthetic algae, for which this study forms an incentive.

Keywords: Blue native PAGE; Chlorophyte; Heterotrophic lifestyle; Mitochondrial cytochrome; Photosynthesis; Respiratory chain

1. Introduction

Unicellular colorless algae are found the world over in a myriad of soils and aqueous habitats. Original descriptions of colorless species can be found as far back as the late 19th century. A few colorless algae have long been described as non-photosynthetic equivalents of photosynthetic algae since they share morphological and reproduction characteristics with green algae and possess plastid-related organelles that contain starch granules. The algae from the genus *Prototheca* for example were proposed to be relatives of the green algae *Chlorella* [1] and the species *Polytoma* and *Polytomella* are regarded as colorless species of the order Volvocales [2]. It has been hypothesized that some colorless algae derived from photosynthetic algal ancestors by losing a functional photosynthetic apparatus [3]. The evolutionary link between some colorless and green species has been

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Abbreviations: AOX, alternative oxidase; BN-PAGE, blue native polyacrylamide gel electrophoresis; Cyt, cytochrome; ISP, iron–sulfur protein; MPP, mitochondrial processing peptidase; OXPHOS, oxidative phosphorylation; TMPD, *N*,*N*,*N*′,*N*′-tetramethyl-*p*-phenylenediamine dihydrochloride

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confirmed by phylogenetic sequence analysis of nuclear small-subunit (SSU) rRNA genes [4,5], mitochondrial DNA or remnant plastid DNA. For instance, fragmentation and scrambling of the mitochondrial ribosomal RNAs occur similarly in the colorless alga *Polytomella parva* [6] and in the green algae *Chlamydomonas reinhardtii* and *C. eugametos* [7,8]. Also, both *Polytomella* and *Chlamydomonas* species have a reduced size mitochondrial DNA which codes for a limited set of respiratory proteins [9,10].

The events that have led to the supposed loss of photosynthesis and the emergence of the colorless algae bear a speculative character in part due to the limited number of studied colorless species. Among the different scenarios invoked are: (i) adaptation to a long-term heterotrophic lifestyle; (ii) appearance of mutations that have irreversibly affected photosynthetic ability or (iii) acquisition of foreign genes by lateral gene transfer.

The loss of the chloroplast is expected to have had an impact on the mitochondrion as a whole. Indeed, mitochondria are crucial for optimal chloroplast metabolism: In photosynthetic organisms, these organelles possess several specific enzymes which play a key role under light conditions in balancing the cellular energy and redox status (for a review, see [11]). So far, comparative studies dedicated to the mitochondrial energy metabolism of the colorless algae and their green counterparts are scarce. At present, the duo of photosynthetic/non-photosynthetic algae best suited for mitochondrial comparative studies is represented by *C. reinhardtii* and *Polytomella* sp. Recent technical advances have allowed comprehensive studies on *C. reinhardtii* mitochondria: purification of mitochondria [12], micro-analytic methods to characterize mitochondrial proteins [13–15] and the sequencing of the *C. reinhardtii* nuclear genome [16,17]. Fact however remains that the study of the mitochondria of the green alga (and of photosynthetic organisms in general) is severely hampered by the presence of a chloroplast. *Polytomella*, which lacks chloroplasts, has proven useful to investigate certain aspects of the structure of respiratory complexes in the chlorophytes. Indeed, the oxidative phosphorylation (OXPHOS) systems in *C. reinhardtii* and *Polytomella* sp. share several unusual structural features: (i) a heterodimeric COX II subunit of complex IV (COXII) [14,18]; (ii) a decreased hydrophobicity of the nucleus-encoded COX III subunit of complex IV that could facilitate import into the mitochondrion [19]; and (iii) subunits α and β of complex V exhibit extensions on the N- and C-terminus, respectively [20–22]. The discovery of these shared unusual traits has strengthened the assumption that *Polytomella* species have evolved from a *Chlamydomonas*-like ancestor. Yet, significant differences between the mitochondrial electron transport chains of *C. reinhardtii* and *Polytomella* have been observed too. Cytochrome *b* (cyt *b*) of *Polytomella* complex III exhibits an α-absorption at 567 nm [23] which is 3–4 nm red-shifted compared to cyt *b* of *C. reinhardtii* [24,25] and of mammals and plants [26,27]. Also, in contrast to *C. reinhardtii* [12,28], *Polytomella* sp. lacks a typical SHAM-sensitive alternative oxidase (AOX) [29]. The origin of AOX is uncertain but its occurrence in a number of prokaryotes is suggestive of an endosymbiotic acquisition [30]. The absence of an AOX in *Polytomella* sp. may be related to the loss of photosynthetic metabolism or could be a consequence of newly acquired metabolic features or regulatory mechanisms. Indeed, unlike *C. reinhardtii*, *Polytomella* sp. is able to grow under a wide range of pH and in the presence of various carbon sources [31].

Here, we extend the investigation on the mitochondrial respiratory chains of the algae *C. reinhardtii* and *Polytomella* sp. Our results revealed unexpected differences in the structure and composition of the cyanide-sensitive respiratory chain of the evolutionary related algae. This interesting divergence provides grounds to further research efforts that aim to assess the impact on mitochondria of on one hand the loss of photosynthetic activity and on the other the consequences of newly acquired metabolic features or regulatory mechanisms.

2. Materials and methods

2.1. Algal strains, growth conditions, and mitochondria isolation

C. reinhardtii cell wall-less mutant strain 84CW15 was grown at 25–26 °C in continuous light and agitation on TAP medium [32] supplemented with 1% (w/v) sorbitol. *Polytomella* sp. (198.80, E.G. Pringsheim) was grown aerobically at room temperature on MAP medium which consists of 30 mM sodium acetate, 35 mM Mes, 1 mM potassium phosphate (pH 7.4), 7.4 mM NH_4Cl, 0.3 mM $CaCl_2$, 0.5 mM $MgSO_4$; trace elements and vitamins were added as in [31]. The pH of MAP medium was adjusted to 5.7–5.9 with KOH. Mitochondria were isolated from *C. reinhardtii* and *Polytomella* sp. cells in their exponential growth phase [12,31]. Due to the significant chlorophyll contamination, the mitochondria of *C. reinhardtii* were further purified on a Percoll gradient [12]. Protein concentration in isolated mitochondria was determined using the modified Lowry procedure [33] after protein precipitation with methanol/chloroform [34].

2.2. Protein electrophoresis and analysis

For BN-PAGE, mitochondrial proteins were solubilized in the presence of *n*-dodecyl-β-maltoside according to [14,15]. Blue native gels consisted of a separating gel (5–12% [w/v] acrylamide) and a stacking gel (4% [w/v] acrylamide) [14]. For second-dimension analysis, BN-PAGE lanes or excised gel pieces corresponding to the protein complexes of interest were applied onto either Glycine- or Tricine-SDS-PAGE. In order to achieve the separation of *Polytomella* subunits COXIIA and COXIIB