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Evolution of mitochondria and
mitochondrion-related organelles with special
reference to the free-living anaerobic
stramenopile *Cantina marsupialis*

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博士学位論文内容要旨
Abstract

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論文題目 Title	Evolution of mitochondria and mitochondrion-related organelles with special reference to the free-living anaerobic stramenopile <i>Cantina marsupialis</i> 嫌気性自由生活型原生生物 <i>Cantina marsupialis</i> を中心としたストラメノパイル生物群のミトコンドリア進化に関する研究		

In this doctoral dissertation, I studied the two following evolutionary subjects regarding mitochondria in the eukaryotic lineage stramenopiles.

1) Metabolic capacity of mitochondrion related organelles in the free-living anaerobic stramenopile *Cantina marsupialis*

Functionally and morphologically degenerate mitochondria, so-called mitochondrion related organelles (MROs), are frequently found in eukaryotes inhabiting hypoxic or anoxic environments. In the last decade, MROs have been discovered from a phylogenetically broad range of eukaryotic lineages and these organelles have been revealed to possess diverse metabolic capacities. In this study, the biochemical characteristics of an MRO in the free-living anaerobic protist *Cantina marsupialis*, which represents an independent lineage in stramenopiles, were inferred based on RNA-seq data. I found transcripts for proteins known to function in one form of MROs, the hydrogenosome, such as pyruvate:ferredoxin oxidoreductase, iron only-hydrogenase, acetate:succinate CoA-transferase, and succinyl-CoA synthase, along with transcripts for acetyl-CoA synthetase (ADP-forming). These proteins possess putative mitochondrial targeting signals at their N-termini, suggesting dual ATP generation systems through anaerobic pyruvate metabolism in *Cantina* MROs. In addition, MROs in *Cantina* were also shown to contain several features of canonical mitochondria, including amino acid metabolism and an “incomplete” tricarboxylic acid cycle. Transcripts for all four subunits of complex II of the electron transport chain were detected, while there was no evidence for the presence of complexes I, III, IV, and F1Fo ATPase. *Cantina* MRO biochemistry raises a question about the categories of mitochondrial organelles recently proposed. The previous study proposed that the mitochondrial family of organelles (i.e., a canonical mitochondrion and its derived organelles) can be divided into five classes in the context of function. Among these classes, a hydrogen-producing (class 3) mitochondrion is defined as an organelle with a proton-pumping electron transport chain and iron-only hydrogenase. MROs in *Cantina*, together with those of the anaerobic protists *Mastigamoeba* and *Pygsumia*, possess iron-only hydrogenase and therefore could produce molecular hydrogen. However, there is no evidence indicating the proton-pumping electron transport chain exists in MROs in *Cantina*, *Mastigamoeba*, and *Pygsumia*, although these organelles seem to have some components of the membrane-associated electron transport system including the complex II of the electron transport chain. Therefore, these MROs cannot be classified into any classes according to the criteria previously proposed. If MROs in *Cantina*, *Mastigamoeba*, and *Pygsumia* are placed in class 3, the definition of this class may have to be slightly revised by omitting the term “proton-pumping”. In the present study, several unique biochemical characteristics were suggested in MROs in *Cantina*. Among these characteristics, it should be especially noted that *Cantina* unlikely possesses the low redox potential electron carrier, rhodoquinone, and fumarate hydratase and that the incomplete oxidative tricarboxylic acid cycle may operate in its organelles. These findings allow us to reconsider the evolution associated with mitochondria that could have occurred during the course of adaptation to hypoxic/anoxic environments. According to the evolutionary scenario for degenerate mitochondria previously suggested, the successive evolutionary steps from an aerobic (class 1)

mitochondrion to a class 3 mitochondrion are as follows: 1) the acquisition of the low redox potential electron carrier, rhodoquinone, and the employment of the rhodoquinone-associated reverse tricarboxylic acid cycle (fumarate respiration); 2) the acquisition of iron-only hydrogenase; and 3) the loss of complexes III, IV, and F1Fo ATPase. However, the order of the two former events could be reversed or the first step could be skipped in the process of evolution, because it is likely that *Cantina* MROs have iron-only hydrogenase, but lack rhodoquinone. Based on my present findings, the evolutionary path leading to degenerate organelles in association with adaptation to oxygen-depleted environments may not necessarily be uniform in different organisms.

2) Complex evolution of two types of cardiolipin synthase in the eukaryotic lineage stramenopiles

The phospholipid cardiolipin is indispensable for eukaryotes to activate mitochondria, and it was previously reported that two phylogenetically distinct types of enzyme synthesizing cardiolipin, one with two phospholipase D domains (CLS_pld) and the other with a CDP-alcohol phosphatidyltransferase domain (CLS_cap), are patchily and complementarily distributed at higher taxonomic (e.g., supergroup) levels of eukaryotes. Stramenopiles, one of the major eukaryotic clades, have been considered to possess CLS_cap exclusively. However, through my present surveys with genome or transcriptome data from a broad range of stramenopile taxa, species with both CLS_cap and CLS_pld and species with only CLS_pld as well as those with only CLS_cap were discovered among this group. Because these homologues of CLS_cap and CLS_pld retrieved from stramenopiles were likely inherited from the last eukaryotic common ancestor, it is reasonable to assume that a common ancestor of all stramenopiles harbored both CLS_cap and CLS_pld. Furthermore, based on the robust organismal phylogeny of stramenopiles unveiled with large-scale phylogenetic analyses, the earliest diverging lineage of stramenopiles (including bicosoecids, placidids, etc.) was found to comprise species with both CLS_cap and CLS_pld along with species with only either CLS_cap or CLS_pld, suggesting that a common ancestor of the most basal stramenopile lineage retained these two vertically inherited enzymes and that differential losses of either CLS_cap or CLS_pld occurred in this lineage. On the other hand, in the other stramenopile lineage composed of Ochrophyta including a wide variety of photosynthetic lineages such as diatoms and brown algae, Pseudofungi including oomycetes and *Devolpayella*, and Labyrinthulomycetes (to the exclusion of the most basal lineage), only CLS_cap was found, and therefore a common ancestor of these three groups likely lost CLS_pld. The previous study argued that each major eukaryotic group (e.g., supergroup) has only one of either CLS_pld or CLS_cap. Nevertheless, in this pioneering study, only CLS_cap was identified in stramenopiles, a subgroup of the supergroup SAR (composed of alveolates and Rhizaria together with stramenopiles), while only CLS_pld was retrieved from alveolates. Considering this situation, the close relationship between stramenopiles and alveolates was doubted. However, we newly found CLS_pld in several species of stramenopiles. In addition, there are multiple lines of reliable evidence that SAR is a monophyletic clade, and therefore I here propose the hypothesis that a common ancestor of stramenopiles and alveolates (or of SAR) possessed both CLS_cap and CLS_pld, with the subsequent loss of CLS_cap in the alveolate lineage. Thus, the doubt regarding the classification and relationship of stramenopiles and alveolates posed could be dispelled. The evolution of CLSs is not as simple even in a single eukaryotic clade, as suggested by the results of this study. Therefore, the evolutionary history of the enzymes in question in the eukaryotic domain as a whole is also possibly more complicated than previously thought, and major eukaryotic groups other than stramenopiles may have to be individually examined with comprehensive taxon sampling. In that case, a robust organismal phylogeny would be the key to trace the evolution of CLS_cap/CLS_pld precisely in each eukaryotic group.