The Organellar Genomes of *Chromera* and *Vitrella*, the Phototrophic Relatives of Apicomplexan Parasites

Miroslav Oborník[1,2,3] and Julius Lukeš[1,2,4]

[1]Institute of Parasitology, Biology Center, Czech Academy of Sciences, 1160/31 České Budějovice, Czech Republic; email: obornik@paru.cas.cz, jula@paru.cas.cz

[2]Faculty of Science, University of South Bohemia, 37005 České Budějovice, Czech Republic

[3]Institute of Microbiology, Czech Academy of Sciences, 379 81 Třeboň, Czech Republic

[4]Canadian Institute for Advanced Research, Toronto, Ontario M5G 1Z8, Canada

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Abstract

Apicomplexa are known to contain greatly reduced organellar genomes. Their mitochondrial genome carries only three protein-coding genes, and their plastid genome is reduced to a 35-kb-long circle. The discovery of coral-endosymbiotic algae *Chromera velia* and *Vitrella brassicaformis*, which share a common ancestry with Apicomplexa, provided an opportunity to study possibly ancestral forms of organellar genomes, a unique glimpse into the evolutionary history of apicomplexan parasites. The structurally similar mitochondrial genomes of *Chromera* and *Vitrella* differ in gene content, which is reflected in the composition of their respiratory chains. Thus, *Chromera* lacks respiratory complexes I and III, whereas *Vitrella* and apicomplexan parasites are missing only complex I. Plastid genomes differ substantially between these algae, particularly in structure: The *Chromera* plastid genome is a linear, 120-kb molecule with large and divergent genes, whereas the plastid genome of *Vitrella* is a highly compact circle that is only 85 kb long but nonetheless contains more genes than that of *Chromera*. It appears that organellar genomes have already been reduced in free-living phototrophic ancestors of apicomplexan parasites, and such reduction is not associated with parasitism.

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INTRODUCTION

Organellar genomes—which are, beyond a reasonable doubt, of endosymbiotic origin—are typical examples of reductive evolution. The engulfed free-living *Alphaproteobacteria* (ancestors of mitochondria), phototrophic cyanobacteria (ancestors of primary plastids), and eukaryotic algae (ancestors of secondary and tertiary plastids) have undergone morphological and genomic reductions that are to some extent still ongoing (34, 38). An inevitable part of the endosymbiotic process that triggered the evolution of organelles is the loss of dispensable organellar genes and transfer of other genes to the exosymbiont's nucleus. As a result of these processes, even the largest mitochondrial (mt) genome, found in the protist *Jacoba libera*, barely exceeds 100 kb (6), thus retaining just 1% to 5% of the original alphaproteobacterial genome. The mt genome of apicomplexan parasites has been reduced to ~6 kb (18), which is only about 0.1% to 0.5% of the genome of its putative free-living bacterial ancestor. In strictly or facultatively anaerobic eukaryotes such as microsporidia, diplomonads, and even apicomplexans of the genus *Cryptosporidium*, the mt genome has been entirely lost (47).

It is therefore not surprising that the genomes of plastids, the phototrophic organelles of eukaryotes, followed a similarly reductive path. Although the genomes of free-living cyanobacteria may exceed 8 Mb, the derived genomes of photosynthetic plastids range between 72 kb, in *Ostreococcus tauri*, and 520 kb, in *Floydiella terrestris* (these are both green algae) (4). However, the average size of a phototrophic plastid genome is approximately 150 kb, or about 3% of the cyanobacterial genome from which it is derived. Furthermore, on their evolutionary trajectory plastids sometimes forfeit photosynthetic ability, and because of the loss of genes associated with photosynthesis, this leads to additional shrinking of their genomes. A prominent example of such a development is the circular, 35-kb genome of the relic apicomplexan plastid, the apicoplast (~0.7% of the ancestral cyanobacterial genome). However, extreme reductions are also evident in the parasitic plant *Conopholis americana*, with a plastid genome just 45 kb long (~0.9% of the ancestral genome), and the parasitic green alga *Helicosporidium* sp., the plastid genome of which does not exceed 37 kb (12). The phototrophic *Euglena gracilis* and the closely related heterotrophic but plastid-retaining *Euglena longa* are excellent examples of the consequences that loss of photosynthesis has for a plastid genome: The former alga carries a 140-kb, circular genome in its plastid (28), whereas the homologous genome of its osmotrophic sister has so far been reduced to a mere 73 kb (25). Still, a single gene associated with light-driven carbon fixation—it encodes the large subunit of ribulose-1,5-bisphosphate carboxylase—is retained in the *E. longa* plastid. Just as with the mt genome, the plastid genome can be lost completely, as was recently reported of both the primary plastid of the parasitic green alga *Polytomella* sp. (73) and the secondary plastid of the parasitic alveolate *Perkinsus marinus* (66). It should be noted that—surprisingly—the most reduced but still functional plastid genome known belongs to phototrophs: In dinoflagellates, the genomes of peridinin-pigmented plastids are fragmented into a set of small circular molecules

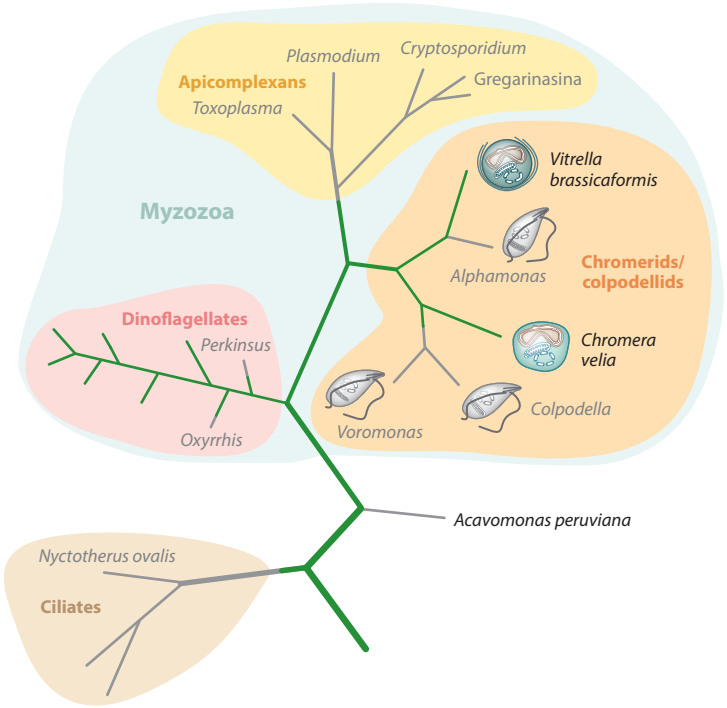


Figure 1

Phylogeny of alveolates, including *Chromera* and *Vitrella*.

called minicircles that together contain only 14 protein-coding genes and 2 reduced rRNA genes (2, 24, 83, 84).

Myzozoans are a subgroup of alveolates that includes apicomplexans, dinoflagellates, and colpodellids (8) (**Figure 1**), and current phylogenies are compatible with *Chromera velia* and *Vitrella brassicaformis* being affiliated with them (22, 31, 53). The common feature of myzozoans is the conoid, a tube that allows feeding through myzocytosis (8). It has been suggested that at least the common myzozoan ancestor passed through a single secondary endosymbiotic event involving a heterotrophic eukaryote (exosymbiont) and a rhodophyte endosymbiont (26, 36, 38, 51, 56). Therefore, a secondary or tertiary plastid surrounded by three or four membranes was expected to be present in members of this group. The phylogenetic structure of myzozoans remains to a large extent unresolved, mainly because of the lack of data for a substantial portion of their diverse members. As obligatory parasites of animals, including humans, the extremely species-rich Apicomplexa is the best-known phylum (1). In particular, *Toxoplasma gondii* and members of the genus *Plasmodium*, the causative agents of widespread and devastating toxoplasmosis and malaria, respectively, are widely studied.

The discovery of a relic plastid in Apicomplexa strongly indicated that all obligatory parasitic apicomplexans have an algal ancestor (52). Consequently, dinoflagellates have been considered their only extant phototrophic relatives, but their plastid genomes are derived beyond comparison (37), and owing to their extreme size their nuclear genomes have yet to be sequenced. A true breakthrough came in 2008 with the report of a photosynthetic secondary alga cultured from Australian corals and named *C. velia* (53). Since then another alga, currently the closest phototrophic relative of *Chromera* that is known—*V. brassicaformis*—has been found in the same environment (58).

Phylogenetic analyses including both algae found that they are genuine phototrophic relatives of the obligatory parasitic apicomplexans (31, 32, 56, 57, 80). Understanding the organellar and nuclear genomes of these evolutionarily uniquely positioned protists will yield important insight into the emergence of the extremely successful parasitic lifestyle of apicomplexans and will certainly also have an impact on our general view of these semiautonomous organelles.

A MITOCHONDRIAL GENOME FULL OF ODDITIES

All alveolates carry a rather conventional mt DNA-containing organelle, with the sole (known) exception of the parasitic genus *Cryptosporidium*, the mitochondrion of which lost its genome altogether and became so functionally reduced that it reached the category of mitosome (39). Most other alveolates have also extensively reduced their mt genomes, as the only conventional gene content is on the linear-mapping mt DNA of ciliates (7) and the early-branching myzozoan *Acavomonas* (33). Mitochondrial genomes of ciliates contain between 20 and 44 protein-coding genes of known and unknown functions, 2 mito-rRNA genes, and just a handful of tRNA genes (71). The most-reduced organellar genome of a ciliate is the mt genome of the hydrogen-producing, anaerobic mitochondrion of *Nyctotherus ovalis*, which has lost all mt DNA-encoded subunits of respiratory complexes III–V (11).

However in both sister clades, apicomplexans and dinoflagellates, mt genomes underwent extensive divergence and reduction from the canonical mt DNA structure, with the latter group accumulating even more oddities. The best-characterized apicomplexan mt genome is that of *Plasmodium falciparum*, which is reduced to cytochrome *c* oxidase subunit 1 (*cox1*), and *cox3*, and cytochrome *b* (*coB*) located on circularly permutated linear molecules (18). The same set of genes was also retained in other apicomplexans, such as eimeriids (45). Moreover, both mito-rRNA genes underwent rampant fragmentation, with several highly conserved domains not yet identified and thus probably missing (17). The full set of tRNAs is, as in kinetoplastid flagellates, imported from the cytosol (16).

The extremely narrow set of just three mt DNA-encoded subunits of respiratory complexes III and IV, polyadenylation of mt transcripts, and extensive fragmentation of mito-rRNAs are features shared between apicomplexans and dinoflagellates. However, the latter highly abundant marine protists developed a number of additional unique mt features (78), relevant here for comparative purposes. The mt genome of dinoflagellates is a pool of linear, heterogeneous molecules of varying sizes that contain a collection of full-size and fragmented genes (translatable *cox3* mRNA must be *trans*-spliced from split fragments) along with inverted repeats and noncoding regions in a chaotic arrangement, making accurate assembly impossible (55). Based on Southern blot analyses and deep sequencing, it can be concluded that these mt genomes exist in seemingly limitless combinations, generated by extensive recombination. One can hardly imagine orderly transcription in a system containing just a handful of genes in hundreds of different sequence contexts. Indeed, it seems that all mt DNA is transcribed, yet most transcripts are subject to quick degradation. Only mature mRNAs generated by cleavages on both ends, followed by stabilization by oligoadenylation, are likely subject to translation (78), although direct evidence for that is missing. Another unique feature is extensive and extremely diverse RNA editing of all mt genes encoding proteins and RNAs; this occurs all along the transcripts and involves at least nine types of posttranscriptional nucleotide changes (46). The mechanisms behind this unprecedentedly versatile editing are completely unknown.

Moreover, dinoflagellates do not use the almost universal start and stop codons. The former are missing from their 5′ transcript regions, and the latter are present in the 3′ ends that are usually preceded by oligoadenylation, implying stop-codon-free termination (78). Despite all

these oddities at the level of nucleic acids, respiratory complexes III and IV seem to be active (35), providing evidence that properly functioning mt subunits are in fact produced by this seemingly messy genetic system. Although studies of the deep-branching *Hematodinium* have shown that the genome scrambling occurred quite early in the dinoflagellate lineage, reasons for inflation of genome size, gene copy number, and gene complexity—apparently departures from an ancestral simplicity—remain obscure (30). Moreover, *Perkinsus*, a representative of another early-branching alveolate lineage, developed its own eccentricities, in particular frequent frameshifts requiring unique translation-decoding mechanisms (50). We know almost nothing about the molecular mechanisms responsible for these oddities.

As they are aerobic relatives of the above-mentioned alveolate groups, *Chromera* and *Vitrella* both harbor several oval, and small mitochondria, usually adjacent to the plastid (58, 59). Its initial misidentification (53) was due to its rather inconspicuous morphology and lack of cristae. Although our knowledge of mt nucleic acids in both chromerids is quite substantial, our understanding of their mt metabolism will remain very limited until we learn more about their nuclear genomes. We can only anticipate that conserved and key components of the alveolate mitochondrion, such as the translocons of the inner and outer mt membranes and the tricarboxylic acid cycle (68, 70, 74), are present and active in *Chromera* and *Vitrella*. Still, due to surprisingly extensive divergence of *Chromera* organellar genomes and respiratory chains from those of *Vitrella* (19, 20), we can expect to see indications of these differences at the metabolic level. Moreover, Sheiner et al. (70) and Hino et al. (29) recently showed that the mt metabolisms of *Plasmodium* and *Toxoplasma* vary substantially at different life cycle stages (29, 70), a feature they share with unrelated parasites such as trypanosomes (77) (and probably other parasitic protists that have not been thoroughly studied). Given that we do not yet know whether chromerids are free-living or have a parasitic and/or symbiotic phase (9, 58, 59), conclusions about organellar changes in the course of development would be premature.

Deep sequencing of the *Chromera* mt genome using 454 and Illumina platforms revealed the presence of just two protein-coding genes (*cox1* and *cox3*) and fragmented mito-rRNAs, qualifying it as the smallest mt genome known, when the coding capacity is considered (20). Whereas *cox1* is well conserved, *cox3*, which is always fused with *cox1*, was initially overlooked owing to little similarity with its homologs (62). In fact, the extremely divergent *cox3* in *Chromera*, which was not found by a BLAST search, questions the unique absence of this gene from the organellar genome of related *Tetrahymena* (5). Because there is no evidence of a mitochondrial targeted homolog in its nuclear genome (15), it is reasonable to speculate that in *Tetrahymena*, *cox3* may have been overlooked because of possible extreme divergence.

Treating the mt DNA-enriched fraction with DNA topoisomerases caused no detectable changes in Southern blots hybridized with *cox1*, and only linear DNA molecules were detected by transmission electron microscopy (20). Therefore, one can conclude that just as in apicomplexans and dinoflagellates (55, 72, 78), the mt genome of *Chromera* comprises numerous short linear molecules that contain approximately 130 copies of either full-size or incomplete *cox1* in a highly variable sequence context (20).

Although it seems that all of the mt genome is transcribed, an unknown processing mechanism is responsible for a single full-length, mature *cox1* mRNA (20). Similarly, the quantitative PCR data indicate that the in-frame *cox1*::*cox3* fusion in *Chromera* is cleaved posttranscriptionally, making the translation of this gene fusion unlikely. This prediction may be extended to the *cox3*::*coB* fusion in *Oxyrrhis marina* (72), although because of the split *cox3* gene in several dinoflagellates, the existence of splicing machinery in their mitochondrion cannot be excluded (71). The selective elimination of transcripts containing incomplete versions of the *Chromera cox1* gene, as monitored by Northern blot analysis, is virtually identical to what has been reported of dinoflagellates (30). Still, we cannot

imagine what mechanism is capable of distinguishing between partial and full-length transcripts in the absence of conserved and reasonably long untranslated regions.

Another feature shared by *Chromera*, *Vitrella*, and dinoflagellates is the oligoadenylation of short mito-rRNA fragments that do not seem to be spliced together (78). The lack of RNA editing in both species correlates with the absence of this posttranscriptional mechanism in the early-branching dinoflagellates. However, the rather complex and nonoverlapping editing patterns imply multiple emergences of RNA editing in the mitochondrion of alveolates (30). Unexpectedly, the *coB* gene was lost by *Chromera* alone, along with its respective complex (see below), yet was retained in the organellar genome of *Vitrella* (20), its closest known phototrophic relative (58). Newly available information from the nucleus is in excellent correlation with this finding, as the nuclear DNA-encoded subunits of respiratory complex III are missing from *Chromera*, whereas this complex is present in *Vitrella* (20). Hence, both algae are predicted to have a strikingly different mt metabolism (**Figure 2**; see *Chromera*'s Unique Respiratory Chain, below).

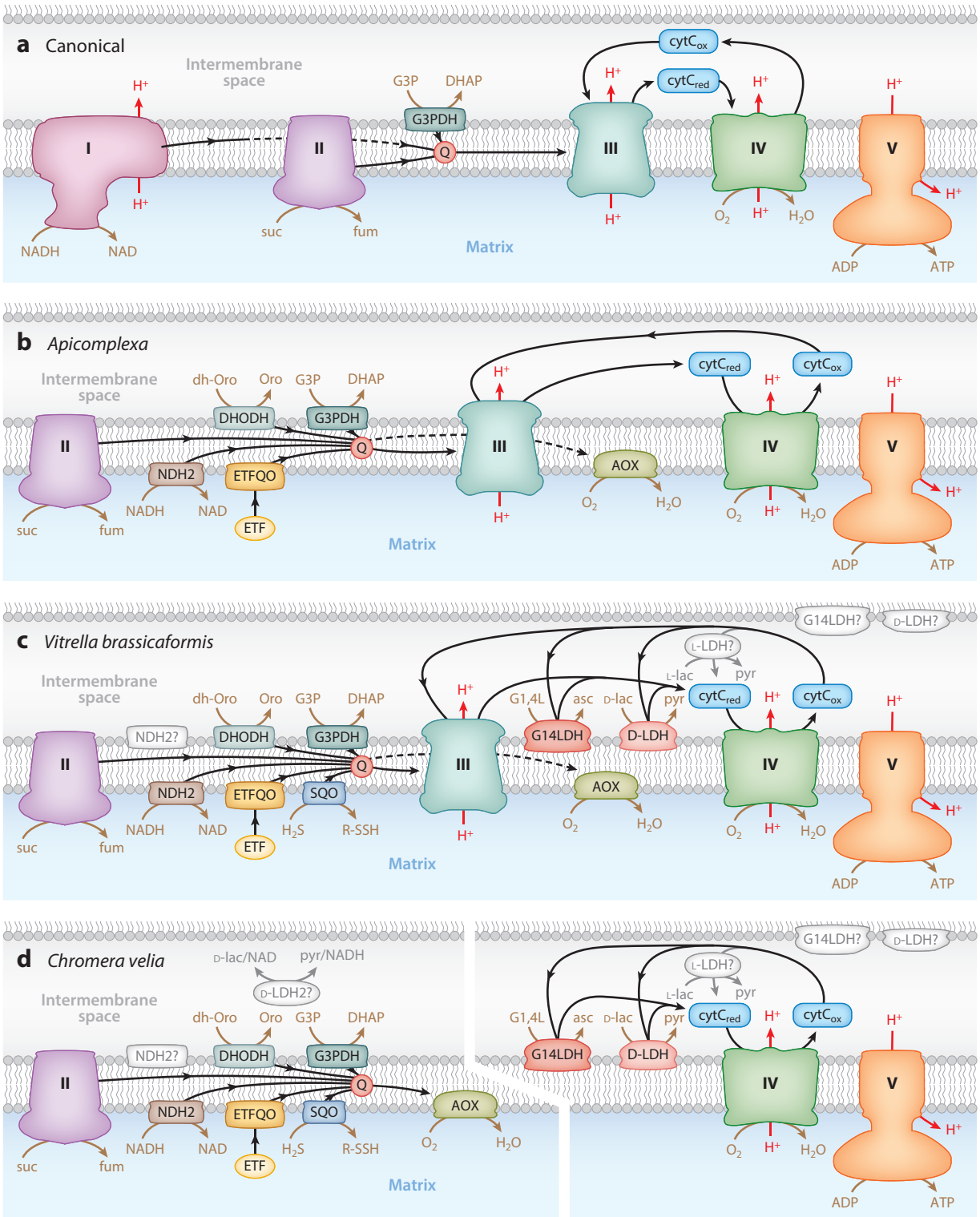
CHROMERA'S UNIQUE RESPIRATORY CHAIN

With its canonical composition of five respiratory complexes, the electron transport chain belongs to the most conserved metabolic pathways. Except for its quite frequent modifications or complete losses by anaerobic eukaryotes (54), the electron transport chain is only very rarely altered in aerobes. In those cases, either complex I (NADH dehydrogenase) is replaced by alternative dehydrogenase(s) (49, 76), or complexes III (cytochrome *c* reductase) and IV (cytochrome *c* oxidase) are concurrently lost (54, 63). Given that the subunits of complexes II (membrane-bound succinate dehydrogenase), IV, and V (ATP synthase)—encoded by mt DNA and/or nuclear DNA—are well conserved in the aerobic mitochondrion of *Chromera*, the available data strongly suggest that complexes I and III are absent from both its mitochondrial and its nuclear genomes (20).

Because *Chromera* lacks complex III, its electron transport chain is unique in that it is divided into two independently operating subchains, and the composition and location of the associated metabolic pathways are also unique (**Figure 2**). The initial subchain includes alternative NADH dehydrogenases, which substitute for the electron transport function of the lost complex I. Electrons from alternative NADH dehydrogenases, complex II, ETF:ubiquinone oxidoreductase, and other donors are channeled to ubiquinone, which passes them to the alternative oxidase. Therefore, the electron transport chain is, in effect, broken into an abortive initial part, with alternative oxidases acting as a universal electron sink (**Figure 2**). The rest of the chain operates independently,

Figure 2

Various arrangements of respiratory chains in aerobic mitochondria of myzozoans. (*a*) The canonical respiratory chain and its modified versions in (*b*) apicomplexans, (*c*) *Vitrella brassicaformis*, and (*d*) *Chromera velia*. Protein localization is shown based on predicted targeting and other available data. Proteins with alternative or uncertain localization are shown in gray with question marks. (*a*) In the canonical respiratory chain, ubiquinone (Q) accepts electrons from complex I (NADH dehydrogenase) and complex II (succinate dehydrogenase) and passes them to complex III (cytochrome *c* reductase). (*b*) In apicomplexans, complex II, alternative NADH dehydrogenase (NDH2), glycerol 3-phosphate dehydrogenase (G3PDH), and electron transfer flavoprotein:ubiquinone oxidoreductase (ETFQO) donate electrons to ubiquinone, which passes them to alternative terminal oxidase (AOX) and complex III. (*c*) The respiratory chain of *V. brassicaformis* is continuous, with alternative ubiquinone reductases, D-lactate:cytochrome *c* oxidoreductase (D-LDH), L-lactate:cytochrome *c* oxidoreductase (L-LDH, also known as cytochrome b_2), sulfide:ubiquinone oxidoreductase (SQO), dihydroorotate dehydrogenase (DHODH), and L-galactono-1,4-lactone dehydrogenase (G14LDH) also present. However, NADH-dependent D-lactate dehydrogenase (D-LDH2) is absent and AOX lacks a robust mitochondrial import signal. The complexes are not depicted to scale. (*d*) In *Chromera velia*, the respiratory chain is split into two disconnected parts, with D-LDH, L-LDH, and G14LDH donating electrons to soluble cytochrome *c*, which is oxidized by complex IV (cytochrome *c* oxidase). DLDH2 probably recycles pyruvate, generated by the cytochrome *c*–dependent D-LDH and L-LDH, into lactate and consumes NADH.


a Canonical
Intermembrane space
H+
G3P
DHAP
G3PDH
Q
I
II
III
IV
V
cytC_ox
cytC_red
NADH
NAD
suc
fum
Matrix
O2
H2O
ADP
ATP
b Apicomplexa
dh-Oro
Oro
DHODH
NDH2
ETFQO
ETF
AOX
c Vitrella brassicaformis
G14LDH?
D-LDH?
L-LDH?
L-lac
pyr
G1,4L
asc
D-lac
G14LDH
D-LDH
NDH2?
SQO
H2S
R-SSH
d Chromera velia
D-lac/NAD
pyr/NADH
D-LDH2?

with complex IV being the only one that is able to pump protons into the mt intermembrane space. Orthologs of both L- and D-lactate:cytochrome *c* oxidoreductases could mediate the transfer of electrons unidirectionally from lactate to cytochrome *c*, bypassing the missing complex III. Regeneration of lactate from pyruvate is probably accomplished by a bidirectional NADH-dependent D-lactate dehydrogenase, which among eukaryotes has otherwise only been found in diatoms, haptophytes, and chlorarachniophytes. In contrast to *Chromera*, *Vitrella* has a respiratory chain that is similar to that of apicomplexans (**Figures 2** and **3**); however, it also contains L- and D-lactate:cytochrome *c* oxidoreductases, although not the bidirectional NADH-dependent D-lactate dehydrogenase (20).

In addition to this novel shuttle and unusually altered respiratory chain, *Chromera* and *Vitrella* possess an extensive set of enzymes that are typical of anaerobic protists. The most prominent among them are a complete set of [FeFe]-hydrogenase maturases and a hydrogenase, pyruvate:NADP oxidoreductase, pyruvate:ferredoxin oxidoreductase, pyruvate-formate lyase, NADH oxidase, acetaldehyde/alcohol dehydrogenase, acetate:succinate CoA-transferase, and acetyl-CoA synthetase (20). Mitochondria of aerobes such as the excavate *Naegleria gruberi* and the green alga *Chlamydomonas reinhardtii* are being found to have greater metabolic versatility than previously appreciated; they contain enzymes previously thought to be confined to the hydrogenosomes (23, 54). Although the full genome sequence will provide more definite answers, based on what can be predicted of its metabolism we speculate that *Chromera* belongs among the trophically extremely versatile protists and is capable of phototrophy, aerobic and strictly anaerobic osmotrophy, and maybe even predation, if the mysterious chromerosome organelle is implied (57, 58).

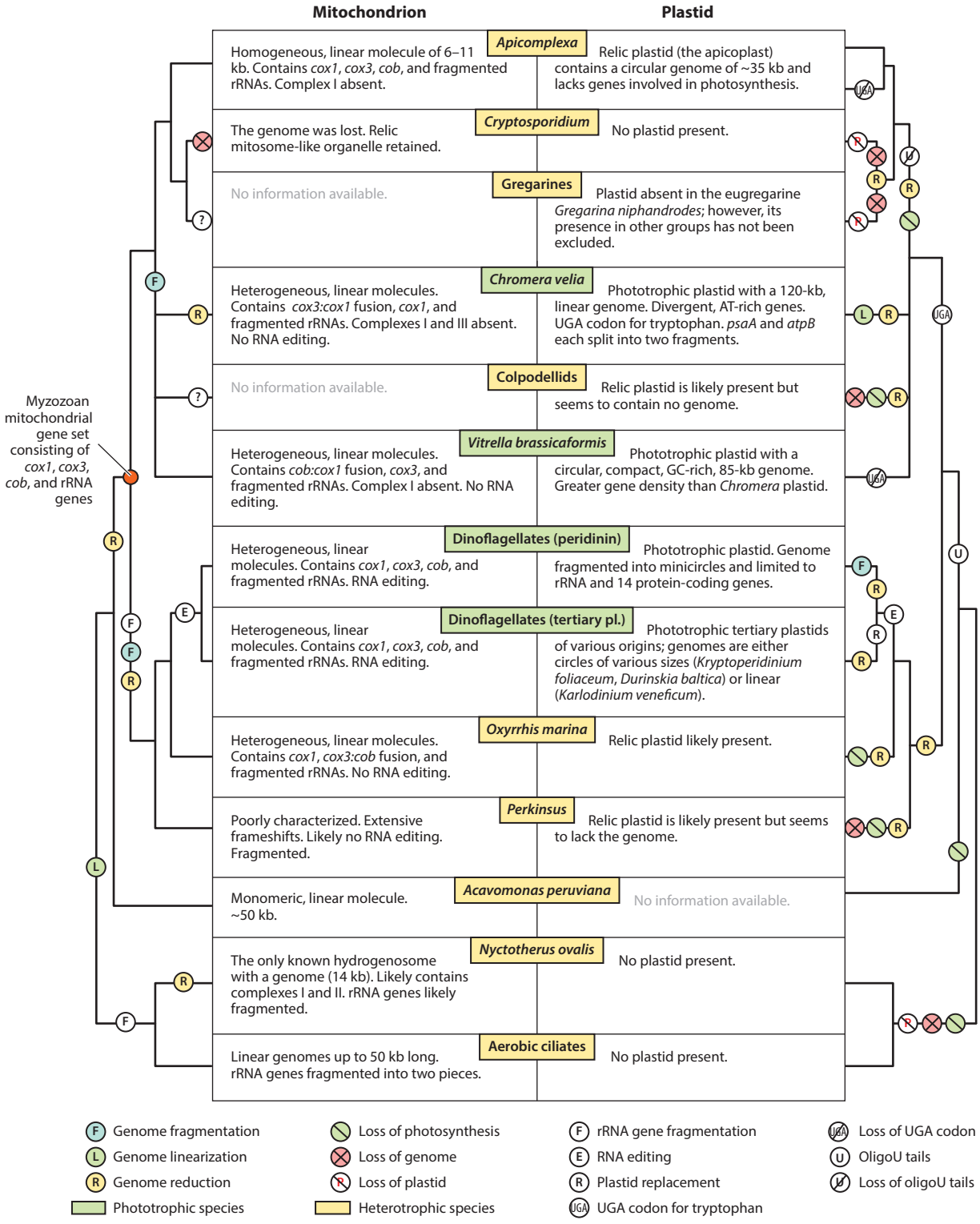
No methods have been successfully developed to isolate particular life stages from *Chromera* and *Vitrella* cultures, preventing thorough investigation of potentially substantial differences between stages on the organellar level; currently, morphology is the only source of evidence. Similarly, with one exception (61), all reported attempts to isolate intact plastids and mitochondria from *Chromera* and *Vitrella* have been unsuccessful. The main hurdle, which will have to be overcome, is the exceptional thickness of the multilayered cell wall, which—especially in *Vitrella*—easily resists even the highest settings of a French press. The only exception is the motile biflagellated zoospore stage, which has a rather thin cell wall. Although these cells can be cultured in sufficient amounts, they encyst under the conditions necessary for purification. It is possible to increase the percentage of these zoospores in a culture (27), but doing so on a large scale and consistently is challenging. Stimulation of the growth of zoospores seems to depend heavily on the health and age of the culture, along with the cell density. The few attempts to genetically modify *Chromera* have failed. Therefore, methodological advancement will be needed to lift the evolutionarily extremely interesting chromerids among model alveolates. This will allow us to experimentally address exciting predictions—for example, that the existence of some rRNA fragments in dinoflagellates and likely also *Chromera* and *Vitrella* is compatible with the existence of more than a single ribosome class (30).

PLASTIDS ON THE ROAD TO OBLIVION

Although the majority of myzozoans are heterotrophs (perkinsids, apicomplexans, colpodellids, and some dinoflagellates), the group has an amazing diversity of plastids. In particular, dinoflagellates are known to frequently replace their original secondary peridinin-pigmented organelle with

Figure 3

Evolution of mitochondrial and plastid genomes in alveolates.


Mitochondrion
Plastid
Apicomplexa
Homogeneous, linear molecule of 6–11 kb. Contains cox1, cox3, cob, and fragmented rRNAs. Complex I absent.
Relic plastid (the apicoplast) contains a circular genome of ~35 kb and lacks genes involved in photosynthesis.
Cryptosporidium
The genome was lost. Relic mitosome-like organelle retained.
No plastid present.
Gregarines
No information available.
Plastid absent in the eugregarine Gregarina niphandrodes; however, its presence in other groups has not been excluded.
Chromera velia
Heterogeneous, linear molecules. Contains cox3:cox1 fusion, cox1, and fragmented rRNAs. Complexes I and III absent. No RNA editing.
Phototrophic plastid with a 120-kb, linear genome. Divergent, AT-rich genes. UGA codon for tryptophan. psaA and atpB each split into two fragments.
Colpodellids
No information available.
Relic plastid is likely present but seems to contain no genome.
Vitrella brassicaformis
Heterogeneous, linear molecules. Contains cob:cox1 fusion, cox3, and fragmented rRNAs. Complex I absent. No RNA editing.
Phototrophic plastid with a circular, compact, GC-rich, 85-kb genome. Greater gene density than Chromera plastid.
Dinoflagellates (peridinin)
Heterogeneous, linear molecules. Contains cox1, cox3, cob, and fragmented rRNAs. RNA editing.
Phototrophic plastid. Genome fragmented into minicircles and limited to rRNA and 14 protein-coding genes.
Dinoflagellates (tertiary pl.)
Heterogeneous, linear molecules. Contains cox1, cox3, cob, and fragmented rRNAs. RNA editing.
Phototrophic tertiary plastids of various origins; genomes are either circles of various sizes (Kryptoperidinium foliaceum, Durinskia baltica) or linear (Karlodinium veneficum).
Oxyrrhis marina
Heterogeneous, linear molecules. Contains cox1, cox3:cob fusion, and fragmented rRNAs. No RNA editing.
Relic plastid likely present.
Perkinsus
Poorly characterized. Extensive frameshifts. Likely no RNA editing. Fragmented.
Relic plastid is likely present but seems to lack the genome.
Acavomonas peruviana
Monomeric, linear molecule. ~50 kb.
No information available.
Nyctotherus ovalis
The only known hydrogenosome with a genome (14 kb). Likely contains complexes I and II. rRNA genes likely fragmented.
No plastid present.
Aerobic ciliates
Linear genomes up to 50 kb long. rRNA genes fragmented into two pieces.
No plastid present.
Myzozoan mitochondrial gene set consisting of cox1, cox3, cob, and rRNA genes
Genome fragmentation
Genome linearization
Genome reduction
Phototrophic species
Loss of photosynthesis
Loss of genome
Loss of plastid
Heterotrophic species
rRNA gene fragmentation
RNA editing
Plastid replacement
UGA codon for tryptophan
Loss of UGA codon
OligoU tails
Loss of oligoU tails

a tertiary acquired plastid (26, 36, 38, 51, 56). Moreover, even heterotrophic myzozoan lineages, such as colpodellids and perkinsids, likely contain nonphotosynthetic relic plastids (22, 66). The origin of the apicoplast was first addressed via comparative analyses with dinoflagellates as the closest phototrophic relatives of apicomplexans. However, with the exception of highly variable rRNA genes (84) their genomes turned out to be useless for evolutionary comparison, mainly because of nonoverlapping gene sets (37). Given that the *Chromera* and *Vitrella* plastids contain both housekeeping and photosynthesis-related genes, their genomes could reliably be compared with that of the apicoplast, which reveals its rhodophyte ancestry (31). The structure of the super-operon in particular strongly supports a common ancestry of plastids in apicomplexans, *Chromera*, *Vitrella*, dinoflagellates, and heterokont algae (**Figures 1** and **3**) (31, 32).

Moreover, a nonphylogenetic support of a common origin of Apicomplexa and *Chromera* came from the analysis of their heme pathways. Apicomplexans are known to synthesize aminolevulinic acid (ALA) in the mitochondrion via the so-called C4 pathway, yet the synthesis in apicomplexan parasites is unique in that it continues in the plastid via enzymes related to their phototrophic rather than heterotrophic counterparts (75). The last step of the synthesis catalyzed by ferrochelatase occurs in the mitochondrion (40, 65, 75). *Chromera*, although a phototroph, synthesizes ALA by the C4 pathway in the mitochondrion, just as the apicomplexans and all other eukaryotic heterotrophs (41). Given that ALA synthase, an enzyme of the heterotrophic C4 pathway, and enzymes of the plastid heme route were recently found in the colpodellid *Voromonas pontica* (22), this metabolic peculiarity likely predates the split of Apicomplexa, colpodellids, *Chromera*, and *Vitrella*. At the same time, thanks to this peculiarity *Chromera* qualifies as the only phototroph synthesizing chlorophyll from glycine instead of glutamate (41).

Although *Chromera* and *Vitrella* are the only known phototrophs closely related to apicomplexan parasites, their plastids differ to a surprising extent (31, 32). The same applies to their nuclear genomes (Y.H. Woo, C. Bowler, P.J. Keeling, D.S. Roos, J.B. Dacks, T.J. Templeton, R.F. Waller, J. Lukeš, M. Oborník & A. Pain, unpublished data). Phylogeny inferred from the nuclear 18S rRNA gene indicates that these algae very likely represent independent phototrophic lineages of colpodellids, with *Chromera* branching on the root of the *Voromonas-Colpodella* clade, whereas *Vitrella* seems to be related to *Alphamonas* (22). In fact, *Chromera* and *Vitrella* are quite different both at the level visible by light microscopy and at the ultrastructural level, their life cycles differ, and the former alga grows much faster than the latter (58, 59). Although both species lack chlorophyll *c*, they display relatively diverse pigment compositions: The plastid of *Chromera* is pigmented with chlorophyll *a*; violaxanthin; a novel type of isofucoxanthin; and β,β-carotene, which is a minor component (53). In contrast, the plastid of *Vitrella* lacks isofucoxanthin and possesses vaucheriaxanthin; thus, it has virtually the same pigment composition as the *Nannochloropsis* plastid (58). Moreover, *Vitrella* contains a morphologically conspicuous pyrenoid visible even by light microscopy, whereas a similar structure is lacking in *Chromera* (59).

When compared with the genomes of the other phototrophic plastids, the plastid genome of *Chromera* appears to be exceptionally modified. It is a linear molecule with inverted repeats of *orf264*, *psbA*, and *atpH2* at the ends, whereas the inverted repeats of rRNA genes found in the canonical plastids are missing (31). Indeed, numerous attempts to circularize this plastid genome using the genomic data and to complete the circle by various PCR-based approaches failed. The linear conformation is also apparent when DNA from intact cells is separated by pulsed-field gel electrophoresis and hybridized with the *psbA* probe. Although a major signal migrated at approximately 120 kb (as specified by a linear DNA marker), an extensive diffuse signal was also recorded between 50 kb and 5 kb (31). To explain this observation, one has to assume the presence of various circular forms or genome fragments; however, no such fragments were found by sequencing. This contrasts with the usually circular plastid genomes, where linear molecules seem

to comprise only replication intermediates (67, 69, 81), although the tertiary plastid genome of the dinoflagellate *Karlodinium veneficum* is also linear and reduced (21). Its gene set overlaps a great deal with that of the *Chromera* plastid, yet it is missing the inverted terminal repeats. Such structural similarity likely resulted from convergent evolution in plastids of various alveolate lineages (21). However, it may also indicate that the *Chromera* plastid, which contains an unexpectedly large and diverse set of genes as well as their fragments and pseudogenes, is of tertiary instead of secondary origin. The generally conserved genes *psaA* and *atpB* are each split into two fragments, a unique characteristic that produces separate transcripts and consequently separate proteins that assemble into a functional photosystem and ATP synthase (32).

Chromera uses a noncanonical code for tryptophan (UGA instead of UGG) to translate some of the plastid-DNA-encoded transcripts. This feature is exceedingly rare among phototrophic plastids and has so far been found only in various mitochondria and nonphotosynthetic plastids of coccidians, such as *Toxoplasma* and *Neospora* (44, 53). However, in both *Chromera* and coccidians, the UGA triplet specifies tryptophan in only some proteins, and the fraction of such noncanonically encoded proteins substantially varies among species (53). Interestingly, this coding is absent from the plastid genome of *Vitrella* and apicomplexans other than coccidians (**Figure 3**) (31).

Several mature mRNAs derived from the primary polycistronic transcripts are oligouridylylated at their 3′ end, a characteristic that likely has a function in their processing (**Figure 3**) (32). Furthermore, the oligoU tails are preferentially attached to transcripts encoding proteins involved in photosynthetic electron transfer. Because transcripts are not oligouridylylated in the apicoplast of *Plasmodium*, an intriguing link between the oligoU-tailed transcripts of phototrophic genes in *Chromera* and the loss of photosynthesis in Apicomplexa was recently proposed (13). Despite a greatly mutated plastid genome (31) and a reduced set of photosystem proteins (H. Esson, A. Horák, P. Dufková, R. Sobotka, P. Komenda & M. Oborník, unpublished data), *Chromera* is highly efficient at photosynthesis—likely because it can acclimatize to different light conditions (3, 42, 43, 48, 64) and because nonphotochemical quenching protects it very effectively from excessive radiation (43, 48). The main light-harvesting complex of *Chromera* contains chlorophyll *a*, violaxanthin, and a yet-unidentified carbonyl isofucoxanthin–related carotenoid, and its energy transfer from carotenoid to chlorophyll *a* is more efficient than any seen among the investigated FCP (fucoxanthin chlorophyll protein)-like proteins (14). Phylogenetic analysis of light-harvesting complexes revealed 23 homologs in *Chromera*, with 17 of them forming a compact clade related to dinoflagellates and diatoms. Three of the investigated proteins are phylogenetically positioned near rhodophytes (60).

In contrast to that of *Chromera*, the plastid genome of *Vitrella* is a compact, 85-kb circle with high gene density and canonical organization. In fact, it contains more genes than the substantially bigger (120 kb) plastid genome of *Chromera*. Whereas the *Vitrella* plastid is unexpectedly GC rich (47.7% of GC), there is a preponderance of AT base pairs (36% of GC) in its homolog in *Chromera*. Unlike dinoflagellates (10, 79, 82), *Chromera* and *Vitrella* seem to lack RNA editing in their plastid-encoded transcripts (32).

CONCLUDING REMARKS

The closest known phototrophic relatives of Apicomplexa, *Chromera* and *Vitrella*, have organelles with extensively reduced genomes. The mt genome of *Chromera* contains (in addition to fragmented rRNA genes) only *cox1* and *cox3* and has lost *coB*, which is present in *Vitrella*, apicomplexans, and dinoflagellates, together with the entire respiratory complex III. In the same vein, plastid genomes substantially differ between *Chromera* and *Vitrella*: The former is linear with low gene density, whereas the latter forms a compact circle with canonical organization. Given that

pervasive organellar genome reduction occurs in both free-living, phototrophic species and the apicomplexan parasites, it is apparent that this streamlining was not caused by parasitism. We therefore postulate that it was primarily the reduction of the plastid genome, which triggered secondary heterotrophy and parasitism in myzozoans. It also seems that with respect to colpodellids, *Chromera* and *Vitrella* represent quite distant photosynthetic lineages, suggesting photosynthesis was lost multiple times within this group. From the evolutionary perspective, it appears that even an alveolate ancestor possessed a linear mt genome that was independently reduced in myzozoans and *Nyctotherus* (**Figure 3**). All myzozoans contain a tiny set of three protein-coding genes that was further reduced to just two in *Chromera* and that was lost altogether in *Cryptosporidium*. The mt genome has been independently fragmented at least twice: in colpodellids, including *Chromera* and *Vitrella*, and in dinoflagellates and related lineages. No traces of a plastid were found in the sister group of ciliates, and no information is available supporting its presence in *Acavomonas* (33). Yet the other myzozoans seem to contain either a photosynthetic or a relic plastid, the genome of which has been fragmented in the peridinin-containing dinoflagellates, linearized in *Chromera*, and likely lost in *Perkinsus*.

One can conclude that in the alveolates, organellar genomes are either subject to extreme reduction or undergoing modifications to an extent never seen in any other eukaryotes. Whether this is a sign of their coming demise or just a tinkering stimulated by often extreme environments inhabited by highly ecologically significant organisms that carry them will be the subject of further research.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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