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REVIEW

CONTRASTING MITOCHONDRIAL GENOME ORGANIZATIONS AND SEQUENCE AFFILIATIONS AMONG GREEN ALGAE: POTENTIAL FACTORS, MECHANISMS, AND EVOLUTIONARY SCENARIOS[1]

Aurora M. Nedelcu[2]

Department of Biology, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4J1

ABSTRACT

The three green algal mitochondrial genomes completely sequenced to date—those of Chlamydomonas reinhardtii *Dangeard,* Chlamydomonas eugametos *Gerloff,* and Prototheca wickerhamii *Soneda & Tubaki—revealed very different mitochondrial genome organizations and sequence affiliations. The* Chlamydomonas *genomes resemble the ciliate/fungal/animal counterparts, and the* Prototheca *genome resembles land plant homologues. This review points out that all the green algal mitochondrial genomes examined to date resemble either the* Chlamydomonas *or the* Prototheca *mitochondrial genome; the* Chlamydomonas*-like mitochondrial genomes are small and have a reduced gene content (no ribosomal protein or 5S rRNA genes and only a few protein-coding and tRNA genes) and fragmented and scrambled rRNA coding regions, whereas the* Prototheca*-like mitochondrial genomes are larger and have a larger set of protein-coding genes (including ribosomal protein genes), more tRNA genes, and 5S rRNA and conventional continuous small-subunit (SSU) and large-subunit (LSU) rRNA coding regions. It appears, therefore, that the differences previously observed between the mitochondrial genomes of* C. reinhardtii *and* P. wickerhamii *extend to the two green algal mitochondrial lineages to which they belong and are significant enough to raise questions about the causes and mechanisms responsible for such contrasting evolutionary strategies among green algae. This review suggests an integrative approach in explaining the occurrence of distinct evolutionary strategies and apparent phylogenetic affiliations among the known green algal mitochondrial lineages. The observed differences could be the result of distinct genetic potentials differentiated during the previous evolutionary history of the flagellate ancestors and/or of subsequent changes in habitat and life history of the more advanced green algal lineages.*

Key index words: Chlamydomonas; Prototheca; *green algae; mitochondrial genome origin and evolution*

The eukaryotic cell is an associative system comprising at least two or three main subsystems with different evolutionary histories (Margulis 1981, Gray 1992). Well accepted now is the eubacterial (alphaproteobacterial and cyanobacterial, respectively) endosymbiotic origin of two of the eukaryotic cell's organelles, namely, the mitochondria and the plastids, although their mono- or polyphyletic origin is still debated (Dayhoff and Schwartz 1981, Stewart and Mattox 1984, Gray et al. 1989, Lockhart et al. 1992, Morden et al. 1992).

Single endosymbiotic events accounting for the origin of mitochondria and plastids, respectively, would imply that some common ancestral characters should be present in all the extant lineages and that distinct derived traits should be developed within and shared among related lineages. In addition, monophyletic origins for the mitochondria and plastids would require that phylogenies based on organellar traits be consistent with those based on nuclear or nucleus-encoded features; in other words, all the compartments within an eukaryotic cell should resemble their corresponding counterparts in the same compared lineage.

However, there are reports of lineages in which the organelles and the nucleo-cytosolic compartment do not display the same phylogenetic affiliations, specifically, the plastids of the cryptomonads (Douglas 1992, 1994, McFadden and Gilson 1995) and the protist *Euglena gracilis* Klebs (Gibbs 1978, 1981, Morden et al. 1992) and the mitochondria of land plants (Gray 1989), the protozoan *Acanthamoeba castellanii* (Lonergan and Gray 1994), and the green alga *Chlamydomonas reinhardtii* Dangeard (Gray 1992). The question to be addressed in such cases is: Are these examples of incongruence between the nuclear and organelle phylogenies the result of very divergent evolutionary strategies among closely related lineages or, rather, of different evolutionary origins?

An interesting case with which to address these types of evolutionary questions is represented by the green algae. The only three green algal mitochondrial genomes completely sequenced to date—namely, those of *C. reinhardtii* (Boer and Gray 1988a, b, c, Gray and Boer 1988, Michaelis et al. 1990), *Chlamydomonas eugametos* Gerloff (Denovan-Wright et al. 1998), and *Prototheca wickerhamii* Soneda & Tubaki (Wolff et al. 1994) revealed very different mitochondrial genome organizations and sequence affiliations. The mitochondrial genomes of the two *Chlamydomonas* taxa on the one hand and that of *Prototheca* on the other resemble more the ciliate/fungal/animal and land plant mitochondrial types,

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[2] Author for reprint request; e-mail amned@is2.dal.ca.

respectively, than one another. In other words, there is an unexpected incongruence between the phylogenetic relationships suggested by the nucleocytosolic compartments of these two green algal lineages and those suggested by their mitochondria.

The phylogeny of the green algal group is progressively being deciphered, and the new information gathered through molecular approaches will probably trigger the reconsideration of the traditional green algal systematics (Chapman and Buchheim 1991, Friedl 1995). *Chlamydomonas* and *P. wickerhamii* are traditionally considered members of different orders (the Chlamydomonadales and the Chlorococcales, respectively) that belong to the same class (i.e. the Chlorophyceae; sensu Mattox and Stewart 1984). However, phylogenies based on nuclear rDNA sequences (Wilcox et al. 1992, Steinkötter et al. 1994, Friedl 1995) suggest that although some members of the Chlorococcales, such as *Scenedesmus obliquus* (Turp.) Kutz, do indeed affiliate with chlorophycean taxa, other members, including *P. wickerhamii,* form a monophyletic group with advanced lineages of the Pleurastrophyceae class (sensu Mattox and Stewart 1984). This latter group was recently defined by Friedl (1995) as a new class: the Trebouxiophyceae. In this context, it appears that *Chlamydomonas* and *Prototheca,* the two green algal lineages whose mitochondrial genomes appear unexpectedly different, may not be as closely related as previously thought; in fact, they are members of two distinct green algal evolutionary lineages, namely, the chlorophycean and the trebouxiophycean (sensu Friedl 1995), whose divergence is probably very old.

Although the phylogenetic relationships among green algal lineages are not fully deciphered, both *Chlamydomonas* and *Prototheca* are unquestionably green algae (as suggested by ultrastructural, biochemical, and molecular data from both the nucleocytosolic and the chloroplast compartments; Devereux et al. 1990, Chapman and Buchheim 1992, Morden et al. 1992), and green algae are the closest relatives of land plants (McCourt 1995). However, in an archaebacterial-eubacterial-chloroplast-mitochondrial-nuclear phylogenetic tree inferred from small-subunit (SSU) rDNA sequence data, the *C. reinhardtii* nuclear and mitochondrial sequences occupied different positions relative to land plants (Gray et al. 1989). In the nuclear subtree, *C. reinhardtii* formed a clade with the plant sequences (as it did also in the chloroplast subtree) and branched off at about the same point as animals and fungi. In contrast, in the mitochondrial subtree, *C. reinhardtii* branched with the ciliate/fungal/animal sequences, far away from higher plants, which clustered very near the root, close to the alpha-proteobacterial clade. The affiliation of the nuclear SSU rDNA sequences of higher plants and *C. reinhardtii* was seen as consistent with traditional phylogenies that consider green algae the closest relatives of land plants (Ragan and Chapman 1978, Chapman and Buchheim 1991), whereas the green algal/land plant dichotomy in the mitochondrial tree was interpreted as an anomaly. However, this anomaly in branching topology was attributed to the plant rather than *C. reinhardtii* mitochondrial sequences and was shown not to be a consequence of differential rate of sequence divergence. Moreover, the authors indicated that plant mitochondrial rRNAs resemble more their eubacterial/chloroplast counterparts than they do their homologues in other mitochondria (Gray et al. 1989).

To explain the different branching position of plants within the nuclear and mitochondrial lineages, respectively, and to account for the strong eubacterial features of their mitochondrial rRNAs, Gray et al. (1989) suggested two possibilities: either 1) the mitochondrial rRNA genes of plants have diverged relatively little from the rRNA genes of the ancient eubacterial ancestor of all mitochondria (monophyletic origin) or 2) the higher plant mitochondrial rRNA genes or the mitochondria itself have been acquired more recently than those of other eukaryotic lineages (biphyletic origin). In addition, because of the very different way in which genes are organized and expressed in the *C. reinhardtii* and plant mitochondrial genomes, the authors concluded that there is no indication that the two shared a common mitochondrial ancestor as recently as they shared a common nuclear (or chloroplast) ancestor.

The input of other green algal mitochondrial rDNA sequences, namely, of *P. wickerhamii* (Wolff and Kück 1990, Wolff et al. 1993) and *C. eugametos* (Denovan-Wright et al. 1996) did not contribute to the dissolution of the land plant/nonplant split observed in the mitochondrial rRNA trees (Gray et al. 1989), nor did these sequences resolve *Chlamydomonas* and *P. wickerhamii* as a green algal clade. However, the fact that the *Prototheca* sequences are relatively AT rich and rapidly evolving in comparison to their land plant counterparts was considered to possibly account for this green algal taxon branching apart from land plants (Gray 1995). On the other hand, although the number of transitional substitutions is probably saturated in the relatively rapidly evolving mitochondrial rRNA sequences, it was suggested that the apparent affiliation of the *Chlamydomonas* sequences with ciliate/fungal/yeast counterparts and therefore their separation from the land plant sequences is not due to a ''long-branch length attract'' artifact (Denovan-Wright et al. 1996).

However, phylogenetic trees based on COX1 amino acid sequences indicated that the plant and green algal mitochondrial lineage, including *P. wickerhamii* (Wolff et al. 1993) and the prasinophyte *Platymonas* (*Tetraselmis*) *subcordiformis* (Wille) Hazen (Kessler and Zetsche 1995), do form a monophyletic group. Therefore, the expected congruency of nu-

TABLE 1. *Mitochondrial genome size, map (structure), gene content, and GC composition among green algal lineages. Cr* = Chlamydomonas reinhardtii, *Cp* = Chlamydomonas pitschmanii, *Cs* = Chlamydomonas smithii, *Pm* = Pandorina morum, *Ce* = Chlamydomonas eugametos, *Cm* = Chlamydomonas moewusii, *Pa* = Polytomella agilis, *Ps* = Platymonas subcordiformis, *So* = Scenedesmus obliquus, *Pw* = Prototheca wickerhamii, *C-l* = Chlorella-*like ex-symbiont, Cpy* = Chlorella pyrenoidosa *(circ = circular, lin = linear).*

Mitochondrial traits	*Chlamydomonas*-like taxa								*Prototheca*-like taxa			
	Cr[a]	Cp[b]	Cs[c]	Pm[d]	Ce[e]	Cm[f]	Pa[g]	So[h]	Ps[i]	Pw[j]	C-l[k]	Cpy[l]
Size (kb)	15.7	16.5	17	20	22.9	22		45	42.8	55.3	76	80
Genome map	lin	circ	lin	lin	circ	circ		circ	circ	circ	circ	circ
Gene content												
cob	+		+		+	+			+	+	+	
*cox*1	+		+		+	+	+	+	+	+	+	
*cox*2	−		−		−	−			+	+	+	
*cox*3	−		−		−	−			+	+		
*atp*1	−		−		−	−			+	+	+	
*atp*6	−		−		−	−			+	+	+	
*atp*9	−		−		−	−				+	+	
*nad*1	+		+		+	+				+		
*nad*2	+		+		+	+			+	+		
*nad*3	−		−		−	−			+	+		
*nad*4	+		+		+	+	+		+	+		
*nad*4L	−		−		−	−				+		
*nad*5	+		+		+	+			+	+		
*nad*6	+		+		+	+			+	+		
rnl	+		+	+	+	+	+	+	+	+	+	
rns	+		+		+			+	+	+	+	
rrn5	−		−		−	−				+		
trn	3				3				7	26		
rpl + *rps*	0		+		0				2	13		
Fragmented rRNAs	+		+	+	+	+	+	+	−	−		
GC content (%)	47.5				34.6				25.8	32.5	40.0	

[a] Grant and Chiang 1980, Michaelis et al. 1990.
[b] Boudreau and Turmel 1995.
[c] Boynton et al. 1987.
[d] Moore and Coleman 1989.
[e] Denovan-Wright et al. 1998.
[f] Lee et al. 1991.
[g] Antamarian et al. 1996.
[h] Kück 1989.
[i] Kessler and Zetsche 1995.
[j] Wolff et al. 1994.
[k] Waddle et al. 1990.
[l] Bayen and Rode 1973.

clear, plastid, and mitochondrial phylogenetic trees is verified in the case of trebouxiophycean (sensu Friedl 1995) and prasinophycean (sensu Moestrup and Throndsen 1988) green algae as well as land plant COX1 amino acid sequences but remains to be demonstrated for the chlamydomonadalean lineage, which branches with embryophytes in nuclear trees and with unrelated taxa in COX1 and rRNA mitochondrial trees (Gray et al. 1989, Wolff et al. 1993, Antamarian et al. 1996, Denovan-Wright et al. 1996). The constant affiliation between the *Chlamydomonas* and the ciliate/fungal/animal mitochondrial sequences was suggested to be consistent with a polyphyletic origin for the green algal mitochondria (Van de Peer et al. 1990, Wolff and Kück 1993).

The present review 1) analyzes the information available on green algal mitochondrial genomes, 2) compares the mitochondrial genomes of green algae to the land plant and nonplant counterparts, and 3) suggests factors, mechanisms, and evolutionary scenarios to explain the two very distinct evolutionary strategies undertaken by the green algal mitochondrial genomes.

TWO VERY DISTINCT TYPES AMONG THE KNOWN GREEN ALGAL MITOCHONDRIAL GENOMES

Information on green algal mitochondrial genomes, although limited and incomplete, suggests a rather great diversity in genome structure and organization among green algae. Current data indicate a fairly large range of genome sizes (from 15.7 kb in *C. reinhardtii* to 80 kb in *Chlorella pyrenoidosa* Shihira et Krauss) and both linear- and circular-mapping genomes (Table 1). In addition, the GC content varies from 25.8% in *P. wickerhamii* to 47.5% in *C. reinhardtii.* The gene content is very reduced (i.e. 13 genes) in *C. reinhardtii* (Michaelis et al. 1990) and *C. eugametos* (Denovan-Wright et al., in press) (Fig. 1) but much larger (i.e. 53 genes) in *P. wickerhamii* (Wolff et al. 1994) (Table 1). It is interesting that the genes coding for the subunits 2 and 3 of cytochrome *c* oxidase (*cox*2 and *cox*3) as well as

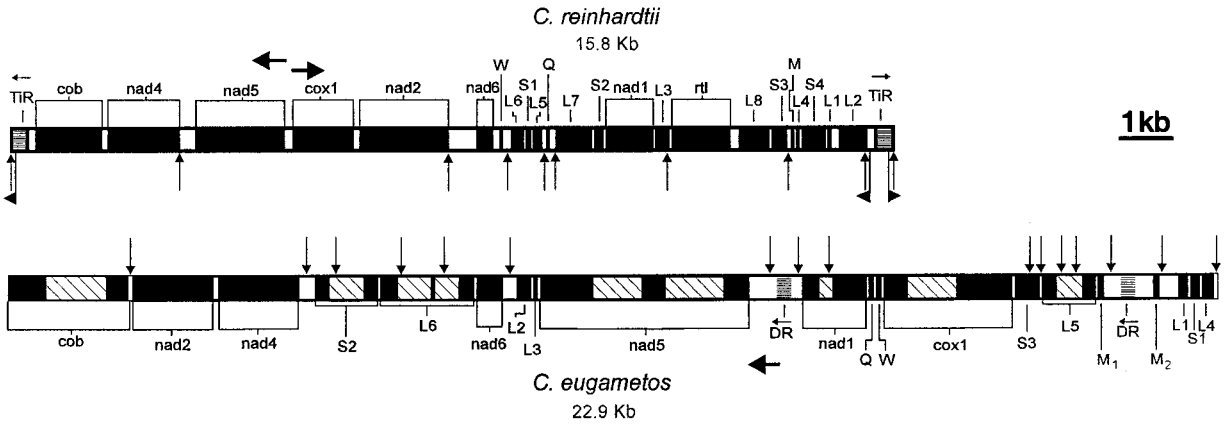

FIG. 1. Linear map and linearized map of the linear- and circular-mapping mtDNA of *Chlamydomonas reinhardtii* and *Chamydomonas eugametos,* respectively. S1–S4 and L1–L8 are SSU rRNA- and LSU rRNA-coding modules, respectively; *cob* and *cox*1 are coding regions for cytochrome *b* and subunit 1 of cytochrome oxidase, respectively; *nad*1, 2, 4, 5, and 6 are the genes coding for subunits 1, 2, 4, 5, and 6, respectively, of the NADH dehydrogenase; M_1, M_2, Q, and W are coding regions for $tRNA^{Met-1}$, $tRNA^{Met-2}$, $tRNA^{Gln}$, and $tRNA^{Trp}$, respectively; solid, cross-hatched, and open boxes denote coding regions, introns, and intergenic spacers, respectively; DR and TIR denote large direct and terminal inverted repeats, respectively; thick horizontal arrows indicate the direction of transcription; thin vertical arrows indicate the position of the short repetitive sequences; and flags indicate the position and orientation of small repeated sequences in the mtDNA of *C. reinhardtii.*

subunits 1, 6, and 9 of the ATP synthase complex (*atp*1, *atp*6, and *atp*9) are missing in the *C. reinhardtii* and *C. eugametos* mtDNA but are present in the mtDNA of *P. wickerhamii, P. subcordiformis,* and a *Chlorella*-like taxon (Michaelis et al. 1990, Waddle et al. 1990, Wolff et al. 1994, Kessler and Zetsche 1995, Denovan-Wright et al., 1998). Moreover, only three tRNAs are mitochondrially encoded in *C. reinhardtii* (Boer and Gray 1988c) and *C. eugametos* (Denovan-Wright et al. 1998), whereas 26 are coded by the *P. wickerhamii* mitochondrial genome (Wolff et al. 1994). The presence (or absence) of the mitochondrial 5S rRNA and ribosomal protein genes is an additional distinctive feature among green algal lineages; *P. wickerhamii* has both a 5S rRNA gene and many ribosomal protein-coding genes, *P. subcordiformis* has at least a few ribosomal protein genes, and *C. reinhardtii* and *C. eugametos* have neither ribosomal protein nor 5S rRNA genes (Michaelis et al. 1990, Wolff et al. 1994, Kessler and Zetsche 1995, Denovan-Wright et al. 1998).

It is noteworthy that the mitochondrial rRNA-coding regions are continuous in *P. wickerhamii* and *P. subcordiformis* (Wolff et al. 1993, Kessler and Zetsche 1995) but fragmented into coding modules that are scrambled along the genome in *C. reinhardtii, C. eugametos,* and *S. obliquus* (Boer and Gray 1988b, Denovan-Wright and Lee 1994, Nedelcu 1997a). However, the coding information and the order of the rRNA-coding modules are different between the *C. reinhardtii* and the *C. eugametos* mtDNAs (Fig. 1). Furthermore, in pairwise rDNA nucleotide sequence comparisons, the *P. wickerhamii* mitochondrial genes appear more related to their land plant homologues (84%–86% sequence identity) than do the *Chlamydomonas* counterparts (only 65% sequence identity) (Gray 1995). Similarly, pairwise comparisons of an incomplete sequence (about 400 nucleotides within the 3′-half of the mitochondrial large-subunit [LSU] rRNA) of another chlorophycean taxon, *S. obliquus,* show only 67% sequence identity of this algal sequence with the wheat or *P. wickerhamii* mitochondrial LSU rRNA counterparts (Gray 1995).

The analysis of the current data suggests that the known green algal mitochondrial genomes fall into two very distinct types: a *Chlamydomonas*-like genome and a *Prototheca*-like genome (Table 1). The *Chlamydomonas*-like mitochondrial genomes are small, have a reduced gene content (no ribosomal protein and 5S rRNA genes and only a few protein-coding and tRNA genes), and fragmented and scrambled rRNA-coding regions, whereas the *Prototheca*-like mitochondrial genomes are larger, have a larger set of protein-coding genes (including ribosomal protein genes), a higher number of tRNA genes as well as continuous conventional SSU and LSU rRNA-coding regions, and a 5S rRNA gene (*rns, rnl,* and *rrn*5, respectively). Features of mitochondrial genome organization (discussed earlier) as well as the presence of discontinuous mitochondrial rRNAs (Denovan-Wright et al. 1996, Nedelcu et al. 1996, Nedelcu 1997a) suggest that chlorophycean lineages—such as several *Chlamydomonas* taxa, *Pandorina morum* (Müller) Bory, *Polytomella agilis* Prings., *Carteria crucifera* Korsch, *Carteria olivieri* G. S. West, *Planophila terrestris* Groover & Hofstetter, *Hormotilopsis gelatinosa* Trainor & Bold, *Neochloris aquatica* Starr, and *S. obliquus*—most likely possess mitochondrial genomes that resemble the *Chlamydomonas* type. In contrast, the mitochondrial genome of some prasinophycean (sensu Moestrup and Throndsen 1988) and trebouxiophycean (sensu Friedl 1995) lineages—such as *Platymonas subcordiformis, Pyramimonas parkae* Nor-

TABLE 2. *Mitochondrial genome size, map (structure), gene content among* Chlamydomonas*-like, protist, fungal, metazoan, plant and* Prototheca*-like lineages (/ and [ ] indicate that both traits, or only exceptions, respectively, have been reported; circ = circular, lin = linear).*

Mitochondrial traits	Lineages					
	Chlamydomonas-like[a]	Protists[b]	Fungi[c]	Metazoans[d]	Plants[e]	*Prototheca*-like[f]
Size (kb)	15.7–45	6–69	19.4–100	14–42	200–2500	42.8–80
Genome map	circ/lin	circ/lin	circ [lin]	circ [lin]	circ	circ
Gene content						
cob	+	+	+	+	+	+
*cox*1	+	+	+	+	+	+
*cox*2	−	+ [−]	+	+	+	+
*cox*3	−	+ [−]	+	+	+	+
*atp*1	−	− [+]	−	−	+	+
*atp*6	−	−/+	+	+	+	+
*atp*9	−	−/+	+/−	−	+	+
*nad*1	+	+ [−]	+ [−]	+	+	+
*nad*2	+	+/−	+ [−]	+	+	+
*nad*3	−	+/−	+ [−]	+	+	+
*nad*4	+	+ [−]	+ [−]	+	+	+
*nad*4L	−	− [+]	+ [−]	+	+	+
*nad*5	+	+ [−]	+ [−]	+	+	+
*nad*6	+	− [+]	+ [−]	+	+	+
rnl	+	+	+	+	+	+
rns	+	+	+	+	+	+
rrn5	−	− [+]	−	−	+	+
trn	3	0–3 [26]	25–28	22 [1–2]	16–29	26
rpl + *rps*	0	0–4 [53]	0–1	0	16	13
Fragmented rRNA coding regions	+	+/−	−	−	−	−

[a] Grant and Chiang 1980, Moore and Coleman 1989, Michaelis et al. 1990, Lee et al. 1991, Denovan-Wright and Lee 1994, Boudreau and Turmel 1995, Denovan-Wright et al. 1998.
[b] For references, see Wolstenholme and Fauron 1995; Burger et al. 1995, Lang et al. 1996.
[c] For references, see Wolstenholme and Fauron 1995.
[d] For references, see Wolstenholme and Fauron 1995; Gjetvaj et al. 1992.
[e] For references, see Wolstenholme and Fauron 1995.
[f] Bayen and Rode 1973, Waddle et al. 1990, Coleman and Goff 1991, Wolff et al. 1994, Kessler and Zetsche 1995.

ris & Pearson, *Hafniomonas montana* Ettl & Moestrup, *Chlorella vulgaris* Beij., and *Pleurastrum terrestre* Fritsch & John (Kessler and Zetsche 1995, Nedelcu et al. 1996)—appear to share some of the distinctive features of the *Prototheca* mitochondrial genome type. Therefore, it appears that the previously observed great evolutionary distance between the mitochondrial genomes of *C. reinhardtii* and *P. wickerhamii* extends in fact to the two green algal mitochondrial lineages to which they belong.

GREEN ALGAL MITOCHONDRIAL GENOMES RESEMBLE DISTINCT COUNTERPARTS

The dichotomy in mitochondrial genome organization between the *Chlamydomonas*-like and *Prototheca*-like types is also reflected in different degrees of resemblance to their land plant counterparts (Table 2), considered their closest relatives at the nucleo-cytosolic level (Ragan and Chapman 1978, Chapman and Buchheim 1992). The mitochondrial genomes of *C. reinhardtii* and *C. eugametos* (and, most likely, of all the chlorophycean taxa) share no specific features with their land plant counterparts. However, the mitochondrial genome of *P. wickerhamii* (and, most likely, of other trebouxiophycean taxa) display many of the embryophyte traits, such as 1) the presence of *atp*1 and *rrn*5 (the only other nonplant taxa that possess *atp*1 or both *atp*1 and *rrn*5 are the protozoans *Acanthamoeba castellanii* and *Reclinomonas americana,* respectively; Burger et al. 1995, Lang et al. 1996, 1997), 2) a virtually identical set of ribosomal protein genes arrayed in a similar manner, 3) three *orf* homologues, 4) conventional continuous rRNA genes, and 5) group I introns in the *cox*1 gene at the same positions as in the liverwort mitochondrial DNA (Gray 1995).

The same molecular data that support the split between the land plant/*Prototheca* and *Chlamydomonas* mitochondrial genome types also emphasize the resemblance between the *Chlamydomonas* and nonplant mitochondrial lineages. The available data from kinetoplastids, apicomplexans, and ciliates (see Wolstenholme and Fauron 1995 for a review and references) suggest, surprisingly, that their mitochondrial genomes share with *Chlamydomonas* mitochondrial type traits that are absent in both the *Prototheca* and the land plant counterparts.

Of the 23 features of mitochondrial genome organization compared in Table 2, 13 are different between the *Chlamydomonas*-like and *Prototheca*-like genomes, and in each of these cases the character status present in *Chlamydomonas*-like mitochondrial

genomes is also shared by at least one of the non-land-plant lineages but not by the land plant homologues (see Table 2). For example, *cox*2 and *cox*3 are missing in the mitochondrial genomes of *C. reinhardtii* and *C. eugametos* but are present in those of *P. wickerhamii, Platymonas subcordiformis,* and land plants; interestingly, *cox*2 and *cox*3 are also missing in the mitochondrial genomes of apicomplexans (*Plasmodium falciparum* and *Theileria parva*) and ciliates (*Paramecium aurelia* and *Tetrahymena pyriformis*), respectively. Likewise, whereas the mitochondrial genomes of *P. wickerhamii* and land plants encode three of the ATPase subunits, those of *C. reinhardtii, C. eugametos,* and *Plasmodium falciparum* do not encode any, and only one is encoded in the mitochondria of *Paramecium aurelia* and kinetoplastids. Moreover, the gene coding for the subunit 4L of the NADH dehydrogenase (*nad*4L) is missing in the *C. reinhardtii, C. eugametos, Paramecium aurelia,* apicomplexan, and kinetoplastid mtDNAs but present in the *P. wickerhamii* and land plant counterparts. Another striking resemblance is the lack of *atp*1 and *rrn*5 in the mitochondrial genome of *C. reinhardtii, C. eugametos, Paramecium aurelia,* apicomplexans, kinetoplastids, fungi, and animals in contrast to its presence in *P. wickerhamii* and land plants. Only three tRNAs are encoded in the mitochondrial genomes of *C. reinhardtii, C. eugametos,* and *Paramecium aurelia,* one or two in those of cnidarians, and none in those of kinetoplastids. However, the *P. wickerhamii* and liverwort mitochondrial genomes encode 26 and 29 tRNAs, respectively. Furthermore, none of the mitochondrial ribosomal proteins is mitochondrially encoded in *Chlamydomonas*; none or one in kinetoplastids, apicomplexans, fungi, and animals; and only seven in *Paramecium aurelia.* On the other hand, *P. wickerhamii* and liverwort mitochondrial genomes code for 13 and 16 ribosomal proteins, respectively. It is noteworthy that fragmented and scrambled mitochondrial rRNA-coding regions present in the *Chlamydomonas* genome type have been found only in the ciliate *Tetrahymena pyriformis,* the apicomplexans *Plasmodium falciparum, Plasmodium* sp., and *Theileria parva* (for references, see Nedelcu 1997a). In addition, the 5′- and 3′-ends of mitochondrial LSU rRNA-coding regions are missing in both *Chlamydomonas* and animals but are present in *P. wickerhamii* and land plants.

It is intriguing that the *C. reinhardtii* mitochondrial genome resembles the metazoan mitochondrial genomes in showing extensive physical (Fig. 1) and transcriptional linkage of genes, processing of long cotranscripts by discrete endonucleolytic scissions, and the absence of 5′-untranslated regions in the mature mRNAs (Gray et al. 1989). It is also interesting that sequences similar to those associated with the origin of mtDNA replication in vertebrates have been found in mtDNA of both *C. reinhardtii* and *C. eugametos* (Nedelcu 1997b). Moreover, the distribution, base composition, and nucleotide sequence of the short GC-rich repeat clusters identified in the *C. reinhardtii* (Boer and Gray 1991) and *C. eugametos* (Nedelcu 1997b, Denovan-Wright et al. 1998) mitochondrial intergenic spacers (Fig. 1) are very similar to those of various fungal counterparts (Nedelcu 1997b).

POTENTIAL FACTORS AND MECHANISMS INVOLVED IN THE EVOLUTION OF CHLAMYDOMONAS-LIKE MITOCHONDRIAL GENOMES

If all the extant green algal mitochondria evolved from a single common ancestor (monophyletic origin), the questions to be addressed are 1) Why are the *Chlamydomonas*-like and *Prototheca*-like mitochondrial lineages so different in so many respects? and 2) Why does the evolutionary trend in the *Chlamydomonas*-like mitochondrial lineage seem so similar, at least as far as the end result (i.e. reduced genome size and gene content, similar gene organization and expression, and DNA sequence), to the ciliate/fungal/animal lineage? If the *Chlamydomonas*-like mitochondrial lineage had a different evolutionary origin than the rest of the green algal group, is this an example of secondary acquisition (as previously proposed by Gray et al. 1989 for the land plant mitochondrial lineage) or independent primary endosymbiosis (i.e. polyphyletic origin)?

Whether the two distinct green algal mitochondrial lineage, (i.e. *Chlamydomonas*-like and *Prototheca*-like) do or do not share a more recent common ancestor with one another and with land plants than they do with other lineages remains to be answered. However, it is quite obvious that the two green algal mitochondrial lineages followed two very distinct evolutionary strategies after their divergence from either the common green algal or the unknown ancestor. Therefore, the questions to be addressed are how and why these changes occurred and evolved.

The most distinctive characteristics of genome organization between the *Chlamydomonas* and *Prototheca* mitochondrial genome types include a reduced gene content and fragmented and scrambled rRNA-coding regions. Although more data are needed before the factors and mechanisms responsible for the occurrence and development of these two features are deciphered, a few suggestions can be made.

The accumulation of short direct and inverted repeated sequences in the intergenic spacers of *Chlamydomonas*-like mitochondrial genomes has been proposed to have triggered intramolecular recombination events, resulting in both the deletion of protein-, tRNA-, or rRNA-coding regions and the fragmentation and scrambling of the rRNA genes in this lineage (Nedelcu 1997a, b) (Fig. 2). Although short repeated sequences were found in the intergenic spacers of both *Chlamydomonas*-like and *P. wickerhamii* mitochondrial genomes, the repeated elements are mostly GC rich in the first (Boer and Gray 1991, Nedelcu 1997b, Denovan-Wright et al. 1998) but highly AT rich in the second (Wolff et al.

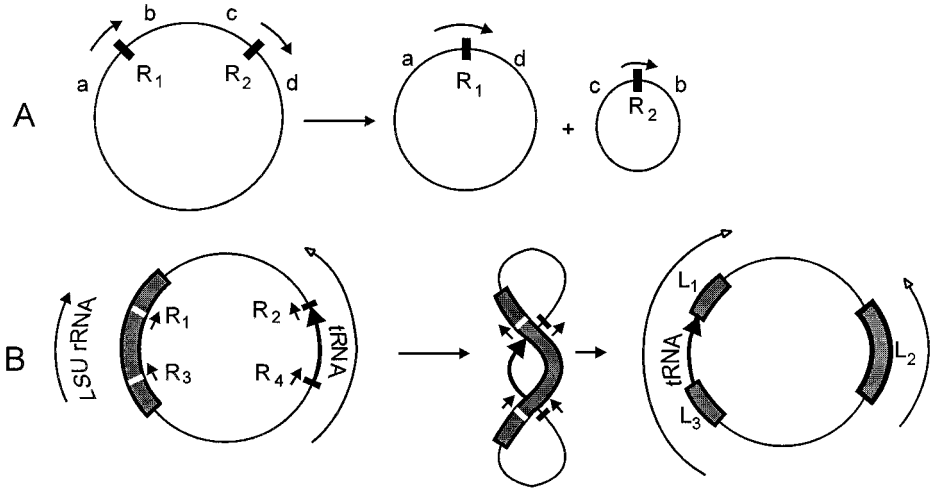

FIG. 2. Recombination events between A) a two-copy (R_1/R_2) short direct repeat and B) two sets of two-copy short inverted repeats (R_1/R_2 and R_3/R_4) resulting in the excision of a coding region and the fragmentation and scrambling of an LSU rRNA-coding region, respectively. Arrows outside the circles indicate the transcriptional orientation of that region; a, b, c, and d are sequences flanking the short repeats, and L_1, L_2, and L_3, are LSU rRNA-coding modules.

1994). The potential recombinogenic activity of the GC-rich short repeated sequences and, subsequently, their involvement in the evolution of the *Chlamydomonas* mitochondrial genome type would be supported by the finding of such repetitive elements in all the *Chlamydomonas*-like mitochondrial genomes but not in the *Prototheca*-like counterparts. It is noteworthy that some of the GC-rich palindromic sequences present in the intergenic spacers of *C. reinhardtii* and *C. eugametos* might act as surrogate origins of mtDNA replication (Nedelcu 1997b). Such structures can initiate the light-strand replication at multiple sites and promote gene disruptions in a manner similar to that proposed to explain the displacement of the origin of light-strand replication associated with a higher level of gene rearrangement among vertebrate mitochondrial genomes (Macey et al. 1997).

Another factor potentially involved in the different evolutionary strategies undertaken by the *Chlamydomonas* and *Prototheca* mitochondrial genome types is the acquisition of a reverse transcriptase-like (*rtl*) coding region in the *Chlamydomonas* mitochondrial lineage. A putative role for such an enzyme in the evolution of the *Chlamydomonas*-like mitochondrial genomes would be supported by the presence of the corresponding gene in *Chlamydomonas*-like mitochondrial genomes but not *Prototheca*-like counterparts. To date, although rather few green algal mitochondrial genomes have been investigated, an *rtl* has been found only in *C. reinhardtii* (Boer and Gray 1988a); however, the overexpressed product of the *C. reinhardtii rtl* does not appear to have a reverse transcriptase activity (Faßbender et al. 1994). The finding that the *C. reinhardtii* mitochondrial *rtl* coding region might in fact be the remnant of an intronic open reading frame previously harbored by a group II intron argues for a potential involvement of mobile group II introns in the evolution of chlorophycean mitochondrial genomes (Nedelcu 1997b). It is interesting that another chlorophycean taxon, *S. obliquus,* contains in its mitochondrial *rnl* a group II intron that is, however, devoid of an *rtl* (Kück et al. 1990). On the other hand, neither *P. wickerhamii* nor *P. subcordiformis* harbors group II introns or open reading frames with *rtl* similarity in their mitochondrial genes.

As the information from other green algal lineages becomes available, the comparisons among the distinct patterns of mitochondrial genome organization will hopefully provide insights into the mechanisms involved in the evolution of the mitochondrial genomes in the green algal group. Furthermore, distinguishing among the possible mechanisms involved in the evolution of the mitochondrial genomes in the *Chlamydomonas*-like lineage would indicate why only this lineage underwent such dramatic evolutionary changes, provided that in the *Prototheca*-like lineage those particular mechanisms were not functional either because the substrate (e.g. recombinogenic repeats or reverse transcriptase-like genes) or additional features required for such mechanisms (e.g. enzymes involved in recombination or facilitating the transfer of genetic information into the nucleus) were absent.

POTENTIAL EVOLUTIONARY SCENARIOS

Distinct evolutionary origins. On the basis of mitochondrial gene content and genome organization comparisons, Gray and Spencer (1996:121) concluded that ''there is little or no evidence from these data that the land plant *M. polymorpha* and the green alga *C. reinhardtii* shared a common mitochondrial ancestor as recently as they shared a common chloroplast or nuclear ancestor.'' Nevertheless, a poly-

phyletic origin for the green algal mitochondria has not been favored.

If the green algal mitochondria were monophyletic, the mitochondrial genome in the most recent common ancestor of the chlorophycean group should retain ancestral (symplesiomorphic) traits present in the common green algal/land plant ancestor but also possess distinctive derived (synapomorphic) characters that are unique to the *Chlamydomonas* mitochondrial type. For example, the presence of *atp*1 and *rrn*5 seems to be a symplesiomorphic character because it is shared by both *Prototheca* and land plant mitochondrial lineages. However, the presence of fragmented mitochondrial rRNA genes is most likely a synapomorphic character, given that it seems to be derived within, and therefore shared by, the members of the chlorophycean lineage only (Nedelcu et al. 1996). The finding of a primitive green algal taxon whose mitochondrial genome both contains the *atp*1 and/or *rrn*5 and possesses fragmented and scrambled mitochondrial rRNA genes whose nucleotide sequences affiliate the *Chlamydomonas*-like and *Prototheca*-like mitochondrial lineages would argue for the evolution of these two mitochondrial genome types from a recent common ancestor. However, if that taxon does not have any of the ancestral features of the green algal/land plant mitochondrial lineage and its rDNA sequences fail to connect the *Prototheca*-like and *Chlamydomonas*-like lineages to the exclusion of other non-green-algal groups, a separate evolutionary origin for the *Chlamydomonas*-like mitochondria might have to be considered.

Although theories of multiple independent endosymbiotic events leading to the organelle genetic diversity in the present eukaryotic lineages are not in favor, they should not be disregarded. The following need to be considered: 1) similarities in organelle genome organization among unrelated lineages as well as striking differences among lineages within the same group, 2) the difficulty in explaining the distribution of many traits other than by implying multiple independent losses or acquisitions of characters in distant evolutionary lineages (e.g. the mitochondrial 5S rRNA gene; see Gray 1995), 3) the diversity and dynamic of present endosymbiotic associations not only in terms of eukaryotic host and prokaryotic symbiont but also in terms of the interactions evolved (see Corliss 1990), and 4) the diversity and most likely genetic instability of prokaryotic life in ancient times. There is no obvious reason to assume that only one individual or even only one population of alpha-proteobacteria invaded only one particular protoeukaryotic cell, with the present mitochondrial diversity being the consequence of different selective pressures acting on the same genetic potential. It seems as likely that several populations of different alpha-proteobacterial strains invaded distinct host populations and that the subsequent interactions between the two co-evolving components were shaped by the distinct genetic potential carried by both partners as well as by new adaptive pressures. For the chloroplast, such a polyphyletic origin from closely related cyanobacteria has been considered to be ''very difficult, if not impossible, to discern'' from a monophyletic ancestry (Delwiche et al. 1995, cited by Gray and Spencer 1996:113).

The main argument against a polyphyletic origin of the mitochondria seems to be the fact that all the mitochondrial rRNA sequences available to date affiliate with only one subgroup of alpha-proteobacteria (Gray and Spencer 1996). Nevertheless, there are two aspects that could be challenged.

First, the fact that only one alpha-proteobacterial subgroup was found with which all the mitochondrial rRNA sequences cluster does not necessarily mean that another subgroup to which some rRNA sequences would more closely affiliate does or did not exist. Although considered as evidence in favor of the importance of slowly evolving sequences in properly positioning the more rapidly evolving rRNA sequences, it is noteworthy that the mitochondrial rRNA sequences from animals, fungi, and ciliates cluster with the alpha-proteobacterial counterparts only when their land plant homologues are included in the analysis (Gray 1995). Moreover, the inclusion of more mitochondrial and alpha-proteobacterial rRNA sequences as well as of the sequences from two ciliate alpha-proteobacterial endosymbionts, *Holospora* and *Caedibacter*, decreased the bootstrap value of the mitochondrial/rickettsial alpha-proteobacterial node from 95% to 73% and 41%, respectively (Gray and Spencer 1996). Theoretically, there is no reason to disregard the possibility that the inclusion of new alpha-proteobacterial sequences will decrease the support value of the rickettsial/mitochondrial node to the point that some of the mitochondrial sequences would affiliate more closely to another alpha-proteobacterial subgroup.

Second, the fact that all the mitochondrial rRNA gene sequences cluster together does not necessarily mean that the respective mitochondria evolved from one single alpha-proteobacterial ancestor; it may as well be that the present mitochondria are the descendants of a few slightly different invading alpha-proteobacterial strains that shared a common ancestor, as recorded in their rRNA sequences, but whose genomes already developed some of the distinctive features (e.g. error-prone replication mechanisms, deficient postreplication repair systems or copy-correction mechanisms, or a novel gene or molecular mechanism) that triggered the changes (e.g. a high rate of nucleotide substitution or increased efficiency of recombination) responsible for the further evolution of their respective mitochondrial-to-be genomes.

Early divergence. To explain the observed differences in mitochondrial genome organization and af-

filiation between *C. reinhardtii* and *P. wickerhamii,* Gray (1995) suggested that a detailed characterization of mitochondrial DNA from other chlorococcalean taxa occupying a phylogenetic position between the two green algal mitochondrial lineages is needed. Nevertheless, such an approach may not provide the information necessary, at least because the two taxa most likely belong to two distinct evolutionary lineages: the chlorophycean and the trebouxiophycean (sensu Friedl 1995), respectively, whose divergence is probably very old. It was proposed (Mattox and Stewart 1984) 1) that the different lines of evolution of higher green algae are different from each other because they had independent origins from different types of green flagellates and 2) that the most fundamental evolutionary changes occurred among green flagellates rather than after the origin of the higher green algal groups. Therefore, the answer most likely will come from among prasinophycean taxa, a pool of primitive green algae that are considered similar to forms from which higher green algae evolved (Mattox and Stewart 1984). Which prasinophycean lineages might yield evidence about the phylogenetic relationships among the extant green algal lineages is difficult to assess because the members of this group have different evolutionary origins and the phylogenetic relationships among the taxa are not fully understood.

There are two pieces of evidence that could argue for an early divergence of the *Chlamydomonas* lineage. First, from the observation that the *Chlamydomonas* nuclear 5S rRNAs are sufficiently distinct in both primary and secondary structure from those of other green algae and plants (Darlix and Rochaix 1981) and diverge much deeper than expected, Devereux et al. (1990) proposed that *Chlamydomonas* may be diverged enough to be considered a major group of green algae. Second, phylogenetic analyses using different nuclear SSU and LSU rRNA data sets assigned chlamydomonadalean lineages to various positions among the other green algal taxa available without being able to definitely resolve their phylogenetic position (Buchheim et al. 1990, 1996, Chapman and Buchheim 1991). In addition, comparisons among *Chlamydomonas* species revealed differences in their nuclear 18S rDNA sequences comparable to the sequence divergence between horsetail and maize (Jupe et al. 1988). Furthermore, because the position of some *Chlamydomonas* lineages was difficult to resolve, Buchheim and Chapman (1992) suggested that it is possible that these divergences have been ancient and rapid, and that the period of shared ancestry has been too short, relative to the time since divergence, to be adequately recorded in the rRNA sequences.

It is noteworthy that the flagellate algal groups are considered to have evolved from distinct zooflagellate ancestors (e.g. Prymnesiophyceae from the unusual zooflagellate *Colponema* and Chrysophyceae probably from a *Bicoseca*-like protozoan; Stewart and Mattox 1980). No known zooflagellate that can be closely linked to the green algal group has been found, although O'Kelly (1992) suggested that members of the genus *Jakoba* might share a distant common ancestry with the green algal group. Furthermore, O'Kelly (1992) presented a speculative phylogenetic scenario suggesting that the green algae have an independent origin from all other photosynthetic organisms: a large predatory zooflagellate having mitochondria with flattened cristae (which evolved from an amitochondrial phagotrophic zooflagellate) acquired a Chl *a,b*–containing chloroplast and developed into the ancestral prasinophytes from which the green flagellate ancestors of the various green algal lineages evolved. However, one cannot exclude the possibility that not only the different flagellate algal groups but also the primitive green flagellates themselves might have evolved from distinct zooflagellate lineages. Such a scenario is consistent with the fact that the extant primitive green flagellates do not seem to cluster together in phylogenetic trees based on nuclear rDNA sequences (Kantz et al. 1990, Steinkötter et al. 1994, Friedl 1995) and that in a recent chloroplast SSU rRNA tree the *Chlamydomonas* sequences do not branch as expected with the other green algae but, rather, suggest a very early divergence relative to all plastid sequences (Gray and Spencer 1996).

Different rates of evolution. As the best explanation for the extreme differences between the mitochondrial genomes of *Marchantia, Prototheca,* and *Acanthamoeba* on the one hand and of *Chlamydomonas* on the other, Gray and Spencer (1996:122) proposed a ''relatively rapid and extreme evolution of the latter genome away from the ancestral pattern represented by the more conservative mtDNAs in the former three organisms.''

It should be mentioned that no extensive studies on point mutation level in *Chlamydomonas* mitochondrial genes have yet been done. Nevertheless, the number of nucleotide substitutions in *Chlamydomonas* mitochondrial SSU and LSU rRNA genes was shown to be severalfold higher than the accumulated substitutions in land plant mitochondrial counterparts (Denovan-Wright et al. 1996). However, mitochondrial rRNA sequences of *P. wickerhamii* also seem to have a high rate of nucleotide substitution and, together with the *Chlamydomonas,* ciliate, fungal, and yeast counterparts, constitute a rapidly evolving group (associated with long branches in phylogenetic analyses) in marked contrast to the slowly evolving land plant mitochondrial rRNA sequences. Moreover, both *Chlamydomonas* and *Prototheca* mitochondrial genome types seem to have undergone extensive gene rearrangements, suggesting a rather high rate of mitochondrial genome evolution in both groups (Nedelcu 1997b, Nedelcu and Lee 1998). Furthermore, it is noteworthy that *Chlamydomonas* chloroplast genomes also display extensive

sequence divergence (at least twice the range of sequence variation seen in all land plants) as well as gene rearrangement (Turmel et al. 1993, Boudreau and Turmel 1996); such observations led Nedelcu and Lee (1998) to suggest that in this group, in contrast to land plants, mitochondrial and chloroplast genomes exhibit concerted modes and tempos of evolution.

The lack of knowledge about the exact time of divergence of different green algal lineages makes it difficult to assess absolute rates of nucleotide substitutions in their mitochondrial genomes and to compare their tempo of DNA evolution. Moreover, substitution rates of protein-coding genes from all three genetic compartments from various lineages within the chlorophycean as well as other green algal groups must be available before one can conclude that the mitochondrial DNA in *Chlamydomonas* evolved more rapidly than it did in other green algal lineages. Nevertheless, no indications have yet been made as to the causes and the mechanisms responsible for the postulated rapid evolution in the *Chlamydomonas* lineage. Potential causes that could have triggered the distinct evolutionary strategy undertaken by the *Chlamydomonas* mitochondrial lineage include new adaptive pressures and changes in life history.

Adaptive pressures. It has been proposed that the changes in the mitochondrial rRNA gene organization leading to highly fragmented and scrambled rRNA coding regions in the *Chlamydomonas* mitochondrial lineage may have started around the time of the chlorophycean divergence from a prasinophycean-like green flagellate (Nedelcu et al. 1996). Given that all the prasinophycean taxa are primarily marine and all the chlorophycean species primarily freshwater algae, a prasinophycean-like ancestor presumably had to switch from a marine to a freshwater habitat. Likewise, the trebouxiophycean (sensu Friedl 1995) taxa, including *P. wickerhamii,* are also mainly freshwater algae, whereas the green flagellate genus *Platymonas,* considered ancestral to the trebouxiophycean group (Mattox and Stewart 1984), is marine. In contrast, the ulvophycean green algal group is mainly marine.

It is likely that the freshwater environment presented a strong challenge for the first ''explorers.'' In this connection, it is noteworthy that the marine taxon *Hafniomonas montana* (or *Pyramimonas montana*), previously known as a prasinophycean alga but currently considered related (and retaining ancestral-like features) to the chlorophycean group (Ettl and Moestrup 1980, O'Kelly et al. 1994), possesses continuous mitochondrial rRNAs, whereas all the freshwater chlorophycean taxa revealed fragmented rRNAs (Nedelcu et al. 1996). Furthermore, the level of mitochondrial rRNA gene fragmentation, which was suggested to be a consequence of recombination events, is much lower in one of the very few chlorophycean marine species, *Chlamydomonas pulsatilla* Wollenweber (Nedelcu 1997a), relative to freshwater *Chlamydomonas* counterparts.

It should be mentioned, however, that in land plant long-term tissue culture, the frequency of mitochondrial genomic rearrangements mediated by small repeated sequences is increased (Hartmann et al. 1994, Benslimane et al. 1996). Therefore, one cannot definitely disregard the possibility that many years of culturing affected in some way the mitochondrial genome organization of the algal strains investigated. This issue can be settled by analyzing field specimens.

Life histories. One of the obvious differences between the life histories of the freshwater chlorophycean and the trebouxiophycean green algal groups is the presence in the first of sexual reproduction involving a dormant zygote and zygotic meiosis and the absence in the second of any type of sexual reproduction. On the other hand, the life history of the mainly marine ulvophycean green algae is characterized by 1) the complete absence of a dormant zygote that is considered typical of sexual reproduction in freshwater green algae and 2) the presence of alternation of generations. Mattox and Stewart (1984) suggested that the more stable marine environment fostered the evolution of longer life cycles and alternation of generations in ulvophycean taxa. The authors also proposed that alternation of generations is an ancestral condition that was lost by the species that invaded freshwater secondarily (e.g. the case of some freshwater species of *Cladophora*; Graham 1982).

Therefore, it appears that the freshwater environment, at least because of its lesser stability relative to the marine counterpart, created adaptive pressures to which different ''explorers'' reacted differently by developing distinct mechanisms to counteract the pressure (e.g. sexual reproduction with dormant zygote among chlorophycean taxa and the loss of sexual reproduction and alternation of generations in trebouxiophycean and some ulvophycean green algae, respectively). The high rate of evolution in both nuclear and chloroplast and mitochondrial DNA sequences (Jupe et al. 1988, Turmel et al. 1993, Denovan-Wright et al. 1996) in the freshwater flagellate chlorophycean green algae might be correlated with the presence of sexual reproduction featuring a dormant zygote in the life cycle of these lineages. During the dormant stages, mutational and recombination events are most likely to occur at high rates. It is known that during the dormant stages, the mitochondria in the chlorophycean flagellate *P. agilis* fuse into a single large mitochondrion unit (Burton and Moore 1974). Moreover, in the case of species undergoing sexual reproduction, the zygospore may contain mtDNA molecules acquired from both mating types, which could allow intermolecular recombination events. The transfer of an intronic sequence during interfertile crosses between *Chlamydomonas smithii* Hoshaw et Ettl and *C.*

reinhardtii (Colleaux et al. 1990, Ma et al. 1992) suggests that recombination (in this case, an intermolecular endonuclease-facilitated recombination event) can occur in *Chlamydomonas* mitochondria during the sexual phase of its life cycle. It is interesting that recombination mechanisms are even more active in the chloroplast of *Chlamydomonas* (Dürrenberger et al. 1996) and are thought to have been responsible for the high level of gene rearrangement observed among lineages.

It is also interesting that the mechanisms responsible for the evolution of the higher-plant mitochondrial genome probably developed after the bryophyte divergence because the mitochondrial genome of the liverwort *M. polymorpha* does not share the distinctive characteristics of the land plant counterparts, such as a very large size, high frequency of recombination, and acquisition of foreign DNA. The distinct and relatively new evolutionary strategy adopted by the higher-plant mitochondrial lineage may have been related to changes in the life history of land plants from a dominant haploid generation in bryophytes to a dominant diploid generation in flowering plants. The changes in the alternation of generation pattern may also be correlated to new adaptive pressures created by the transition from a wet to a dry environment.

CONCLUSIONS

We are probably still far from understanding the evolutionary forces that acted on the primitive mitochondrial genome(s) and determined such a great diversity in the contemporary mitochondria. As much as we would like to define a prototype for an ancestral mitochondrial genome from which all the present mitochondrial lineages diverged and similar modes and tempos of mitochondrial genome evolution in related eukaryotic lineages, it may not be possible. Although the vertebrate and higher-plant mitochondrial genome types seem to be quite easy to define, it is difficult to define a mitochondrial type for the lower eukaryotic groups. The generally accepted view is that these groups are ancient and their divergence is older; therefore, they have evolved for a longer time so that the present forms are more diverse. However, we should not disregard the fact that at the time of vertebrate or land plant divergence, the endosymbiotic associations were well established and the interactions between the cellular compartments quite stable, whereas at the time of protist and algal divergence, the genetic and environmental diversity and instability were probably still significant evolutionary forces. In this context, adaptive pressures represented by changes of habitat correlated with changes in the life history of the primitive eukaryotic lineages acting on less stable genetic potentials may have been important factors in determining different evolutionary strategies reflected in the great diversity we notice among the present lower eukaryotic mitochondrial lineages. However, the present diversity may be the result not only of evolutionary changes that occurred after the divergence of each of the major eukaryotic lineages but also of differences already established in their protoeukaryotic ancestors.

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Antamarian, A., Coria, R., Ramirez, J. & Gonzalez-Halphen, D. 1996. The deduced primary structure of subunit I from cytochrome *c* oxidase suggests that the genus *Polytomella* shares a common mitochondrial origin with *Chlamydomonas. Biochim. Biophys. Acta* 1273:198–202.

Bayen, M. & Rode, A. 1973. The 1,700 DNA of *Chlorella pyrenoidosa:* heterogeneity and complexity. *Plant Sci. Lett.* 1:385–9.

Benslimane, A. A., Hartmann, C., Ouenzar, B. & Rode, A. 1996. Intramolecular recombination of a mitochondrial minicircular plasmid-like DNA of date-palm mediated by a set of short direct-repeat sequences. *Curr. Genet.* 29:591–4.

Boer, P. H. & Gray, M. W. 1988a. Genes encoding a subunit of respiratory NADH dehydrogenase (ND1) and a reverse transcriptase-like protein (RTL) are linked to ribosomal RNA gene pieces in *Chlamydomonas reinhardtii* mitochondrial DNA. *EMBO J.* 7:3501–8.

——— 1988b. Scrambled ribosomal RNA gene pieces in *Chlamydomonas reinhardtii* mitochondrial DNA. *Cell* 55:399–411.

——— 1988c. Transfer RNA genes and the genetic code in *Chlamydomonas reinhardtii* mitochondria. *Curr. Genet.* 14:583–90.

——— 1991. Short dispersed repeats localized in spacer regions of *Chlamydomonas reinhardtii* mitochondrial DNA. *Curr. Genet.* 19:309–12.

Boudreau, E., Otis, C. & Turmel, M. 1995. Gene rearrangements in *Chlamydomonas* chloroplast DNAs are accounted for by the expansion/contraction of the inverted repeat. *Plant Mol. Biol.* 27:351–64.

Boudreau, E. & Turmel, M. 1996. Extensive gene rearrangements in the chloroplast DNAs of *Chlamydomonas* species featuring multiple dispersed repeats. *Mol. Biol. Evol.* 13:233–43.

Boynton, J. E., Harris, E. H., Burkhart, B. D. & Lamerson, P. M. 1987. Transmission of mitochondrial and chloroplast genomes in crosses of *Chlamydomonas. Proc. Natl. Acad. Sci. USA* 84:2391–5.

Buchheim, M. A., & Chapman, R. L. 1992. Phylogeny of *Carteria* (Chlorophyceae) inferred from molecular and organismal data. *J. Phycol.* 28:362–74.

Buchheim, M. A., Lemieux, C., Otis, C., Guttel, R. R., Chapman, R. L. & Turmel, M. 1996. Phylogeny of the Chlamydomonadales (Chlorophyceae): a comparison of ribosomal RNA gene sequences from the nucleus and the chloroplast. *Mol. Phylogenet. Evol.* 3:322–43.

Buchheim, M. A., Turmel, M., Zimmer, E. A. & Chapman, R. L. 1990. Phylogenetic systematics of *Chlamydomonas* based on cladistic analysis of nuclear 18S rRNA sequence data. *J. Phycol.* 26:688–99.

Burger, G., Plante, I., Lonergan, K. M. & Gray, M. W. 1995. The mitochondrial DNA of the amoeboid protozoon, *A canthamoeba castellanii:* complete sequence, gene content and genome organization. *J. Mol. Biol.* 245:522–37.

Burton, M. D. & Moore, J. 1974. The mitochondrion of the flagellate, *Polytomella agilis. J. Ultrastruct. Res.* 48:414–9.

Chapman, R. L. & Buchheim, M. A. 1991. Ribosomal RNA gene sequences: analysis and significance in the phylogeny and taxonomy of green algae. *Crit. Rev. Plant Sci.* 10:343–68.

Chapman, R. L. & Buchheim, M. A. 1992. Green algae and the

evolution of land plants: inferences from nuclear-encoded rRNA gene sequences. *BioSystems* 28:127–37.

Coleman, A. W. & Goff, L. J. 1991. DNA analysis of eukaryotic algal species. *J. Phycol.* 27:463–73.

Colleaux, L., Michel-Wolwertz, M.-R., Matagne, R. F. & Dujon, B. 1990. The apocytochrome *b* gene of *Chlamydomonas smithii* contains a mobile intron related to both *Saccharomyces* and *Neurospora* introns. *Mol. Gen. Genet.* 233:288–96.

Corliss, J. O. 1990. Endosymbionts of Protozoa. *Zool. Sci.* 7(Suppl.):167–77.

Darlix, J.-L. & Rochaix, J.-D. 1981. Nucleotide sequence and structure of cytoplasmic 5S RNA and 5.8S RNA of *Chlamydomonas reinhardii*. *Nucleic Acids Res.* 9:1291–9.

Dayhoff, M. O. & Schwartz, R. M. 1981. Evidence on the origin of eukaryotic mitochondria from protein and nucleic acid sequences. *Ann. NY Acad. Sci.* 361:92–104.

Delwiche, C. F., Kushel, M. & Palmer, J. D. 1995. Phylogenetic analysis of *tuf*A sequences indicates a cyanobacterial origin of all plastids. *Mol. Phylogenet. Evol.* 4:110–28.

Denovan-Wright, E. M. & Lee, R. W. 1994. Comparative structure and genomic organization of the discontinuous mitochondrial ribosomal RNA genes of *Chlamydomonas eugametos* and *Chlamydomonas reinhardtii*. *J. Mol. Biol.* 241:298–311.

Denovan-Wright, E. M., Nedelcu, A. M. & Lee, R. W. 1998. Complete sequence of the mitochondrial DNA of *Chlamydomonas eugametos*. *Plant Mol. Biol.* 36:285–95.

Denovan-Wright, E. M., Sankoff, D., Spencer, D. F. & Lee, R. W. 1996. Evolution of fragmented mitochondrial ribosomal RNA Genes in *Chlamydomonas*. *J. Mol. Evol.* 42:382–91.

Devereux, R., Loeblich, A. R. III & Fox, G. E. 1990. Higher plant origins and the phylogeny of green algae. *J. Mol. Evol.* 31:18–24.

Douglas, S. E. 1992. Eukaryote-eukaryote endosymbioses: insights from studies of a cryptomonad alga. *BioSystems* 28:52–68.

——— 1994. Chloroplast origins and evolution. *In* Bryant, D. A. [Ed.] *The Molecular Biology of Cyanobacteria*. Kluwer Academic Publishers, Amsterdam, The Netherlands, pp. 91–118.

Dürrenberger, F., Thompson, A. J., Herrin, D. L. & Rochaix, J.-D. 1996. Double strand break-induced recombination in *Chlamydomonas reinhardtii* chloroplast. *Nucleic Acids Res.* 24: 3323–31.

Ettl, H. & Moestrup, O. 1980. Light and electron microscopical studies on *Hafniomonas* gen. nov. (Chlorophyceae, Volvocales), a genus resembling *Pyramimonas* (Prasinophyceae). *Plant Syst. Evol.* 135:177–210.

Faßbender, S., Brühl, K.-H., Ciriacy, M. & Kück, U. 1994. Reverse transcriptase activity of an intron encoded polypeptide. *EMBO J.* 13:2075–83.

Friedl, T. 1995. Inferring taxonomic positions and testing genus level assignments in coccoid green lichen algae: a phylogenetic analysis of 18S ribosomal RNA sequences from *Dictyochloropsis reticulata* and from members of the genus *Myrmecia* (Chlorophyta, Trebouxiophyceae cl. nov.). *J. Phycol.* 31: 632–9.

Gibbs, S. P. 1978. The chloroplasts of *Euglena gracilis* may have evolved from symbiotic green algae. *Can. J. Bot.* 56:2883–9.

——— 1981. Chloroplasts of some groups may have evolved from endosymbiotic eukaryotic green algae. *Ann. NY Acad. Sci.* 361:193–207.

Gjetvaj, B., Cook, D. I. & Zouros, E. 1992. Repeated sequences and large-scale variation of mitochondrial DNA: a common feature among scallops (Bivalvia:Pectinidae). *Mol. Biol. Evol.* 9:106–24.

Graham, L. E. 1982. Cytology, ultrastructure, taxonomy, and phylogenetic relationships of Great Lakes filamentous algae. *J. Gt. Lakes Res.* 8:3–9.

Grant, D. & Chiang, K.-S. 1980. Physical mapping and characterization of *Chlamydomonas* mitochondrial molecules: their unique ends, sequence homogeneity, and conservation. *Plasmid.* 4:82–96.

Gray, M. W. 1989. Origin and evolution of mitochondrial DNA. *Annu. Rev. Cell Biol.* 5:25–50.

——— 1992. The endosymbiont hypothesis revisited. *Intl. Rev. Cytol.* 141:233–358.

——— 1995. Mitochondrial evolution. *In* Levings, C. S. III & Vasil, I. K. [Eds.] *The Molecular Biology of Plant Mitochondria*. Kluwer Academic Publishers, Amsterdam, The Netherlands, pp. 635–59.

Gray, M. W. & Boer, P. H. 1988. Organization and expression of algal (*Chlamydomonas reinhardtii*) mitochondrial DNA. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 319:135–47.

Gray, M. W., Cedergren, R., Abel, Y. & Sankoff, D. 1989. On the evolutionary origin of the plant mitochondrion and its genome. *Proc. Natl. Acad. Sci. USA* 86:2267–71.

Gray, M. W. & Spencer, D. F. 1996. Organellar evolution. *In* Roberts, D. M., Sharp, P., Alderson, G. & Collins, M. [Eds.] *Evolution of Microbial Life*. Society for General Microbiology Symposium 54. Cambridge University Press, Cambridge; United Kingdom, pp. 109–26.

Hartmann, C., Recipon, H., Jubier, M.-F., Valon, C., Delcher-Besin, E., Henry, Y., de Buyser, J., Lejeune, B. & Rode, A. 1994. Mitochondrial DNA variability detected in a single wheat regenerant involves a rare recombination event across a short repeat. *Curr. Genet.* 25:456–64.

Jupe, E. R., Chapman, R. L. & Zimmer, E. A. 1988. Nuclear ribosomal RNA genes and algal phylogeny—the *Chlamydomonas* example. *BioSystems* 21:223–30.

Kantz, T. S., Theriot, E. C., Zimmer, E. A. & Chapman, R. L. 1990. The Pleurastrophyceae and Micromonadophyceae: a cladistic analysis of nuclear rRNA sequence data. *J. Phycol.* 26: 711–21.

Kessler, U. & Zetsche, K. 1995. Physical map and gene organization of the mitochondrial genome from the unicellular green alga *Platymonas (Tetraselmis) subcordiformis (Prasynophyceae)*. *Plant Mol. Biol.* 29:1081–6.

Kück, U. 1989. The intron of a plastid gene from a green alga contains an open reading frame for a reverse transcriptase-like enzyme. *Mol. Gen. Genet.* 218:257–65.

Kück, U., Godenhardt, I. & Schmidt U. 1990. A self-splicing group II intron in the mitochondrial large subunit rRNA (LSU rRNA) gene of the eukaryotic alga *Scenedesmus obliquus*. *Nucleic Acids Res.* 18:2691–7.

Lang, B. F., Burger, G., O'Kelly, C. J., Cedergren, R., Golding, G. B., Lemieux, C., Sankoff, D., Turmel, M. & Gray, M. W. 1997. An ancestral mitochondrial DNA resembling a eubacterial genome in miniature. *Nature* 387:493–7.

Lang, B. F., Goff, L. J. & Gray, M. W. 1996. A 5S rRNA gene is present in the mitochondrial genome of the protist *Reclinomonas americana* but is absent from the red algal mitochondrial DNA. *J. Mol. Biol.* 61:607–13.

Lee, R. W., Dumas, C., Lemieux, C. & Turmel, M. 1991. Cloning and characterization of the *Chlamydomonas moewusii* mitochondrial genome. *Mol. Gen. Genet.* 231:53–8.

Lockhart, P. J., Penny, D., Hendy, M. D., Howe, C. J., Beanland, T. J. & Larkum, A. W. D. 1992. Controversy on chloroplast origins. *FEBS.* 301:127–31.

Lonergan, K. M. & Gray, M. W. 1994. The ribosomal RNA gene region in *A canthamoeba castellanii* mitochondrial DNA: a case of evolutionary transfer of introns between mitochondria and plastids? *J. Mol. Biol.* 239:476–90.

Ma, D.-P., King, Y.-T., Kim, Y. & Luckett, S. Jr. 1992. The group I intron of apocytochrome *b* gene from *Chlamydomonas smithii* encodes a site-specific endonuclease. *Plant Mol. Biol.* 18: 1001–4.

Macey, J. R., Larson, A., Ananjeva, N. B., Fang, Z. & Papenfuss, T. J. 1997. Two novel gene orders and the role of light-strand replication in rearrangement of the vertebrate mitochondrial genome. *Mol. Biol. Evol.* 14:91–104.

Margulis, L. 1981. *Symbiosis in Cell Evolution*. W. H. Freeman, San Francisco, California.

Mattox, K. R. & Stewart, K. D. 1984. Classification of the green algae: a concept based on comparative cytology. *In* Irvine, D. E. G. & John, D. M. [Eds.] *Systematics of the Green Algae*. Academic Press, London and Orlando, pp. 29–72.

McCourt, R. M. 1995. Green algal phylogeny. *TREE* 10:159–63.

McFadden, G. & Gilson, P. 1995. Something borrowed, something green: lateral transfer of chloroplasts by secondary endosymbiosis. *TREE* 10:12–8.

Michaelis, G., Varenholz, C. & Pratje, E. 1990. Mitochondrial DNA of *Chlamydomonas reinhardtii:* the gene for apocytochrome *b* and the complete functional map of the 15.8 kb DNA. *Mol. Gen. Genet.* 223:211–6.

Moestrup, O. & Throndsen, J. 1988. Light and electron microscopical studies on *Pseudoscourfieldia marina,* a primitive scaly green flagellate (Prasinophyceae) with posterior flagella. *Can. J. Bot.* 66:1415–34.

Moore, L. J. & Coleman, A. W. 1989. The linear 20 kb mitochondrial genome of *Pandorina morum* (Volvocaceae, Chlorophyta). *Plant Mol. Biol.* 13:459–65.

Morden, C. W., Delwiche, C. F., Kuhsel, M. & Palmer, J. D. 1992. Gene phylogenies and the endosymbiotic origin of plastids. *BioSystems* 28:75–90.

Nedelcu, A. M. 1997a. Fragmented and scrambled mitochondrial ribosomal RNA coding regions among green algae: a model for their origin and evolution. *Mol. Biol. Evol.* 5:506–17.

——— 1997b. Mitochondrial and chloroplast genome evolution among green algae. Ph.D. thesis, Dalhousie University, Halifax, Nova Scotia, Canada, 257 pp.

Nedelcu, A. M. & Lee, R. W. 1998. Mitochondrial and chloroplast genome evolution in *Chlamydomonas:* a comparative analysis. *In* Rochaix, J.-D. [Ed.] *Molecular Biology of Chlamydomonas: Chloroplast and Mitochondria.* Kluwer Academic Publishers, Amsterdam, The Netherlands, (in press).

Nedelcu, A. M., Spencer, D. F., Denovan-Wright, E. M. & Lee, R. W. 1996. Discontinuous mitochondrial and chloroplast large subunit ribosomal RNAs among green algae: phylogenetic implications. *J. Phycol.* 32:103–11.

O'Kelly, C. J. 1992. Flagellar apparatus architecture and the phylogeny of ''green'' algae: chlorophytes, euglenoids, glaucophytes. *In* Menzel, D. [Ed.] *Cytoskeleton of the Algae.* CRC Press, Boca Raton, Florida, pp. 315–45.

O'Kelly, C. J., Watanabe, S. & Floyd, G. L. 1994. Ultrastructure and phylogenetic relationships of Chaetopeltidales ord. nov. (Chlorophyta, Chlorophyceae). *J. Phycol.* 30:118–28.

Ragan, M. A. & Chapman, D. J. 1978. *A Biochemical Phylogeny of the Protists.* Academic Press, New York.

Steinkötter, J., Bhattacharya, D., Semmelroth, I., Bibeau, C. & Melkonian, M. 1994. Prasinophytes form independent lineages within the Chlorophyta: evidence from ribosomal RNA sequence comparisons. *J. Phycol.* 30:340–5.

Stewart, K. D. & Mattox, K. R. 1980. Phylogeny of phytoflagellates. *In* Cox, E. R. [Ed.] *Phytophlagellates,* Vol 2. Elsevier/North-Holland, New York, pp. 433–62.

——— 1984. The case for a polyphyletic origin of mitochondria: morphological and molecular comparisons. *J. Mol. Evol.* 21: 54–7.

Turmel, M., Gutell, R. R., Otis, J. P. & Lemieux, C. 1993. Analysis of the chloroplast large subunit ribosomal RNA gene from 17 *Chlamydomonas* taxa: three internal transcribed spacers and 12 group I intron insertion sites. *J. Mol. Biol.* 232:446–67.

Van de Peer, Y., Neefs, J.-M. & De Watcher, R. 1990. Small subunit ribosomal RNA sequences, evolutionary relationships among different life forms, and mitochondrial origins. *J. Mol. Evol.* 30:463–76.

Waddle, J. A., Schuster, A. M., Lee, K. W. & Meints, R. H. 1990. The mitochondrial genome of an exosymbiotic *Chlorella*-like green alga. *Plant Mol. Biol.* 14:187–95.

Wilcox, L. W., Lewis, L. A., Fuerst, P. A. & Floyd, G. L. 1992. Assessing the relationships of autosporic and zoosporic chlorococcalean green algae with 18S rDNA sequence data. *J. Phycol.* 28:381–6.

Wolff, G. & Kück, U. 1990. The structural analysis of the mitochondrial SSU rRNA implies a close phylogenetic relationship between mitochondria from plants and from the heterotrophic alga *Prototheca wickerhamii. Curr. Genet.* 17:347–51.

——— 1993. Organization and coding capacity of mitochondrial genomes of algae. *In* Brennicke, A. & Kück, U. [Eds.] *Plant Mitochondria—With Emphasis on RNA Editing and Cytoplasmic Male Sterility.* VCH Verlagsgesellschaft mbH, Weinheim, Germany, pp. 103–15.

Wolff, G., Burger, G., Lang, B. F. & Kück, U. 1993. Mitochondrial genes in the colourless alga *Prototheca wickerhamii* resemble plant genes in their exons but fungal genes in their introns. *Nucleic Acids Res.* 21:719–26.

Wolff, G., Plante, I., Lang, B. F., Kück, U. & Burger, G. 1994. Complete sequence of the mitochondrial DNA of the chlorophyte alga *Prototheca wickerhamii. J. Mol. Biol.* 237:75–86.

Wolstenholme, D. R. & Fauron, C. M.-R. 1995. Mitochondrial genome organization. *In* Levings, C. S. III & Vasil, I. K. [Eds.] *The Molecular Biology of Plant Mitochondria.* Kluwer Academic Publishers, Amsterdam, The Netherlands, pp. 1–59.