Evidence for Horizontal Gene Transfer from Bacteroidetes Bacteria to Dinoflagellate Minicircles

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Abstract

Dinoflagellate protists harbor a characteristic peridinin-containing plastid that evolved from a red or haptophyte alga. In contrast to typical plastids that have ~100–200 kb circular genomes, the dinoflagellate plastid genome is composed of minicircles that each encode 0–5 genes. It is commonly assumed that dinoflagellate minicircles are derived from a standard plastid genome through drastic reduction and fragmentation. However, we demonstrate that the *ycf16* and *ycf24* genes (encoded on the *Ceratium* AF490364 minicircle), as well as *rpl28* and *rpl33* (encoded on the *Pyrocystis* AF490367 minicircle), are related to sequences from *Algoriphagus* and/or *Cytophaga* bacteria belonging to the Bacteroidetes clade. Moreover, we identified a new open reading frame on the *Pyrocystis* minicircle encoding a SRP54 N domain, which is typical of FtsY proteins. Because neither of these minicircles share sequence similarity with any other dinoflagellate minicircles, and their genes resemble bacterial operons, we propose that these *Ceratium* and *Pyrocystis* minicircles resulted from a horizontal gene transfer (HGT) from a Bacteroidetes donor. Our findings are the first indication of HGT to dinoflagellate minicircles, highlighting yet another peculiar aspect of this plastid genome.

Key words: dinoflagellate plastid, horizontal gene transfer, minicircle, *Ceratium*, *Pyrocystis*, *rpl28*, *rpl33*, *ycf16*, *ycf24*.

Introduction

Dinoflagellates are a peculiar group of protists with significant ecological and evolutionary importance (Delwiche 2007). They are closely related to parasitic apicomplexans (containing a nonphotosynthetic plastid called apicoplast) and heterotrophic ciliates (entirely aplastidic), forming the super-assemblage Alveolata (Keeling 2009). During their evolution, dinoflagellates have acquired several plastid types from distinct algal sources; however, most harbor the so-called peridinin plastid surrounded by three membranes and containing the carotenoid peridinin (Delwiche 2007). This plastid certainly evolved from a eukaryotic alga, but it still is debated whether it was a red alga (Keeling 2009) or a haptophyte (Bodył et al. 2009).

The peridinin plastid is characterized by the presence of a peculiar genome composed of small circular chromosomes called minicircles (Zhang et al. 1999; Howe et al. 2008). At present, complete sequences are known for more than 100 minicircles isolated from numerous dinoflagellate genera, including *Adenoides*, *Amphidinium*, *Ceratium*, *Heterocapsa*, *Protoceratium*, *Pyrocystis*, and *Symbiodinium* (Howe et al. 2008). They range in size from 0.4 to 10 kb and contain noncoding regions that can be conserved among minicircles within a given species (Howe et al. 2008). These regions, consisting of repetitive sequences, exhibit an ability to form secondary structures that could be involved in recombination, replication, and segregation of minicircles (Zhang et al. 1999; Nelson and Green 2005). Although some dinoflagellate minicircles encode up to five genes, the overall coding capacity of the peridinin plastid is surprisingly poor and does not exceed ~20 genes in total (Howe et al. 2008). In addition to minicircles, a 50–150 kb DNA molecule, in the size range of typical plastid genomes, was identified in some peridinin plastids (Laatsch et al. 2004; Wang and Morse 2006). This DNA contains mainly noncoding sequences, although some genes, including *psbA*, were present as well (Wang and Morse 2006).

To explain the astonishing genome organization of the peridinin plastid, one could assume reduction of an ancestral population of typical plastid chromosomes to form the currently observed minicircles, a process driven by the short times needed to replicate smaller genomes (Zhang et al. 2002). To date, two models have been advanced to explain the reduction process of the ancestral dinoflagellate plastid genome. The first assumes duplication and transposition of replication initiation sites; the additional sites made the genome recombinationally unstable, resulting in its drastic reduction by numerous intra- and interchromosomal recombinations (Zhang et al. 2002). The second model postulates that many deletion episodes, probably initiated within the tandem repeats, led to a differentiation of the multiple plastid chromosomes and finally to their unprecedented reduction into minicircles (Zhang et al. 2002).

Both of these evolutionary scenarios assume that all dinoflagellate minicircles are derived from an ancestral plastid genome and, consequently, presume a common origin

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of their noncoding and coding regions; however, these predictions have yet to be tested. To this end, we performed BLASTN searches querying with all 103 minicircle sequences (for accession numbers, see supplementary table S1, Supplementary Material online). Interestingly, the only sequences that did not show similarity to regions of other dinoflagellate minicircles were found in *Ceratium horridum* (AF490364) and *Pyrocystis lunula* (AF490367) (see supplementary fig. S1, Supplementary Material online). The lack of hits to the *Pyrocystis* AF490367 minicircle could be explained by poor sequence sampling in available databases, which contain only two of its minicircles. This caution does not apply to *Ceratium*, however, which is represented by as many as 18 minicircles. In order to verify whether genes encoded on these minicircles belong to the red algal plastid lineage (as was demonstrated for other minicircle genes), we performed additional similarity searches and phylogenetic analyses (see Materials and Methods in Supplementary Material online) focusing on four protein-encoding genes: "hypothetical chloroplast open reading frames 16 (*ycf16*)" and *ycf24* (on the *Ceratium* minicircle) as well as *rpl28* and *rpl33* (on the *Pyrocystis* minicircle).

Results

Origin of *ycf16* and *ycf24*

Genes *ycf16* and *ycf24*, respectively, are similar to bacterial *sufC* and *sufB* that encode proteins constituting part of the bacterial SUF (mobilization of sulfur) system; SUF is responsible for assembly and repair of iron-sulfur [Fe-S] clusters under oxidative stress conditions (Ellis et al. 2001). The bacterial SUF system is encoded by the *sufABCDSE* operon, whereas eukaryotic *suf* homologs are dispersed between plastid and nuclear genomes (Balk and Lobréaux 2005). *SufC* and *sufB* appear to be the most conserved *suf* genes; they are present in Archaea, Bacteria, and plastid-containing eukaryotes (Ellis et al. 2001; Watanabe et al. 2005; Xu et al. 2005). The *ycf16* (*sufC*) and *ycf24* (*sufB*) genes are adjacent to each other in the plastid genomes of red algae, stramenopiles, and cryptophytes (based on the gene annotations in the databases listed in the Supplementary Material online). The plastid genome of the haptophyte *Emiliania huxleyi* encodes only *ycf24* (Sánchez-Puerta et al. 2005). Given the commonly assumed origin of the peridinin plastid from the red algal plastid lineage (Bodył et al. 2009; Keeling 2009), the presence of *ycf16* and *ycf24* genes on the *Ceratium* minicircle could be regarded as an example of vertical inheritance; however, our results put this assumption in doubt.

The closest BLAST hits to translated *Ceratium ycf16* and *ycf24* genes are from bacterial sequences belonging to the Bacteroidetes group, which suggests horizontal gene transfer (HGT) of these sequences. Further supporting this hypothesis, phylogenies for each gene (supplementary figs. S2 and S3, Supplementary Material online), as well as for concatenated alignments of Ycf16 and Ycf24 (fig. 1), all indicate bacterial origins of the *Ceratium* sequences. Trees inferred using different algorithms (see Materials and Methods in the Supplementary Material online) had very similar topologies, with the *Ceratium* Ycf16 and Ycf24 sequences emerging from within the Bacteroidetes clade with high bootstrap support. The sequences closest to the *Ceratium* genes are from *Cytophaga* and *Algoriphagus*, suggesting that related bacteria donated *ycf16* and *ycf24* genes to the dinoflagellate minicircles via the HGT.

The sequences from *Ceratium* clearly are separated from a well-defined clade of plastid-containing protists, including those with red alga-derived plastids (fig. 1 and supplementary figs. S2 and S3, Supplementary Material online). This plastid clade also includes Ycf16 from *Amphidinium carterae*, another peridinin-containing dinoflagellate (supplementary fig. S2, Supplementary Material online); this gene likely was transferred from the plastid to the host nuclear genome (Bachvaroff et al. 2004). The strongly supported and distinct positions of *Ceratium* and *Amphidinium* sequences indicate they arrived in the two dinoflagellates through independent evolutionary pathways. All alternative topologies that assume close relationships between the *Ceratium* sequence and putative homologs from *Amphidinium*, apicomplexans, or haptophytes were firmly rejected (see supplementary table S3, Supplementary Material online).

Interestingly, some β-proteobacteria sequences also group with *Amphidinium* Ycf16 (supplementary fig. S2, Supplementary Material online); however, this clade has poor support suggesting that the positions of these bacterial sequences could be an artifact of their higher divergence rates. Nevertheless, we cannot exclude that these bacteria actually acquired their *ycf16* gene from an alga via HGT.

Origin of *rpl28* and *rpl33*

The *rpl28* and *rpl33* genes encode proteins associated with the large subunit of bacterial and plastid ribosomes (Yamaguchi et al. 2003). Bacterial versions of *rpl28* (annotated as *rpmB*) and *rpl33* (annotated as *rpmG*) are encoded by the *rpmBG* operon (Maguire and Wild 1997). In red algae, both *rpl28* and *rpl33* are present in plastid genomes (separated by 30 to 90 open reading frames [ORFs]), whereas in cryptophytes, stramenopiles, and haptophytes, these genes are either plastid (*rpl33*) or nuclear (*rpl28*) encoded (see the gene annotations in the Supplementary Material online). In contrast to red algal plastid genomes, *rpl28* and *rpl33* genes are adjacent on the *Pyrocystis* AF490367 minicircle, resembling the bacterial *rpmBG* operon. In support of this bacteria-like syntenic association, homology searches showed much higher similarity of *Pyrocystis* Rpl28 and Rpl33 sequences to bacterial proteins than to plastid-derived homologs. As with the *Ceratium* Ycf16 and Ycf24 sequences, the best hits were to the Bacteroidetes group. A significant association of *Pyrocystis* Rpl33 with Bacteroidetes sequences is further supported by phylogenetic analyses (supplementary fig. S4, Supplementary Material online), although the tree is poorly resolved because of the very short alignment. Nevertheless, nuclear-encoded *A. carterae* Rpl33 (Bachvaroff et al. 2004) clusters with nuclear-encoded sequences from Apicomplexa (supplementary fig. S4, Supplementary Material online)

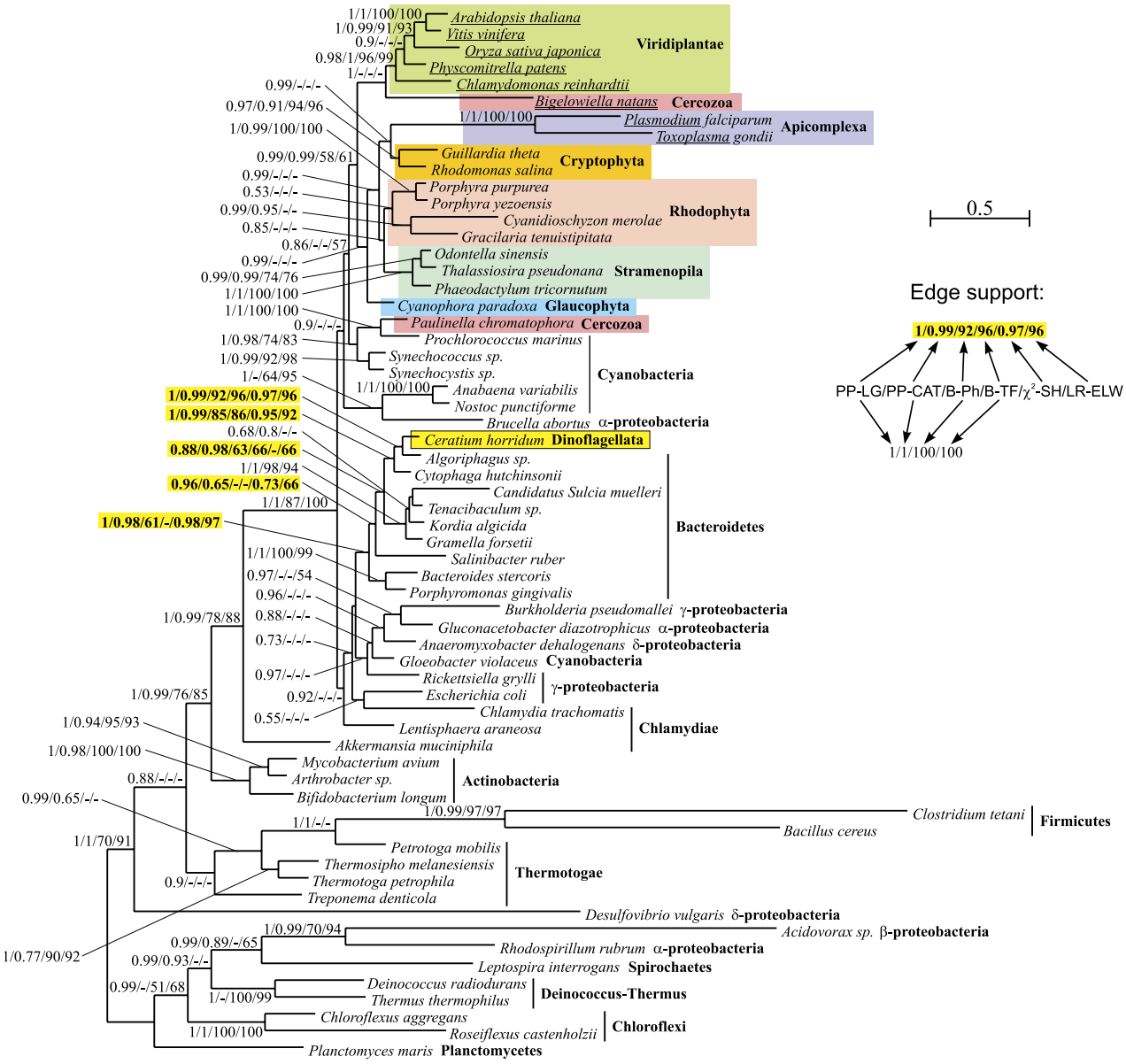

FIG. 1. The Bayesian tree for the Ycf16 + Ycf24 set obtained in PhyloBayes under the LG + Γ model. Plastid-derived sequences from eukaryotic taxa are in colored rectangles. Sequences encoded in the nuclear genome are underlined. The nucleomorph-encoded sequence from the chlorarachniophyte *Bigelowiella natans* is double underlined. In the case of two apicomplexan sequences, Ycf16 is encoded by the nuclear genome, and Ycf24 by the plastid genome; therefore only part of the name is underlined. Sequences of unknown localization are indicated by an asterisk (*). Numbers at nodes, in the order shown, correspond to posterior probabilities estimated in Phylobayes for LG + Γ model (PP-LG) and CAT + Γ model (PP-CAT), as well as support values resulting from bootstrap analysis in PhyMl (B-Ph) and TreeFinder (B-TF). Additionally, minimum support values calculated in PhyMl by χ^2 and Shimodaira-Hasegawa–like procedure (χ^2-SH), and support values obtained by Local Rearrangements-Expected Likelihood Weights method in TreeFinder (LR-EWL) are shown for selected nodes that refer to the position of the dinoflagellate *Ceratium horridum* sequences. Values of the posterior probabilities and bootstrap percentages lower or equal to 0.50 and 50%, respectively, are omitted or indicated by a dash (–).

with moderate support, indicating a different evolutionary history from the *Pyrocystis* gene. Moreover, alternative topologies grouping the *Pyrocystis* Rpl33 with putative homologs from *Amphidinium*, apicomplexans or haptophytes all were significantly worse than the best tree recovered (supplementary table S3, Supplementary Material online). The third dinoflagellate gene, *rpl33* from *Karlodinium micrum*, groups with sequences from the haptophyte *E. huxleyi*, consistent with a haptophyte origin of its fucoxanthin plastid (Shalchian-Tabrizi et al. 2006).

The *Pyrocystis* Rpl28 protein also clusters with bacterial sequences (including those from Bacteroidetes) on the PhyloBayes tree (supplementary fig. S5, Supplementary Material online). Although not all phylogenetic methods provide significant support for this grouping, the *Pyrocystis* sequence clearly is separated from a well-supported clade

of plastid-derived sequences and their cyanobacterial homologs. Alternative topologies grouping the *Pyrocystis* Rpl28 sequence with putative homologs from apicomplexans or haptophytes were rejected, whereas trees that considered different positions of this sequence among the Bacteroidetes were not significantly worse than the best tree (supplementary table S3, Supplementary Material online).

One ostensible plastid-derived sequence ascribed to the diatom *Phaeodactylum tricornutum* clusters with the *Pyrocystis* Rpl28 on the PhyloBayes tree, but without significant support (supplementary fig. S5, Supplementary Material online). Moreover, the position of this diatom sequence was not stable across phylogenetic methods; the TreeFinder tree grouped it with other bacterial sequences, away from the *P. lunula* homolog (tree not shown).

In some bacterial genomes (e.g. *Gramella forsetii* KT0803 belonging to the Bacteroidetes clade) an additional *ftsY* gene resides 299 bp downstream of the *rpmBG* operon. FtsY participates in co-translational protein transport as a receptor for the SRP (Signal Recognition Particle) complex associated with a nascent polypeptide and its ribosome (Chandrasekar et al. 2008). The cyanobacterial ortholog of *ftsY*, annotated as *cpftsY* in plastid-containing eukaryotes, is nuclear-encoded and its protein product is imported into the plastid (Chandrasekar et al. 2008). Interestingly, we identified an unannoted ORF on the *Pyrocystis* minicircle located 493 bp downstream of the *rpl33* gene that overlaps with the *rpl28* if recircularization of the minicircle is assumed (see supplementary fig. S6, Supplementary Material online). Protein homology searches recovered Bacteroidetes FtsY as top hits to this ORF sequence. According to Pfam database searches and eight programs that predict protein secondary structures, the translated ORF contains a SRP54 N domain with characteristic α-helices typical of FtsY proteins (supplementary fig. S7, Supplementary Material online). However, the inferred translation product is only 70 amino acids long and lacks a G domain with GTPase properties; in contrast, bacterial FtsY and plastid cpFtsY proteins contain more than 300 residues (Chandrasekar et al. 2008). Because the newly identified ORF contains only a part of the original *ftsY* sequence, it could be hypothesized to be a pseudogene; however, the part of the sequence that is retained is relatively conserved, suggesting that some function remains, as was hypothesized previously for truncated *Amphidinium* rRNA minicircle genes (Barbrook et al. 2006).

Discussion

Results of our phylogenetic analyses indicate a bacterial origin of the genes encoded on *Ceratium* AF490364 and *Pyrocystis* AF490367 minicircles. This hypothesis is further supported by the presence of *rpmBG*-like region along with an adjacent *fts Y* on the *Pyrocystis* minicircle. Our results suggest that bacteria related to *Cytophaga* and/or *Algoriphagus* donated this genetic material (for data and arguments against bacterial sequence contamination, see the Discussion, section "Evidence against bacterial sequence contamination of *Ceratium* and *Pyrocystis* minicircles" in the Supplementary Material online). Both of these Gram-negative genera are cosmopolitan obligate aerobes belonging to one of the more abundant Cytophaga–Flavobacteria groups within the Bacteroidetes clade (Kirchman 2002). Many members of *Cytophaga* are pathogens of cyanobacteria, green algae, and red algae, whereas *Algoriphagus* species have been isolated from the green algae (*Acrosiphonia sonderi*) and the stramenopiles (*Chorda filum*) (Nedashkovskaya et al. 2007). Moreover, bacteria classified in the Bacteroidetes also have been identified as endosymbionts in the dinoflagellates *Heterocapsa circularisquama* (Maki et al. 2004) and *Alexandrium minutum* (Palacios and Marín 2008). These observed associations provide a reasonable explanation for how minicircle genes in *Ceratium* and *Pyrocystis* could have been acquired from intracellular bacteria. Because *Ceratium* and *Pyrocystis* are closely related members of the order Gonyaulacales (Zhang et al. 2007), it is reasonable to postulate that HGT could have occurred in a common ancestor of these genera. In support of this view, their minicircle genes group with the same or closely related bacteria in the phylogenetic trees. At least 16 cases of HGT from bacteria to dinoflagellate nuclei have been identified to date (e.g., Waller, Patron, et al. 2006; Waller, Slamovits, et al., 2006; Nosenko and Bhattacharya 2007). Interestingly, in some instances, the donors were members of the Bacteroidetes group (Nosenko and Bhattacharya 2007), further supporting our hypothesis of a bacteroidetal origin of the *Ceratium* and *Pyrocystis* minicircle genes.

Until now, only Laatsch et al. (2004) have reported the presence of *Ceratium* minicircles (including AF490364) in nuclear DNA fractions and assumed they are present only in the nucleus. If the same were to be true of the *Pyrocystis* AF490367 minicircle (as suggested by the database description), then *ycf16*, *ycf24*, *rpl28*, *rpl33*, and *FtsY* all would be examples of HGT from bacteria to the dinoflagellate nuclear genome. However, it should be stressed that Laatsch et al. (2004) localized only one minicircle gene, *psbB*, to the nucleus. Moreover, their in situ hybridization experiments also showed plastid localization of *psbB* (Laatsch et al. 2004), thereby indicating that the *Ceratium psbB* is present in both the nucleus and the plastid. In addition, some Southern blot data (fig. 1, lane F* in Laatsch et al. 2004) show a weak signal from the hybridization of *psbB* probe with the *Ceratium* plastid DNA. This suggests that the other Southern blots that did not confirm the plastid residence of *psbB* (Laatsch et al. 2004) could have resulted from insufficient target DNA due to very low copy numbers of minicircles. In support of this view, Southern blot analysis of *Amphidinium operculatum* minicircles showed that copy number is very low during the exponential growth stage but increases during later growth (Koumandou and Howe 2007) (see also the Discussion, section "Are *Ceratium* minicircles recovered in higher-molecular weight fractions of plastid DNA?" in the Supplementary Material online).

Nuclear localization of the active genes we analyzed on the *Ceratium* and *Pyrocystis* minicircles appears to be rather improbable for three reasons. First, none of these

genes encodes a bi- or tripartite presequence required for import of nuclear-encoded proteins into the peridinin plastid (Patron et al. 2005). Second, Zauner et al. (2004) found that transcripts generated in vivo from *psbB* and other *C. horridum* minicircle genes are edited. Because RNA editing is known to occur in the dinoflagellate peridinin plastid (Lin 2011), it is most parsimonious to assume a plastid residence of *Ceratium* minicircles. Third, plastid localization of the four *Ceratium* and *Pyrocystis* minicircle genes analyzed also is supported by their 37–41% G + C content, which overlaps the range typical of other minicircle genes (33–51%, mean = 40% calculated from 85 minicircle-encoded genes) and is well below the 55–62% G + C content range of nuclear-encoded genes in dinoflagellates (e.g., Kii et al. 2007; McEwan et al. 2008).

The movement of the plastid DNA into the nucleus, and the presence of both organelle- and nucleus-localized gene copies are characteristic of the initial stages of endosymbiotic gene transfer (EGT) (Selosse et al. 2001). EGT has occurred on a massive scale in dinoflagellates (Hackett et al. 2004), and we hypothesize that it is an ongoing process, which accounts for the presence of *psbB* gene in the *Ceratium* nucleus. This is consistent with the nonintegrated state of *psbB* and its residence in the nuclear matrix between chromosomes (Laatsch et al. 2004). Our hypothesis presents a holistic and dynamic synthesis accounting for all available data regarding minicircle localization in the *Ceratium* cell. It reconciles nuclear (but extrachromosomal) and plastid localizations of *C. horridum psbB* with data demonstrating the common plastid residence of minicircles in other dinoflagellate species (Howe et al. 2008).

All available data are consistent with the hypothesis that the bacterium-derived genes on the *Ceratium* and *Pyrocystis* minicircles are the result of HGT from the Bacteroidetes bacteria to the peridinin plastid. Unlike mitochondria, however, plastid genomes have been transformed very rarely by HGT in the course of evolution (Rice and Palmer 2006). In eukaryotic alga-derived plastids, which are represented by at least 10 distinct lineages and originated through an undetermined number of independent endosymbioses (Bodył et al. 2009), only a few examples of such gene transfers are known. A proteobacterium- or planctomycete-derived ribosomal protein gene (*rpl36*) was found in the plastid genomes of cryptophytes and haptophytes (Rice and Palmer 2006). In turn, DNA polymerase III-encoding *dnaX* was transferred from Firmicutes bacteria specifically to the *Rhodomonas* lineage within the Cryptophyta (Khan et al. 2007). Two independent transfers to the plastid genomes of apicomplexans were identified: one was from spirochetes (*clpC* encoding ATPase class III) (Zhu 2004) and the second involved three genes (*rpl2*, *rpl14*, and *rps12*) from the mitochondrion (Oborník et al. 2002). Our phylogenetic investigation demonstrates, for the first time, HGT to dinoflagellate minicircles and implies a chimeric origin of the peridinin plastid genome. Therefore, the future reconstructions that its evolutionary history should take into account are possible interactions of the ancestral plastid genome with the foreign DNA molecules, and the current models should be revised accordingly.

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References

Bachvaroff TR, Concepcion GT, Rogers CR, Herman EM, Delwiche CF. 2004. Dinoflagellate expressed sequence tag data indicate massive transfer of chloroplast genes to the nuclear genome. *Protist* 155:65–78.

Balk J, Lobréaux S. 2005. Biogenesis of iron-sulfur proteins in plants. *Trends Plant Sci.* 7:324–331.

Barbrook AC, Santucci N, Plenderleith LJ, Hiller RG, Howe CJ. 2006. Comparative analysis of dinoflagellate chloroplast genomes reveals rRNA and tRNA genes. *BMC Genomics* 7:297.

Bodył A, Stiller JW, Mackiewicz P. 2009. Chromalveolate plastids: direct descent or multiple endosymbioses? *Trends Ecol Evol.* 24:119–121.

Chandrasekar S, Chartron J, Jaru-Ampornpan P, Shan S. 2008. Structure of the chloroplast signal recognition particle (SRP) receptor: domain arrangement modulates SRP-receptor interaction. *J Mol Biol.* 375:425–436.

Delwiche CF. 2007. The origin and evolution of dinoflagellates. In: Falkowski PG, Knoll AH, editors. Evolution of primary producers in the sea. Oxford: Elsevier Academic Press. p. 191–205.

Ellis KE, Clough B, Saldanha JW, Wilson RJ. 2001. Nifs and Sufs in malaria. *Mol Microbiol.* 41:973–981.

Hackett JD, Yoon HS, Soares MB, Bonaldo MF, Casavant TL, Scheetz TE, Nosenko T, Bhattacharya D. 2004. Migration of the plastid genome to the nucleus in a peridinin dinoflagellate. *Curr Biol.* 14:213–218.

Howe CJ, Nisbet RE, Barbrook AC. 2008. The remarkable chloroplast genome of dinoflagellates. *J Exp Bot.* 59:1035–1045.

Keeling PJ. 2009. Chromalveolates and the evolution of plastids by secondary endosymbiosis. *J Eukaryot Microbiol.* 56:1–8.

Khan H, Parks N, Kozera C, Curtis BA, Parsons BJ, Bowman S, Archibald JM. 2007. Plastid genome sequence of the cryptophyte alga *Rhodomonas salina* CCMP1319: lateral transfer of putative DNA replication machinery and a test of chromist plastid phylogeny. *Mol Biol Evol.* 24:1832–1842.

Kii S-I, Tanaka J, Watanabe T. 2007. Guanine-cytosine contents of the host and symbiont cDNA in a symbiotic coral. *Fish Sci.* 73:1362–1372.

Kirchman DL. 2002. The ecology of Cytophaga-Flavobacteria in aquatic environments. *FEMS Microbiol Ecol.* 39:91–100.

Koumandou VL, Howe CJ. 2007. The copy number of chloroplast gene minicircles changes dramatically with growth phase in the dinoflagellate *Amphidinium operculatum*. *Protist* 158:89–103.

Laatsch T, Zauner S, Stoebe-Maier B, Kowallik KV, Maier UG. 2004. Plastid-derived single gene minicircles of the dinoflagellate *Ceratium horridum* are localized in the nucleus. *Mol Biol Evol.* 21:1318–1322.

Lin S. 2011. Genomic understanding of dinoflagellates. *Res Microbiol.* 162:551–569.

Maguire BA, Wild DG. 1997. Mutations in the *rpmBG* operon of *Escherichia coli* that affect ribosome assembly. *J Bacteriol.* 179:2486–2493.

Maki T, Yoshinaga I, Katanozaka N, Imai I. 2004. Phylogenetic analysis of intracellular bacteria of a harmful marine microalga, *Heterocapsa circularisquama* (Dinophyceae). *Aquat Microb Ecol.* 36:123–135.

McEwan M, Humayun R, Slamovits CH, Keeling PJ. 2008. Nuclear genome sequence survey of the dinoflagellate *Heterocapsa triquetra*. *J Eukaryot Microbiol.* 55:530–535.

Nedashkovskaya OI, Kim SB, Kwon KK, Shin DS, Luo X, Kim SJ, Mikhailov VV. 2007. Proposal of *Algoriphagus vanfongensis* sp. nov., transfer of members of the genera *Hongiella* Yi and Chun 2004 emend. Nedashkovskaya et al. 2004 and *Chimaereicella* Tiago et al. 2006 to the genus *Algoriphagus*, and emended description of the genus *Algoriphagus* Bowman et al. 2003 emend. Nedashkovskaya et al. 2004. *Int J Syst Evol Microbiol.* 57:1988–1994.

Nelson MJ, Green BR. 2005. Double hairpin elements and tandem repeats in the non-coding region of *Adenoides eludens* chloroplast gene minicircles. *Gene* 358:102–110.

Nosenko T, Bhattacharya D. 2007. Horizontal gene transfer in chromalveolates. *BMC Evol Biol.* 7:173.

Oborník M, Van de Peer Y, Hypsa V, Frickey T, Slapeta JR, Meyer A, Lukes J. 2002. Phylogenetic analyses suggest lateral gene transfer from the mitochondrion to the apicoplast. *Gene* 285:109–118.

Palacios L, Marín I. 2008. Enzymatic permeabilization of the thecate dinoflagellate *Alexandrium minutum* (Dinophyceae) yields detection of intracellularly associated bacteria via catalyzed reporter deposition-fluorescence in situ hybridization. *Appl Environ Microbiol.* 74:2244–2247.

Patron NJ, Waller RF, Archibald JM, Keeling PJ. 2005. Complex protein targeting to dinoflagellate plastids. *J Mol Biol.* 348:1015–1024.

Rice DW, Palmer JD. 2006. An exceptional horizontal gene transfer in plastids: gene replacement by a distant bacterial paralog and evidence that haptophyte and cryptophyte plastids are sisters. *BMC Biol.* 4:31.

Sánchez-Puerta MV, Bachvaroff TR, Delwiche CF. 2005. The complete plastid genome sequence of the haptophyte *Emiliania huxleyi*: a comparison to other plastid genomes. *DNA Res.* 12:151–156.

Selosse M, Albert B, Godelle B. 2001. Reducing the genome size of organelles favours gene transfer to the nucleus. *Trends Ecol Evol.* 16:135–141.

Shalchian-Tabrizi K, Skånseng M, Ronquist F, Klaveness D, Bachvaroff TR, Delwiche CF, Botnen A, Tengs T, Jakobsen KS. 2006. Heterotachy processes in rhodophyte-derived secondhand plastid genes: implications for addressing the origin and evolution of dinoflagellate plastids. *Mol Biol Evol.* 23:1504–1515.

Waller RF, Patron NJ, Keeling PJ. 2006. Phylogenetic history of plastid-targeted proteins in the peridinin-containing dinoflagellate *Heterocapsa triquetra*. *Int J Syst Evol Microbiol.* 56:1439–1447.

Waller RF, Slamovits CH, Keeling PJ. 2006. Lateral gene transfer of a multigene region from cyanobacteria to dinoflagellates resulting in a novel plastid-targeted fusion protein. *Mol Biol Evol.* 23:1437–1443.

Wang Y, Morse D. 2006. The plastid-encoded *psbA* gene in the dinoflagellate *Gonyaulax* is not encoded on a minicircle. *Gene* 371:206–210.

Watanabe S, Kita A, Miki K. 2005. Crystal structure of atypical cytoplasmic ABC-ATPase SufC from *Thermus thermophilus* HB8. *J Mol Biol.* 353:1043–1054.

Xu XM, Adams S, Chua NH, Møller SG. 2005. AtNAP1 represents an atypical SufB protein in *Arabidopsis* plastids. *J Biol Chem.* 280:6648–6654.

Yamaguchi K, Beligni MV, Prieto S, Haynes PA, McDonald WH, Yates JR 3rd, Mayfield SP. 2003. Proteomic characterization of the *Chlamydomonas reinhardtii* chloroplast ribosome. Identification of proteins unique to the 70S ribosome. *J Biol Chem.* 278:33774–33785.

Zauner S, Greilinger D, Laatsch T, Kowallik KV, Maier UG. 2004. Substitutional editing of transcripts from genes of cyanobacterial origin in the dinoflagellate *Ceratium horridum*. *FEBS Lett.* 577:535–538.

Zhang H, Bhattacharya D, Lin S. 2007. A three-gene dinoflagellate phylogeny suggests monophyly of prorocentrales and a basal position for *Amphidinium* and *Heterocapsa*. *J Mol Evol.* 65:463–474.

Zhang Z, Cavalier-Smith T, Green BR. 2002. Evolution of dinoflagellate unigenic minicircles and the partially concerted divergence of their putative replicon origins. *Mol Biol Evol.* 19:489–500.

Zhang Z, Green BR, Cavalier-Smith T. 1999. Single gene circles in dinoflagellate chloroplast genomes. *Nature* 400:155–159.

Zhu XY. 2004. Phylogenetic analysis indicates bacteria-to-apicoplast lateral gene transfer. *Yi Chuan Xue Bao* 31:1316–1320.