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Origins of plastids and glyceraldehyde-3-phosphate dehydrogenase genes in the green-colored dinoflagellate *Lepidodinium chlorophorum*

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Abstract

The dinoflagellate *Lepidodinium chlorophorum* possesses "green" plastids containing chlorophylls *a* and *b* (Chl *a*+*b*), unlike most dinoflagellate plastids with Chl *a*+*c* plus a carotenoid peridinin (peridinin-containing plastids). In the present study we determined 8 plastid-encoded genes from *Lepidodinium* to investigate the origin of the Chl *a*+*b*-containing dinoflagellate plastids. The plastid-encoded gene phylogeny clearly showed that *Lepidodinium* plastids were derived from a member of Chlorophyta, consistent with pigment composition. We also isolated three different glyceraldehyde-3-phosphate dehydrogenase (GAPDH) genes from *Lepidodinium*—one encoding the putative cytosolic "GapC" enzyme and the remaining two showing affinities to the "plastid-targeted GapC" genes. In a GAPDH phylogeny, one of the plastid-targeted GapC-like sequences robustly grouped with those of dinoflagellates bearing peridinin-containing plastids, while the other was nested in a clade of the homologues of haptophytes and dinoflagellate genera *Karenia* and *Karlodinium* bearing "haptophyte-derived" plastids. Since neither host nor plastid phylogeny suggested an evolutionary connection between *Lepidodinium* and *Karenia*/*Karlodinium*, a lateral transfer of a plastid-targeted GapC gene most likely took place from a haptophyte or a dinoflagellate with haptophyte-derived plastids to *Lepidodinium*. The plastid-targeted GapC data can be considered as an evidence for the single origin of plastids in haptophytes, cryptophytes, stramenopiles, and alveolates. However, in the light of *Lepidodinium* GAPDH data, we need to closely examine whether the monophyly of the plastids in the above lineages inferred from plastid-targeted GapC genes truly reflects that of the host lineages.

Keywords: Chromalveolate hypothesis; Dinoflagellates; GAPDH; Lateral gene transfer; Plastid replacement

1. Introduction

Plastids in major photosynthetic dinoflagellates contain chlorophylls *a* and *c* (Chl *a*+*c*) plus a unique carotenoid peridinin (peridinin-containing plastids). Molecular phylogenetic analyses of peridinin-containing plastids have consistently displayed an evolutionary affinity to red algal and red alga-derived plastids (e.g., Takishita and Uchida, 1999; Zhang et al., 2000; Inagaki et al., 2004; Bachvaroff et al., 2005; Shalchian-

Abbreviations: AU, approximately unbiased; EGT, endosymbiotic gene transfer; Chl, chlorophylls; GAPDH, glyceraldehydes-3-phosphate dehydrogenase; LGT, lateral gene transfer; LSU rRNA, large subunit of ribosomal RNA; ML, maximum-likelihood; ER, endoplasmic reticulum; ASRV, among-sites rate variation; BP, bootstrap probability.

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Tabrizi et al., 2006b; Iida et al., 2007; Sanchez Puerta et al., 2007). However, a few groups of dinoflagellates have plastids in which pigmentation patterns are different from the canonical (i.e. peridinin-containing) plastids. Such "non-canonical" dinoflagellate plastids are similar to those of other eukaryotic algae in pigment composition and/or ultrastructural characteristics, implying that these types of plastids are the remnants of endosymbiotic eukaryotic algae (Archibald and Keeling, 2004; Bhattacharya et al., 2004). In nucleus-encoded gene phylogenies, the dinoflagellate species with non-canonical plastids are scattered amongst the species with peridinin-containing plastids, suggesting that peridinin-containing plastids were ancestral, but were replaced by endosymbiotic algae in multiple lineages in dinoflagellate evolution (Saldarriaga et al., 2001; Shalchian-Tabrizi et al., 2006a).

At least three types of non-canonical plastids are known in dinoflagellates, not including transiently acquired plastids (so-called kleptoplastids) from cryptophytes in *Dinophysis* spp. (Takishita et al., 2002; Park et al., 2006). Firstly, the genera *Karenia* and *Karlodinium* possess plastids with Chl *a*+*c* plus fucoxanthin and its derivatives (19' hexanoyloxyfucoxanthin and/or 19' butanoyloxyfucoxanthin) as carotenoids, which are otherwise found in haptophyte plastids. Indeed, plastid-encoded gene phylogenies clearly show that the plastids in *Karenia* and *Karlodinium* were derived from a haptophyte (haptophyte-derived plastids) (Takishita et al., 1999, 2000; Tengs et al., 2000). Secondly, permanent diatom or diatom-like endosymbionts are observed in *Durinskia baltica*, *Kryptoperdinium foliaceum*, and *Peridinium quinquecorne*, and plastid-encoded gene data later confirmed the diatom origin of these dinoflagellate plastids (Chesnick et al., 1996; Horiguchi and Takano, 2006). Finally, the genus *Lepidodinium* (*L. viride* and *L. chlorophorum*) possesses green-colored plastids containing Chl *a*+*b*, indicating a possible green algal origin (Watanabe et al., 1987; Watanabe et al., 1990; Elbrächter and Schnepf, 1996; Hansen et al., 2007). To concretely identify the origin of *Lepidodinium* plastids inferred from the pigment composition data, phylogenetic analyses of plastid-encoded genes of *Lepidodinium* are indispensable.

It has been postulated that plastid replacement events are associated with the drastic modification of the host (dinoflagellate) genomes (e.g., Yoon et al., 2005). In general photosynthetic eukaryotes, the vast majority of plastid proteins are encoded in the nuclear genomes, and gene products synthesized in the cytosol are transported into plastids via protein machineries coupled with intracellular membrane systems. Therefore, when an endosymbiotic eukaryotic alga is integrated into a dinoflagellate cell as a plastid, a massive transfer of plastid-targeted genes from the endosymbiont nuclear genome to the host nuclear genome (endosymbiotic gene transfer or EGT) should occur. Amongst the dinoflagellates bearing non-canonical plastids, the EGT in *Karenia* and *Karlodinium* have been investigated, and several "haptophyte-like" plastid-targeted genes were identified in dinoflagellate nuclear genomes (Ishida and Green, 2002; Yoon et al., 2005; Nosenko et al., 2006; Patron et al., 2006). One of the endosymbiotically transferred genes in *Karenia*/*Karlodinium* is a gene encoding glyceraldehyde-3-phosphate dehydrogenase (GAPDH) that catalyzes the reversible interconversion between glyceraldehyde-3-phosphate and 1,3-diphosphoglycerate (e.g., Takishita et al., 2004). GAPDH is a ubiquitous, highly conserved enzyme, and the gene sequences can be relatively easily obtained by the polymerase chain reaction (PCR) with degenerate primers from phylogenetically diverged organisms. Thus, GAPDH genes may be a good marker to start inspecting the EGT in dinoflagellates bearing non-canonical plastids in general.

Photosynthetic eukaryotes generally possess GAPDH for both cytosol and plastids—involved in glycolysis/gluconeogenesis and Calvin cycle reactions, respectively. In land plants, green algae, red algae, glaucophytes, and euglenids, the nucleus-encoded plastid-targeted GAPDH (GapA/B) bear a clear evolutionary affinity to cyanobacterial homologues, and are distantly related to the cytosolic enzymes (GapC). These findings suggest that the ancestral GapA/B gene was endosymbiotically transferred from a cyanobacterium that gave rise to plastids, and GapA/B and GapC have two phylogenetically distinctive origins (Brinkmann et al., 1989; Martin et al., 1993; Liaud et al., 1994; Henze et al., 1995; Petersen et al., 2006a,b). In sharp contrast, dinoflagellates, cryptophytes, haptophytes, photosynthetic stramenopiles, and the apicomplexan *Toxoplasma gondii* possess no GapA/B, but utilize GapC-related enzymes for plastids (Liaud et al., 1997, 2000; Fagan et al., 1998; Fast et al., 2001; Harper and Keeling, 2003; Takishita et al., 2003). All "plastid-targeted GapC" genes form a robust monophyletic clade in phylogenetic analyses, and this result prompted the hypothesis assuming the single photosynthetic ancestry of haptophytes, cryptophytes, stramenopiles, and alveolates (including dinoflagellates, ciliates, and apicomplexans)—collectively called "chromalveolates" (Cavalier-Smith, 1999, 2002). The robust monophyly of plastid-targeted GapC genes can be interpreted as the plastid-targeted GapC gene being produced by a GapC gene duplication followed by changing the sub-cellular localization from the cytosol-to-plastids in the hypothetical photosynthetic ancestor of "chromalveolates" (Fast et al., 2001). In a subsequent genome evolution, the plastid-targeted GapC enzyme may have replaced the GapA/B enzyme, which likely operated in the initial plastids derived from an endosymbiotic red alga (Fast et al., 2001). Importantly, the chromalveolate hypothesis assumes the vertical inheritance of the plastid-targeted GapC genes during eukaryotic evolution.

In this study, we investigated the origin of Chl *a*+*b*-containing plastids in *Lepidodinium chlorophorum* (Elbrächter and Schnepf, 1996; Hansen et al., 2007). We here determined 8 plastid-encoded gene sequences of *Lepidodinium*. Our plastid-encoded gene phylogeny suggested that this species obtained its current plastids from a member of Chlorophyta. We also identified three different GapC-related genes in *Lepidodinium*, and one of the three genes unexpectedly showed a clear evolutionary affinity to the plastid-targeted GapC genes of haptophytes and dinoflagellates bearing haptophyte-derived plastids. Since the *Lepidodinium*'s host or plastid lineage has no close relationship to haptophytes, we concluded that the plastid-targeted GapC gene was laterally (not endosymbiotically) transferred from a haptophyte or a dinoflagellate bearing haptophyte-derived plastids to *Lepidodinium*. We discuss the impact of the laterally transferred plastid-targeted GapC gene detected in this study on the chromalveolate hypothesis.