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Plastid genomes of the Rhodophyta and Chromophyta constitute a distinct lineage which differs from that of the Chlorophyta and have a composite phylogenetic origin, perhaps like that of the Euglenophyta

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Summary. A phylogenetic tree has been constructed from comparisons of entire 16S rRNA gene sequences from different prokaryotes and from several algal plastids. According to this study, and to previous work on the ribulose-1,5-bisphosphate carboxylase oxygenase (Rubisco) large and small subunit genes, we postulate that: (1) rhodophyte and chromophyte plastid genomes have a common, composite phylogenetic origin which implies at least two different ancestors, a cyanobacterial and a β-proteobacterial ancestor; (2) chlorophyte (green algae and land plants) plastids have a cyanobacterial ancestor which probably differs from that of rhodophyte and chromophyte plastids, and in any case constitute a different lineage; (3) euglenophyte plastid genomes also seem to have a composite phylogenetic origin which involves two different lineages.

Key words: Algae – Molecular phylogeny – Plastid origins – Composite poly-endosymbiosis

Introduction

Recent studies, based on 16S rRNA and ribulose-1,5-bisphosphate carboxylase oxygenase (Rubisco) large and small subunit comparisons, suggest that the plastid genome of the primitive brown alga *Pylaiella littoralis* has a composite phylogenetic origin. While the 16S rRNA gene has a cyanobacterial origin (Markowicz et al. 1988a), Rubisco genes originate from β-proteobacteria (also named β purple bacteria, as defined by Woese 1987) (Assali et al. 1990). Other studies also show that Rubisco genes from the Chromophyta and Rhodophyta evolved from β-proteobacterial genes, and not from cyanobacterial genes as do those of chlorophyll *b*-containing organisms, i.e., Chlorophyta and Euglenophyta (Boczar et al. 1989; Douglas et al. 1990; Valentin and Zetsche 1990a, b).

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One important issue derived from these studies was to determine whether 16S rRNA genes from the Rhodophyta and Chromophyta were closer to other cyanobacteria-like 16S rRNA genes, which would support the plastid composite origin hypothesis (Assali et al. 1990), or whether they were more closely related to analogous genes from β-proteobacteria, which would lead to a completely revised view of plastid and prokaryote phylogeny.

Here, we compare 16S rRNA gene sequences from four α-, β- or γ-proteobacteria, one cyanobacterium (the only one available from the data banks), and the plastids of one primitive rhodophyte, two chromophytes, two euglenophytes and eight chlorophytes. A phylogenetic tree has been inferred from these sequence comparisons. Based on this analysis, and previously published results, we discuss the evolution of algal plastids.

Materials and methods

16S rRNA gene sequences from 18 different species (Table 1) were taken from the EMBL/GenBank data base and aligned using the B.I.S.A.N.C.E. software package (CITI 2, Université René Descartes, Paris, France). Homology calculations and determination of structural distances (Knuc: rate of nucleotide substitution between two homologous sequences) were performed as previously described (Markowicz et al. 1988a). The phylogenetic tree was inferred from numerical data using a Fitch/Margoliash Matrix Construction Program contained in the B.I.S.A.N.C.E. software package.

Results and discussion

The phylogenetic tree presented in Fig. 1 clearly shows that all plastid 16S rRNA genes are derived from cyanobacterial-like ancestors and not from β-proteobacteria. In contrast, plastid Rubisco genes from the Rhodophyta and Chromophyta have been shown to be closer to β-proteobacterial genes than to those from green plants and cyanobacteria. These apparently conflicting observations actually give strong support in favor of the hypothesis for a composite phylogenetic origin of plastid

Table 1. List of the different 16S rRNA gene sequences used for the construction of the phylogenetic tree in Fig. 1

Species	Taxonomic position	Length	Reference
Agrobacterium tumefaciens	α Proteobacteria	1 487	Yang et al. 1985
Alcaligenes eutrophus	β Proteobacteria	1 511*	Woese (unpublished data)
Pseudomonas testosteroni	β Proteobacteria	1 534	Yang et al. 1985
Escherichia coli (rrnD)	γ Proteobacteria	1 541	Carbon et al. 1979
Anacystis nidulans	Cyanobacteria	1 487	Tomioka and Sugiura 1983
Cyanidium caldarium	Rhodophyta	1 492	Maid and Zetsche 1990
Ochromonas danica	Chromophyta (Chrysophyceae)	1 300*	Witt and Stakebrandt 1988
Pylaiella littoralis	Chromophyta (Phaeophyceae)	1 505	Markowicz et al. 1988a
Astasia longa	Euglenophyta	1 520	Siemeister and Hachtel 1990
Euglena gracilis (rrnC)	Euglenophyta	1 491	Graf et al. 1982
Chlamydomonas reinhardtii	Chlorophycophyta	1 492	Dron et al. 1982
Chlorella ellipsoidea	Chlorophycophyta	1 583	Yamada 1988
Chlorella vulgaris	Chlorophycophyta	1 442*	Huss and Giovannoni 1989
Marchantia polymorpha	Chlorophyta (Bryophyta)	1 496	Ohyama et al. 1986
Nicotiana tabacum	Chlorophyta (Angiospermae, Dicotyledoneae)	1 486	Tohdoh and Sugiura 1982
Glycine max	Chlorophyta (Angiospermae, Dicotyledoneae)	1 470	Von Allmen and Stutz 1988
Zea mays	Chlorophyta (Angiospermae, Monocotyledoneae)	1 491	Schwarz and Kössel 1980
Oryza sativa	Chlorophyta (Angiospermae, Monocotyledoneae)	1 491	Hiratsuka et al. 1989

* Incomplete sequence

genomes from the Rhodophyta and Chromophyta, i.e., these plastids have arisen from both vertical and horizontal inheritance (Assali et al. 1990, 1991).

Plastidial 16S rRNA genes from the Chromophyta and Rhodophyta clearly share the same common cyanobacterial ancestor. Thus, it is reasonable to assume that chromophyte plastid genomes have a common origin since two of their essential genome regions, the 16S rRNA gene and the Rubisco operon, although of different prokaryotic origins (from cyanobacteria and β-proteobacteria) share a common history. This observation supports the secondary endosymbiotic theory (reviewed by Gibbs 1990) which implies that chromophyte plastids evolved from red algae engulfed by eukaryotic cells.

16S rRNA sequences also suggest that the chromophyte and rhodophyte putative common ancestor differs from that of green algae and land plants, but that both lineages originate from the cyanobacterial tree in such a close proximity that their order of branching cannot be clearly determined. In a previous study, using only six species (Markowicz et al. 1988a), we postulated that non-green plastid ancestors emerged from the cyanobacterial lineage slightly after green plastid ancestors, but a re-examination of our preceding results indicates that, as in this study, the branching order can not be determined with certainty.

The two prokaryotic lineages leading to the plastidial 16S rRNA genes emerged roughly at the same geological time, as did also the radiations of each of these lineages. From these results we cannot infer at what moment the endosymbiotic events which gave rise to plastids occurred. The different protists which engulfed cyanobacterial-like prokaryotes, giving rise to rhodophytes and to chlorophytes respectively (Douglas et al. 1991), could have taken their cyanobacterial endosymbionts on our tree just before the separation of both plastidial lineages or sometime between the separation of these lineages and their first radiations.

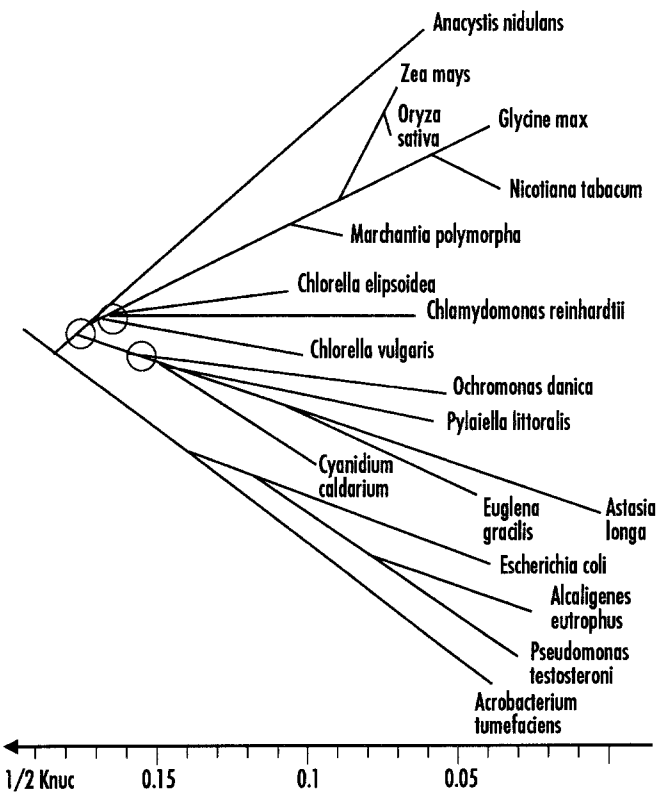

Fig. 1. A rooted phylogenetic tree depicting evolutionary relationships among 16S rRNA genes from *A. nidulans*, a cyanobacteria, chloroplasts from Chlorophyta, Euglenophyta, Chromophyta and Rhodophyta, and microorganisms representative of the α (*A. tumefaciens*), β (*A. eutrophus*, *P. testosteroni*) and γ (*E. coli*) subdivisions of the class proteobacteria. Different data input orders always led to the same result. In this figure, evolutionary distances between nodes of the tree (Knuc) are represented by the horizontal component of their separation. The junction-point between the proteobacterial and the cyanobacterial/plastidial branches was determined as previously described (Markowicz et al. 1988a). Circled regions correspond to the standard deviation: in these regions, the branching order can be randomly changed since the depicted Knuc values lie between (Knuc+σ) and (Knuc−σ)

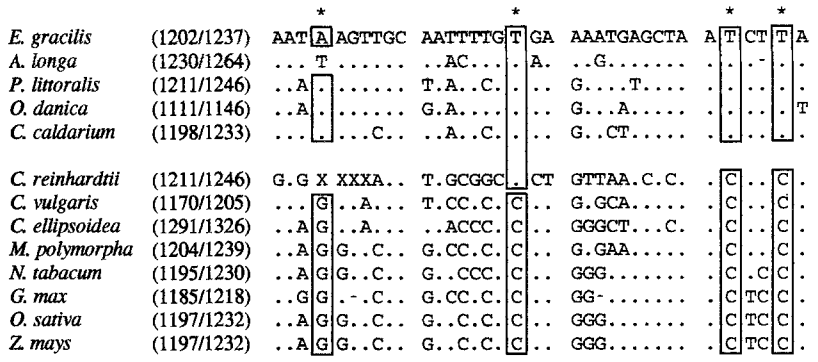

Fig. 2. Alignment of nucleotides from different plastid 16S rRNA gene sequences which correspond to helix 44 (V8 variable region) of the secondary structure model (Neefs et al. 1990). Nucleotide positions are given between *brackets*. *Dashes* correspond to nucleotides which are identical to the analogous position in the *E. gracilis* gene; *asterisks* depict nucleotides which are differently conserved in the two plastidial lineages

In the former case, the cyanobacteria would have been the same or very closely related (but in different hosts, and with a different fate); in the latter case they would have been clearly different.

Another enigmatic plastid genome is that of *Euglena*, which has 16S rRNA genes related to those of the cyanobacterial ancestor leading to the Rhodophyta and Chromophyta, and Rubisco genes clearly related to those of other chlorophyll *b*-containing organisms (Assali et al. 1990; Douglas et al. 1990) and thus to another cyanobacterial lineage. Of course, *E. gracilis* has several common features with green algae and plants, most notably its pigment composition. However, as previously mentioned, "similar pigment compositions do not necessarily reflect close evolutionary relationships" (Turner et al. 1989). On the contrary, *E. gracilis* plastids also share common characteristics with chromophyte plastids, such as poly-β-(1–3) linked glucose polymers as cytoplasmic storage products and one extra membrane around the plastids (Gibbs 1981). We also know that the ribosomal operons of *Euglena* are organised as are those of chromophytes (Markowicz et al. 1988 b; Loiseaux-de Goër et al. 1991).

Figure 2 shows the alignment of a small fragment of the 16S rRNA genes which corresponds to helix 44 of the secondary structure model (located in an evolutionary variable region, V8) (Neefs et al. 1990). Interestingly, nucleotides 1205, 1219, 1233 and 1236 of the *E. gracilis* sequence are conserved when compared to non-green plastid genes and differ from the identical (and equally conserved) positions in green plastid genes. These observations suggest that the position of *Euglena* in the tree is not artefactual (i.e., is not due to stochastic similarities), and that the plastid genome of *Euglena* probably also has a composite origin, with genes coming from two different ancestors.

Another surprising feature of our analysis is the branching of the different Chlorophycophyta which shows a closer relationship between *Chlorella vulgaris* and *Chlamydomonas reinhardtii* than between the two *Chlorella* species. Here, again, the branching orders are too close to be clearly delineated. Phylogenetic studies of *Chlamydomonas*, based on a cladistic analysis of cytoplasmic 18S rRNA sequence data, show that this genus is not monophyletic and thus needs to be revised (Buchheim et al. 1990); the same could be true for *Chlorella* and, in that case, it could explain their respective positions in our phylogenetic tree.

Many questions regarding plastid evolution remain to be answered, such as: how did algal plastid genomes become composite? It has been shown that some bacterial genes have a mosaic structure arising from local gene exchanges between related species (Maynard Smith et al. 1991). It is also known that lateral gene transfers are commonly observed in bacterial populations, both within the same genera and also between taxonomically distant organisms (for example, transfer of proteobacterial plasmids to cyanobacteria), and can even occur between bacteria and eukaryotes (Hirsch 1990). Thus, a possible explanation is that conjugational recombination, i.e., lateral gene transfers followed by recombination processes (and loss of one of the two homologous sets of genes), occurred between cyanobacteria and β-proteobacteria (or perhaps in the case of *E. gracilis* between two different cyanobacteria). This could have happened either before any endosymbiotic event giving rise to plastids took place, or even between different endosymbionts engulfed by the same host cell.

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