

A genomic and phylogenetic perspective on endosymbiosis and algal origin

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Abstract

Accounting for the diversity of photosynthetic eukaryotes is an important challenge in microbial biology. It has now become clear that endosymbiosis explains the origin of the photosynthetic organelle (plastid) in different algal groups. The first plastid originated from a primary endosymbiosis, whereby a previously non-photosynthetic protist engulfed and enslaved a cyanobacterium. This alga then gave rise to the red, green, and glaucophyte lineages. Algae such as the chlorophyll *c*-containing chromists gained their plastid through secondary endosymbiosis, in which an existing eukaryotic alga (in this case, a rhodophyte) was engulfed. Another chlorophyll *c*-containing algal group, the dinoflagellates, is a member of the alveolates that is postulated to be sister to chromists. The plastid in these algae has followed a radically different path of evolution. The peridinin-containing dinoflagellates underwent an unprecedented level of plastid genome reduction with the ca. 16 remaining genes encoded on 1–3 gene minicircles. In this short review, we examine algal plastid diversity using phylogenetic and genomic methods and show endosymbiosis to be a major force in algal evolution. In particular, we focus on the evolution of targeting signals that facilitate the import of nuclear-encoded photosynthetic proteins into the plastid.

Introduction

The eukaryotic photosynthetic organelle (plastid) is critical to life on our planet because of its contribution to global primary production. Ten different types of plastids are known and are found in evolutionarily divergent eukaryotic clades (Baldaif et al., 2000; Bhattacharya et al., 2004). The endosymbiosis hypothesis was put forth to explain the origin of plastids and mitochondria (Margulis, 1970; Mereschkowsky, 1905) and has been extensively supported with modern molecular evolutionary analyses. In plastid primary endosymbiosis, a non-photosynthetic protist engulfed a cyanobacterium and converted it into a permanent photosynthetic organelle. This photosynthetic eukaryote gave rise to the red, green, and glaucophyte algae that have a plastid bound by two membranes. Thereafter, plastids were horizontally spread into the remaining photosynthetic protist groups through secondary endosymbiosis, in which non-photosynthetic cells engulfed an existing (red or green) alga. This process

resulted in the plastid of cryptophytes, haptophytes, stramenopiles, apicomplexans, dinoflagellates (red algal endosymbiont), euglenophytes, and chlorarachniophytes (green algal endosymbiont, Bhattacharya & Medlin, 1995; Cavalier-Smith, 1986; Douglas, 1998; Douglas et al., 1991; Gibbs, 1978; McFadden et al., 1994; Yoon et al., 2002b; Zhang et al., 1999). However, endosymbiosis did not stop there because in dinoflagellates the existing plastid of red algal origin was replaced on multiple independent occasions with this organelle from an alga containing a secondary plastid (a cryptophyte, haptophyte or stramenopile: tertiary endosymbiosis) or a primary plastid (a green alga) (Chesnick et al., 1997; Hackett et al., 2003; Ishida & Green, 2002; Tengs et al., 2000; Watanabe et al., 1990; Yoon et al., 2002a).

The development of large scale sequencing and genomic approaches has greatly augmented our understanding of algal evolution. These methods have been applied to generate complete genome or expressed sequence tag (EST) databases of model algae or protists

as well as to generate broadly sampled multi-gene phylogenies. In this paper, we discuss algal diversity from the perspective of plastid endosymbiosis, and present a brief summary of recent findings from genomic and phylogenetic approaches. In addition, we examine the leader sequences of nuclear-encoded plastid genes that have resulted from intracellular gene transfer and that make possible plastid targeting.

Endosymbiosis is an important driving force in algal evolution

Primary endosymbiosis

Rhodophyta, Viridiplantae (green algae and land plants), and Glaucocystophyta contain plastids surrounded by a double membrane that very likely originated through a single primary endosymbiosis in the common ancestor of these taxa (Bhattacharya & Medlin, 1995; Delwiche et al., 1995; Gray, 1992; McFadden, 2001; Moreira et al., 2000; Matsuzaki et al., 2004; McFadden & van Dooren, 2004). Molecular clock analysis using a concatenated data set of six plastid genes and multi-fossil calibrations suggest that the primary endosymbiosis occurred around 1.6 billion years ago (Yoon et al., 2004). This estimate has been independently confirmed by multi-protein analyses of nuclear loci that suggest a date of 1.6–1.5 BY for primary plastid origin (Hedges et al., 2004; Hackett et al., 2006). Despite their ancient origin, the monophyly of Plantae is moderately supported by recent molecular phylogenetic studies using nuclear and mitochondrial genes (Baldauf et al., 2000; Moreira et al., 2000; Palmer, 2003; Rodriguez-Ezpeleta et al., 2005). A broadly sampled tree of microbial eukaryotes is urgently needed to test the monophyly of Plantae (and other groups – see below).

Secondary endosymbiosis

The putative lineage Chromista, which comprises the cryptophytes, haptophytes, and stramenopiles, contain chlorophyll *c* in their 4-membrane bound plastid (Cavalier-Smith, 1986). The chromist plastid is not located in the cytosol but rather within the rough endoplasmic reticulum (RER), which is connected to the outermost membrane of the plastid and is referred to as the chloroplast endoplasmic reticulum (CER). Secondary endosymbiosis, in which the non-photosynthetic ancestor of chromists engulfed an existing red alga, explains plastid origin in this group

(Bhattacharya & Medlin, 1995; Douglas et al., 1991; Fast et al., 2001; Gibbs, 1981; Harper & Keeling, 2003). Evidence for this secondary endosymbiosis comes from the cryptophytes that retain the remnant nucleus of the red algal endosymbiont, the nucleomorph, between the two inner and two outer plastid membranes. The haptophytes and stramenopiles have presumably lost the nucleomorph after their divergence from the cryptophytes. Our molecular clock analysis suggests a minimum age of 1.3 BY for this secondary endosymbiotic event and around 1.2 BY for the divergence of cryptophytes from the other chromists and 1 BA for the split of haptophytes and stramenopiles (Yoon et al., 2004).

Alveolates, which comprise the dinoflagellates, apicomplexans, and ciliates, are postulated to be sister to the chromists (together, the chromalveolates; Cavalier-Smith, 1999 [see Fast et al., 2001; Harper & Keeling, 2003; Bhattacharya et al., 2004]). The chromalveolate common ancestor most likely contained a red algal secondary endosymbiont (Cavalier-Smith, 1999) that was apparently lost in the ciliates. In the apicomplexans, such as the well-known human parasite *Plasmodium falciparum* Welch, the remnant plastid (called the apicoplast) genome was reduced to a 35 Kb circle (Williamson et al., 1994). However, the phylogenetic history of apicoplasts remains unclear because of the high divergence of the encoded sequences that usually results in long branch artifacts in trees (Funes et al., 2002; Waller et al., 2003; Zhang et al., 1999, 2000). An important data set that supports chromalveolate monophyly is the presence of a unique glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene replacement shared by these taxa (Fast et al., 2001; Harper & Keeling, 2003). In addition, because the plastid genes of dinoflagellates were re-organized into mini-circles and most of the plastid genes were transferred to the nucleus (followed by high sequence divergence rates in many of these coding regions), it is difficult to accurately infer the phylogeny of dinoflagellate plastids (Bachvaroff et al., 2004; Hackett et al., 2004; Zhang et al., 1999). To resolve this issue, we sequenced five minicircle-encoded plastid proteins from a handful of peridinin dinoflagellates and from fucoxanthin-containing taxa. These latter taxa presumably gained their plastid through a haptophyte tertiary endosymbiosis (Ishida & Green, 2002; Tengs et al., 2000; Yoon et al., 2005). In the resulting trees, the evolutionary origin of the peridinin plastids remains unclear but this clade is clearly positioned as a monophyletic lineage within the red algae with a weak sister group