Current Biology *19*, R81–R88, January 27, 2009 ©2009 Elsevier Ltd All rights reserved DOI 10.1016/j.cub.2008.11.067

The Puzzle of Plastid Evolution

Review

John M. Archibald

A comprehensive understanding of the origin and spread of plastids remains an important yet elusive goal in the field of eukaryotic evolution. Combined with the discovery of new photosynthetic and non-photosynthetic protist lineages, the results of recent taxonomically broad phylogenomic studies suggest that a re-shuffling of higher-level eukaryote systematics is in order. Consequently, new models of plastid evolution involving ancient secondary and tertiary endosymbioses are needed to explain the full spectrum of photosynthetic eukaryotes.

Introduction

Our planet is teeming with photosynthetic life. The textbook version of how this came to be is relatively straightforward: oxygenic photosynthesis first evolved in the ancestors of modern cyanobacteria more than two billion years ago [1] and their light-harvesting capabilities were subsequently exploited by eukaryotic (nucleus-containing) cells through the process of endosymbiosis [2,3]. Co-evolving with their opportunistic hosts, these intracellular cyanobacteria were eventually transformed into *bona fide* organelles — plastids — ultimately giving rise to the plants and algae that surround us today. Easy, right?

The basic outline of this evolutionary scenario is correct, but the reality is much, much more complicated. Photosynthetic eukaryotes are astonishingly diverse in form and function, a fact that complicates efforts to discern their evolutionary history. Eukaryotic phototrophs can be macroscopic (e.g., land plants, seaweed) or microscopic (e.g., the unicellular green alga *Chlamydomonas*), sessile or motile (or both), and given a bit of sunlight, they thrive in virtually any habitat imaginable, terrestrial and aquatic, from the equator to the poles. This vast diversity actually makes sense when one considers that the term 'algae' can be applied to organisms that are not specifically related to one another. In addition to simple vertical inheritance, plastids have on multiple occasions spread laterally between distantly related groups of eukaryotes. Having evolved ~one billion years in the past [4], today's plastids weave a tangled web across a very large fraction of the eukaryotic tree. Consequently, large sections of the puzzle of plastid evolution remain unassembled.

This article focuses on the latest advances in our understanding of the origin and spread of plastids. In particular, the merits and shortcomings of competing hypotheses about the evolution of plastids are discussed in light of a flood of new molecular, biochemical, genomic and phylogenomic data. Progress has been swift, but there are still many questions that need to be answered, and many newly discovered protist lineages that need to be investigated, before it can be said that the evolution of eukaryotic photosynthesis is understood with confidence.

The Canadian Institute for Advanced Research, Program in Integrated Microbial Biodiversity, Department of Biochemistry and Molecular Biology, Dalhousie University, Sir Charles Tupper Medical Building, 5850 College Street, Halifax, Nova Scotia, B3H 1X5, Canada.
E-mail: jmarchib@dal.ca

Primary Plastids

Unlike the origin of mitochondria, the details of which are still debated [5], there is no longer any doubt that plastids are derived from once free-living cyanobacteria and that the host cell was a full-blown eukaryote with a nucleus, cytoskeleton and mitochondrion. The so-called 'primary' endosymbiotic origin of plastids (Figure 1A) can be considered the 'launch point' for eukaryotic photosynthesis in the sense that all canonical plastids appear to be derived from this pivotal event, either directly or indirectly. Primary plastids are characterized by the presence of two membranes, both of which are cyanobacterial in nature [2], and are found in red algae, glaucophyte algae and green algae, the latter group being the unicellular lineage that gave rise to land plants. This tripartite assemblage is referred to as the Plantae or Archaeplastida [6] (Figure 2).

The evidence for and against the singular origin of primary plastids has been reviewed extensively elsewhere (e.g., [3,7,8] and references therein). For the purposes of this review, I will simply note that many researchers are reasonably convinced that primary plastids evolved only once, in the common ancestor of green, red and glaucophyte algae (Figure 2; see refs [3,7] for review), recognizing that the technical and conceptual challenges associated with inferring such ancient evolutionary events are considerable [8]. Indeed, there is much debate as to whether red, green and glaucophyte algae really are each other's closest relatives to the exclusion of all other eukaryotes (e.g., [9–13]) and if they are not, how the evolution of their plastids should be interpreted [7,12,14].

What can be said with confidence is that intracellular (or endosymbiotic) gene transfer (EGT) was a major factor in the integration of the cyanobacterial progenitor of the plastid and its eukaryotic host [15,16]. While plastid genomes rarely encode more than ~200 proteins, a thousand or more nucleus-encoded proteins — many but not all of which are demonstrably cyanobacterial in origin — are needed to service a fully functional plastid. The bulk of these proteins are translated on cytoplasmic ribosomes and targeted to the plastid post-translationally by a dedicated protein import apparatus [2,17] whose evolution has been touted as the defining feature of an endosymbiont-turned-organelle [18,19]. The nuclear genomes of primary-plastid-bearing eukaryotes thus possess hundreds of endosymbiont-derived genes, many encoding plastid-targeted proteins as well as others that have evolved non-plastid, host-associated functions [15,16,20]. This endosymbiotic 'footprint' becomes significant when one considers the pervasiveness of plastid loss in eukaryotic evolution, and whether plastid-/algal-derived genes in the nuclear genomes of non-photosynthetic eukaryotes are reliable indicators of a photosynthetic ancestry (below).

As ancient as the primary endosymbiotic origin of plastids was, it is worth noting that several instances of 'recent' cyanobacterium–eukaryote endosymbioses are known, for example, in the testate amoeba *Paulinella chromatophora* [21] and the diatom *Rhopalodia gibba* [22]. Such examples

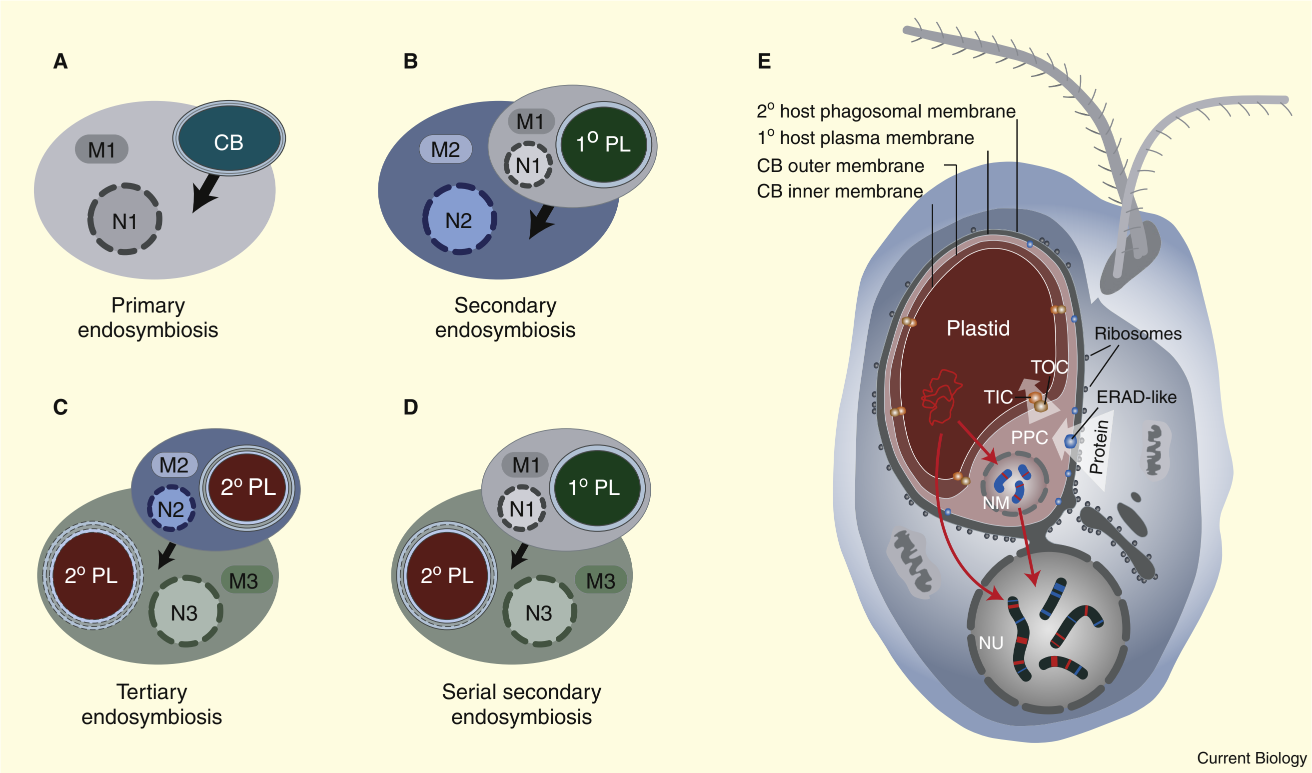

Figure 1. Plastid evolution by primary, secondary, and tertiary endosymbiosis.

(A) A cartoon depicting the primary endosymbiotic origin of plastids through the uptake of a double-membrane-bound cyanobacterium by a non-photosynthetic host eukaryote. (B) Secondary endosymbiosis involves the engulfment of a primary-plastid-containing eukaryote by a second, non-photosynthetic eukaryote. All known primary plastids are surrounded by two membranes and, in the case of glaucophyte algae, a layer of peptidoglycan. (C) Tertiary endosymbiosis occurs when a secondary-plastid-containing endosymbiont is taken up by a eukaryotic host, which may or may not itself possess a plastid. (D) Serial secondary endosymbiosis between a secondary-plastid-containing eukaryotic host and an endosymbiont with a primary plastid. (E) Endosymbiotic gene transfer and protein targeting in secondary-plastid-containing algae. Diagram shows basic cellular structure of a cryptophyte alga with a four-membrane-bound plastid of red-algal origin and a nucleomorph. Plastid and plastid-derived DNA is in red, nucleomorph and nucleomorph-derived DNA is blue, and host nuclear DNA is black (mitochondrial DNA has been omitted for simplicity). As in haptophytes and heterokonts, the outermost plastid membrane of the cryptophyte plastid is continuous with the host cell endomembrane system and is studded with ribosomes. In most secondary-plastid-containing algae, the nucleomorph-to-host-nucleus gene transfer process has gone to completion. See text for further discussion. Abbreviations: CB, cyanobacterium; M, mitochondrion; NU, host nucleus; PL, plastid; NM, nucleomorph; PPC, periplastid compartment; ERAD, endoplasmic reticulum-associated protein degradation machinery; TIC and TOC, translocons of the inner and outer chloroplast membrane, respectively.

do not directly bear on the diversification of canonical plastids, but they are potentially informative in that they may provide clues as to the molecular and cellular events that led to their establishment [23]. Whether the term ‘endosymbiont’ or ‘organelle’ is more appropriate in these cases is debatable and depends in large part on one’s definition of organelle [19,24,25].

Secondary and Tertiary Plastids: Who’s Got’em, Where’d They Get’em?

Speculation that photosynthesis has spread laterally across the eukaryotic tree dates back to the 1970s (e.g., [26,27]). A Canadian, Sarah Gibbs, first noted that the chlorophyll-*a*+*b*-pigmented plastid of the common lab alga *Euglena* was clearly green algal in nature, yet the ultrastructure of the host organism “…could not be more unlike green algae” [26,28]. Coupled with the presence of supernumerary plastid membranes, incongruence between the evolution of the plastid and the host cell in which it resides is now seen as the red flag of ‘secondary’ endosymbiosis, i.e., the movement of plastids from one eukaryote to another (Figure 1B). In the case of *Euglena*, its plastid is indeed derived from a green alga [29], which was engulfed by a non-photosynthetic relative of the euglenids, an important protist group belonging to the eukaryotic ‘supergroup’ Excavata [30] (Figure 2).

The chlorarachniophytes are a group of rhizarian amoeboflagellate algae that also possess green algal plastids of secondary endosymbiotic origin (Figure 2). In this case there is no doubt as to the mechanism of organelle capture: unlike *Euglena*, chlorarachniophyte cells still possess the nucleus of the green algal endosymbiont that came in with the plastid in a vestigial form referred to as a ‘nucleomorph’ [31–33]. Euglenid and chlorarachniophyte plastids possess three and four plastid membranes, respectively, in contrast to the two membranes that envelop all known primary plastids.

No fewer than six algal lineages are known to harbor red-algal-derived plastids: these include the cryptophytes (which also possess a nucleomorph [34,35]), haptophytes, plastid-bearing stramenopiles (e.g., diatoms and kelp), apicomplexans, dinoflagellates and *Chromera velia* (Figure 2). The case for red algal plastids in cryptophytes, haptophytes and stramenopiles is clear-cut (e.g., [34,36–38]), but the