

## PROTIST NEWS

# A Brief History of Plastids and their Hosts

## Introduction

Plastids are photosynthetic organelles and their non-photosynthetic derivatives found in plants and algae. They have many names, depending on their colour or function, including chloroplasts, chromoplasts, leucoplasts, or apicoplasts, but all of these are homologous structures tracing back to a single origin. Plastids are most commonly associated with photosynthesis, but are generally metabolically diverse organelles, having been found to function in controlling oxidative stress as well as the biosynthesis of fatty acids, isoprenoids, heme, amino acids, and other small compounds. The great diversity of plastids, and the organisms that harbour them, have made plastid evolution one of the more interesting challenges in reconstructing eukaryotic macroevolutionary history – a challenge that has been made even more intriguing by the unexpected twists and turns that plastid evolution is now known to have taken. Despite its complexity, the events surrounding the origin and spread of plastids are now unfolding to reveal a complex history with unexpected impacts on eukaryotic evolution and biodiversity (Fig. 1).

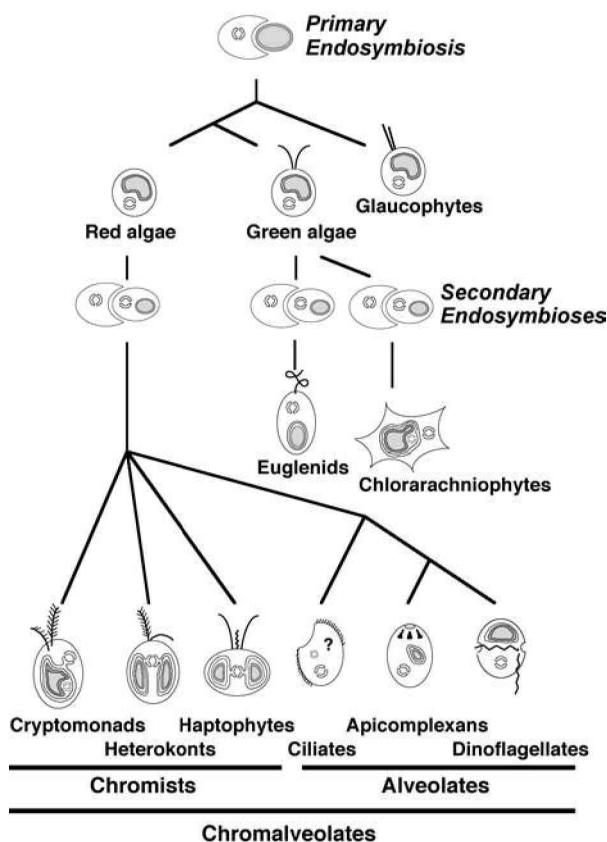
## Primary Plastids

For many years it has been known that plastids are the product of an endosymbiotic partnership between a eukaryotic host and a photosynthetic cyanobacterial symbiont. Briefly, a heterotrophic eukaryote swallowed a cyanobacterium, but failed to digest it. The two cells gradually integrated and the cyanobacterium lost many genes and transferred many others to the host nucleus, eventually becoming the relatively specialized organelle we see today (Gray and Spencer 1996). Plastids descending directly from this event are called “primary plastids”. These organelles are bound by two membranes, which appear to be homologous to the inner and outer membranes of the cyanobacterial endosymbiont (Jarvis and Soll 2001).

Primary plastids are found in glaucophytes, red algae, green algae and their close relatives, the land plants. Whether these were derived from one endosymbiosis or several independent events has been debated extensively. While this debate continues (Stiller et al. 2003), virtually all current evidence supports a single endosymbiosis. By far the strongest evidence for this comes from the plastid itself, where morphology, biochemistry, genomic organization, and molecular phylogenies of numerous plastid RNAs and proteins support their common ancestry (Besendahl et al. 2000; Delwiche et al. 1995; Durnford et al. 1999; Helmchen et al. 1995), although the exact type of cyanobacterium remains unclear (Turner et al. 1999). Nuclear gene phylogenies have frequently failed to group these lineages, but since they fail to resolve any position for them (e.g., Bhattacharya et al. 1995; Keeling et al. 1999), these genes are more likely inadequate rather than contradictory. This is supported by recent analyses of nuclear genes which do show support for the common ancestry of red, green, and (where included) glaucophyte algae (Baldauf et al. 2000; Moreira et al. 2000). Given a relationship between these three groups, the order in which they diverged has also been a subject of controversy. Molecular and morphological data have been used at various times to argue that each lineage diverged first (Cavalier-Smith 1982; Delwiche et al. 1995; Herdman and Stanier 1977; Martin et al. 1998; Valentin and Zetsche 1990). However, there is a growing consensus that this interesting position belongs to the glaucophytes, placing new importance on this intriguing but little-studied group.

## Secondary Plastids

The primary plastid-containing algae, together with plants, make up one of the emerging supergroups of eukaryotes and by any measure are an important part



**Figure 1.** Schematic diagram of plastid evolution (adapted from Archibald and Keeling 2004). Primary endosymbiosis took place once to yield glaucophytes, red algae, green algae and their land plant relatives. Secondary endosymbiosis involving green algae took place twice, once yielding euglenids and once yielding chlorarachniophytes. Secondary endosymbiosis involving a red alga took place once yielding the many groups now called chromalveolates. In each case, except chlorarachniophytes, photosynthesis has been lost extensively.

of eukaryotic biodiversity. However, they still account for only a fraction of plastid and algal diversity. The plastids of most algal groups evolved by “secondary endosymbiosis”, which is when one of the primary algae is itself eaten by another eukaryote, and degenerates into an organelle (Archibald and Keeling 2002). This process also involves transfers and losses of large numbers of genes and the development of a new protein targeting system, in this case derived from the secretory system (McFadden 1999). One of the characteristics of secondary plastids is the presence of more than two membranes around the plastids, which are derived from the endosymbiont plasma membrane (which has been lost in euglenids

and dinoflagellates) and the secondary host endomembrane. In two cases, cryptomonads and chlorarachniophytes, the endosymbiont degeneration has been incomplete, so that a relict nucleus with a highly reduced genome is found between the second and third membranes (McFadden et al. 1997). Secondary plastids are not known to have been derived from a glaucophyte (probably because this group is the least abundant of the primary algae), but they have originated more than once, and have been derived from both red and green algae.

Secondary plastids of green algal origin are found in euglenids and chlorarachniophytes, but these two groups share little else in common and are not closely related in molecular phylogenies based on plastid or nuclear genes. Instead, euglenids are related to diplomonads and kinetoplastids (Leedale and Vickerman 2000), while chlorarachniophytes are related to cercozoans, foraminifera and other amoeboid flagellates (Archibald et al. 2002). Their plastids have nevertheless been proposed to share a common origin (Cavalier-Smith 1999). While there is currently no phylogenetic evidence to support this, only once the closest green algal relatives of both plastid symbionts are unambiguously determined will it be certain whether they share a common origin or not. It has also recently been proposed that the plastids of euglenids originated prior to their split from kinetoplastids based on the presence of several “plant-like” genes in *Trypanosoma* (Hannaert et al. 2003). However, even the most compelling examples are not actually related to plastid homologues when analysed with greater taxonomic diversity (Rogers and Keeling 2004) and close examination of the remaining genes suggests that there is no evidence that trypanosomes had a plastid-bearing ancestor.

Secondary plastids of red algal origin are much more common and diverse, being found in cryptomonads, haptophytes, heterokonts (stramenopiles), dinoflagellates, and apicomplexan parasites. A relationship between these groups was proposed some time ago, when they were dubbed “chromalveolates” (Cavalier-Smith 1999). Hints of such a relationship have appeared in various molecular phylogenies (Baldauf et al. 2000; Van de Peer et al. 1996), but in general gene trees have not united these lineages until recently. Now, a gene duplication and replacement event involving the cytosolic and plastid-targeted glyceraldehyde-3-phosphate dehydrogenase (GAPDH) has been used to argue for a common ancestor for all of these lineages and a single origin of their plastids (Fast et al. 2001; Harper and Keeling 2003). In addition, an analysis of five concatenated plastid-encoded genes unites heterokonts, haptophytes,

phytes and cryptomonads (collectively called chromists) with strong support (Yoon et al. 2002b).

A single origin of the chromalveolate plastids has interesting implications for plastid evolution, particularly since there are a large number of chromalveolates where no plastid is (yet) known. These include some large and well-studied groups like ciliates and oomycetes. The chromalveolate hypothesis suggests that the ancestor of these groups was photosynthetic, and that these groups either lost their plastids or lost photosynthesis and retain a relict plastid (an important distinction). Indeed, the number and distribution of non-photosynthetic chromalveolates remains one of the principle points of contention in this group (although relationships within heterokonts are only partially known), and so it is ironic that we will never fully understand the process of plastid origins until we have a better understanding of plastid loss and whether it even occurs (Williams and Keeling 2003).

### **Distinguishing Between the Evolution of Organelles and their Proteins**

Molecular phylogenies can, at best, reveal the evolution of the gene being analysed: conclusions about the evolution of organisms or organelles derived from molecular data are inferences that assume the evolution of that gene mirrors the evolution of the organism or organelle. In recent years this distinction has become quite important, as it has been shown that genes can be transferred between distantly related genomes in a process called lateral or horizontal gene transfer. The impact of this process in eukaryotes is very poorly understood, but some of the earliest characterised instances have involved organelles. At first, the clean distinction between organellar and host proteins was slightly blurred by the discovery that host genes could replace organelle homologues and vice versa in a process called endosymbiotic gene replacement (Brinkmann and Martin 1996). This process is now thought to have affected secondary as well as primary plastids (Fast et al. 2001; Keeling and Palmer 2001), and has been suggested to have made an important contribution to plant nuclear genomes (Martin et al. 2002).

Presently there is little evidence for lateral transfer to plastid genomes themselves (although recent data suggests it is prevalent in plant mitochondria: Bergthorsson et al. 2003), but there is emerging evidence that it has played a role in the evolution of plastid-targeted proteins. Normally these proteins are considered to have originated with the cyanobacterial endosymbiont and to have tracked the evo-

lution of the plastid. However, it has now been shown that several plastid-targeted genes in the chlorarachniophyte, *Bigelowiella* are not of green-algal origin (Archibald et al. 2003). Interestingly, the same analyses found no such evidence for lateral transfer of plastid-targeted genes in *Chlamydomonas*. Similarly, another recent study showed that both plastid-targeted and cytosolic GAPDH genes in some dinoflagellate species had been acquired from euglenids (Takishita et al. 2003). Altogether, these data indicate that different organisms are likely affected to differing degrees by lateral transfer, but that it can also affect the organelle proteome. Lateral transfer is not a common feature of organelle genes, but caution must nevertheless be used when interpreting organelle evolution based on a single gene. Some of the most contentious issues in plastid evolution today surround the apicomplexan and dinoflagellate plastids, and many of these arguments are based on one or a handful of nuclear-encoded, plastid-targeted genes (Fast et al. 2001; Köhler et al. 1997; Waller et al. 2003; Yoon et al. 2002a). It is unlikely that *all* of these data accurately reflect the evolution of the organelle. The challenge lies in separating those gene trees that actually reflect the history of the organelle from those that do not.

### **Concluding Remarks**

While a number of interesting and important questions remain debated, and new data have raised several novel possibilities for the origin of plastids in some groups, remarkable progress has been made in determining the broad outlines of plastid evolution. What has emerged is a multilayered history of endosymbiotic mergers, intergenomic genetic transfers, and extreme alterations in genome structure. Despite this winding and sometimes confusing path, the evolution of plastids is one of the success stories of modern molecular evolutionary investigation: the essence of most of the major events in this history are now understood, and a complete picture appears to be within our grasp.

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