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Chloroplast Evolution: Secondary Symbiogenesis and Multiple Losses

Dispatch

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Chloroplasts originated from cyanobacteria only once, but have been laterally transferred to other lineages by symbiogenetic cell mergers. Such secondary symbiogenesis is rarer and chloroplast losses commoner than often assumed.

During or following the global warming that thawed the last 'snowball earth' glaciation about 580 million years ago [1], chloroplasts originated from a cyanobacterial symbiont in a biciliate protozoan [2,3]. The resulting cellular chimaera was so successful that it rapidly diversified into two primary lineages of eukaryotic algae: the now rare glaucophytes like *Cyanophora*, which retained the cyanobacterial peptidoglycan wall within their chloroplast envelope, and the green plant/red algal lineage, which lost the peptidoglycan. The latter split into red algae, which retained the cyanobacterial phycobilisome pigments, and green algae, which replaced them by chlorophyll b to adapt to different light frequencies. Soon afterwards, a red alga and two different green algal cells were implanted into yet other biciliate hosts to form three further groups of eukaryotic algae: a process called secondary symbiogenesis (see Figure 1). New light on these early events in eukaryotic evolution comes from host genes encoding proteins that were secondarily imported into the acquired plastids [4] and, as reported by Andersson and Roger [5] in this issue, from symbiont genes that were apparently retained in the host nucleus after the symbiont was lost. Secondary symbiogenesis has also been greatly clarified by the complete sequence of a cryptomonad nucleomorph [6], an evolutionarily miniaturised relic of the red algal nucleus that was enslaved over 500 million years ago.

Several well-established eukaryote groups comprise a mixture of photosynthetic algae and non-photosynthetic heterotrophs, notably dinoflagellates, heterokonts, cryptophytes and Euglenozoa. We now know that all these acquired chloroplasts secondarily from other eukaryotes (red or green algae). Early in the twentieth century, however, it was thought that such groups with mixed nutritional properties were ancestrally photosynthetic and their non-photosynthetic members evolved by losing chloroplasts. After the 1960s revival of Mereschkowsky's symbiogenetic theory of chloroplast origins, the alternative dogma arose that such groups were ancestrally heterotrophic and acquired plastids by numerous independent symbioses. But because each symbiogenetic origin of an organelle is evolutionarily complex, requiring novel organelle-specific protein-targeting machinery and the acquisition by over a thousand genes of appropriate targeting signals, I have long maintained that symbiogenesis is very rare and chloroplast loss distinctly commoner [2,3,7]. Although photosynthesis has been repeatedly lost, complete plastid loss has never been demonstrated within the plant kingdom — glaucophytes, red algae and green plants — which arose by the primary origin of chloroplasts from cyanobacteria. However, molecular phylogeny has established that chloroplast loss is indeed relatively frequent in heterokonts [8] and dinoflagellates [9], and also occurred in euglenoids [10].

Secondary symbiogenesis has left remarkable traces of its evolutionary role in the more complex topology of the membranes surrounding all non-plant chloroplasts. The kingdom Chromista was established in 1981 to embrace all algae — cryptomonads, heterokonts and haptophytes — with chloroplasts located within the lumen of the rough endoplasmic reticulum (ER), plus their heterotrophic relatives like the heterokont oomycetes which are deemed to have evolved from them by chloroplast loss [2]. Chloroplasts of all chromists are separated from the surrounding rough ER by a smooth membrane, the periplastid membrane, the relic of the plasma membrane of the red alga phagocytosed by the ancestor of all chromists. This unique topology probably arose when the former food vacuole membrane fused with the nuclear envelope of the engulfing host. In cryptomonads, the nucleus of the enslaved red alga persists because it has 30 genes encoding proteins essential for the secondarily acquired plastid [6]. But in other chromists — heterokonts and haptophytes, collectively called chromobiotes as they share the brown carotenoid fucoxanthin that colours brown seaweeds and diatoms — these thirty genes were transferred to the host nucleus, allowing the nucleomorph to be lost with its evolutionary burden of hundreds of housekeeping genes (needed only for expressing those 30 genes).

In all chromistan algae, thousands of plastid proteins encoded by nuclear genes must be imported across four topologically and chemically distinct membranes: the rough ER, the periplastid membrane, and the outer and inner chloroplast envelope membranes. Their bipartite amino-terminal targeting sequences direct this: distally is a signal sequence mediating import into the rough ER lumen [11], and proximally a chloroplast transit peptide that mediates import across the chloroplast envelope and possibly also the periplastid membrane [12]. Traditionally, chromistan algae were grouped as 'chromophyte

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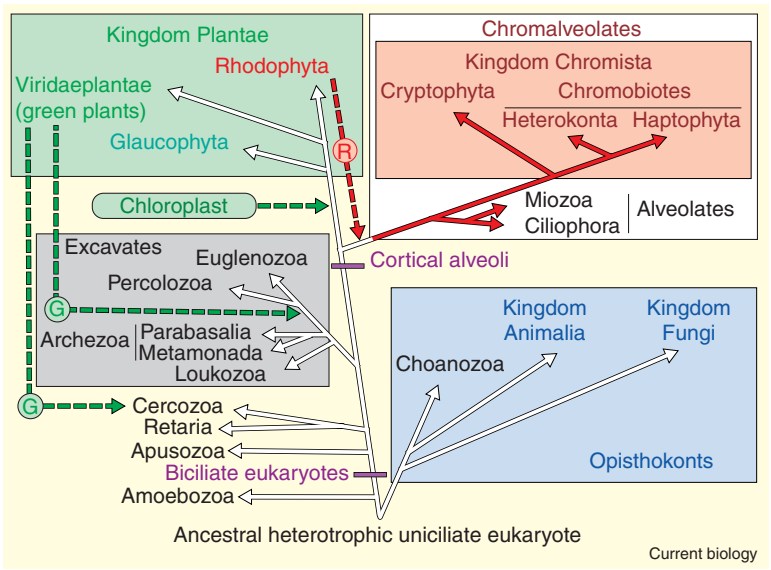

Figure 1.

Phylogeny of the five eukaryote kingdoms, highlighting the primary symbiogenetic origin of chloroplasts from cyanobacteria which created the plant kingdom, and the three secondary symbiogenetic events which created eukaryote–eukaryote chimaeras by laterally implanting pre-existing chloroplasts into other protozoan lineages (dashed lines). A single implantation of a red algal chloroplast (R) created the typically brown chromalveolates, in which several lineages, such as the ciliates (Ciliophora), later lost photosynthesis. Cryptomonad nucleomorphs are relics of the endosymbiotic red alga's nucleus that have persisted in these cellular chimaeras for half a billion years. Two separate implantations of green algal cells (G) took place. One gave rise to euglenoid chloroplasts (probably in the common ancestor of Euglenozoa and Percolozoa or an even earlier excavate). The other was into a cercozoan flagellate giving the chloroplasts and nucleomorphs of chlorarachnean algae. At least two independent tertiary replacements of typical dinoflagellate chloroplasts by phylogenetically different ones also occurred [9,13], but are not shown (dinoflagellates belong in Miozoa with Sporozoa, which are non-photosynthetic but mostly retained plastids for lipid synthesis). The 12 phyla in black belong in the kingdom Protozoa (see [17], where the rationale behind this phylogeny is detailed). Though Percolozoa are shown as sisters to Euglenozoa, some rRNA trees [17] suggest that they may be sisters to Archezoa instead; if this were true, Archezoa also must have had photosynthetic ancestors.

algae' with dinoflagellates, which have a different brown carotenoid, peridinin. But dinoflagellate plastids are apparently not within the rough ER lumen, and they have an envelope of only three smooth membranes. It has therefore long been assumed that their plastids had a separate secondary symbiogenetic origin; many also discounted the arguments for a single symbiogenetic origin for all chromistan plastids [13]. Discovery of the remarkable single-gene circles of dinoflagellate chloroplasts [14] and their phylogenetic analysis [15] led to a radical reappraisal of chromist and dinoflagellate relationships [12]. Not only chromists and dinoflagellates, but also Sporozoa, such as malaria parasites and *Toxoplasma*, apparently had plastids of red algal origin [15].

A substantial simplification of eukaryote phylogeny was effected by proposing that all these groups are related, and that their red algal chloroplasts had been acquired not independently in five separate symbioses, as many then supposed [13], but in a single secondary symbiogenetic event to form a photosynthetic common ancestor of all chromophytes [12]. It was already known that dinoflagellates and Sporozoa are mutually related by descent from an obscure group of flagellates, the protalveolates, grouped with them in the phylum Miozoa [12]. Miozoa are related to the apparently non-photosynthetic ciliate protozoa, the two groups being collectively called alveolates because of related ultrastructural features of their cell cortex. If alveolates and chromists really do have a photosynthetic common ancestor, ciliates and protalveolates must also be derived from it and have lost photosynthesis. That this is almost certainly so is now dramatically shown by a remarkable case of gene replacement in chromists and alveolates [4], collectively called chromalveolates [12].

Fast *et al.* [4] discovered that, in two chromist groups (cryptomonads and heterokonts) and two alveolate groups (dinoflagellates and Sporozoa), the glyceraldehyde phosphate dehydrogenase (GAPDH) gene that originally encoded the chloroplast GAPDH protein has been replaced by a duplicate of the radically different host gene encoding cytosolic GAPDH. Furthermore, they found that the chromalveolate algal replacement genes branch on trees with the ciliate gene for the cytosolic protein, not with those of non-alveolates. If these replacements happened independently, one would have to suppose that the same gene underwent four identical duplications and retargetings from cytosol to plastid by acquiring bipartite targeting signals for import across four (or in dinoflagellates three) membranes. Furthermore, the retargeted cytosolic enzymes would have had to change their cofactor specificity from NAD to NADPH independently.

Such multiple convergence of duplication, acquisition of targeting sequences, and enzymatic remoulding is simply incredible, especially when we bear in mind that the ancestral chimaeric cells also had host and symbiont genes for distinct mitochondrial GAPDH enzymes and symbiont cytosolic genes, any of which in principle could have undergone duplication and retargeting, and that gene replacement is unlikely even to have been physiologically necessary — it was probably just an evolutionary accident. It is far simpler to invoke a single gene replacement in the photosynthetic common ancestor of all chromalveolates [4,12] and accept that all heterotrophic chromalveolates

evolved by losing photosynthesis. This was probably easier in chromalveolates because, unlike plants, many retain a capacity for phagotrophy, often being mixotrophs able both to photosynthesise and phagocytose. Thus, in the protist world they fill a niche like corals among animals, which themselves rely on temporarily cultured intracellular photosynthetic dinoflagellates.

So we must now accept that chromalveolates form a major monophyletic branch of the eukaryotic tree. Andersson and Roger [5] have independently shown that the heterotrophic oomycete heterokonts, once misclassified as fungi, did evolve from algal ancestors: they have a 6-phosphogluconate dehydrogenase (*gnd*) gene closely related to that of diatoms and brown algae, which must have come from the chromalveolate red algal symbiont. As glaucophytes, the most divergent plant group, have cortical alveoli like those of alveolates, plants and chromalveolates may actually be sister groups [12]. Though collectively called photokaryotes [12], it is usually thought that their common ancestor was non-photosynthetic. However, Andersson and Roger [5] also found *gnd* genes clearly related to those of plants and chromists in the purely heterotrophic amoeboflagellate Heterolobosea (phylum Percolozoa); the simplest interpretation is that Percolozoa evolved by plastid loss from photosynthetic ancestors.

Either the primary origin of chloroplasts or a lateral transfer by secondary symbiogenesis occurred earlier than usually thought. I favour the latter, as Percolozoa are probably sisters to Euglenozoa [12,16,17], which acquired plastids from a green alga. If this took place in the common ancestor of Euglenozoa and Percolozoa, we need not postulate primary plastid loss (never clearly demonstrated) in any eukaryotic lineage, merely additional losses of secondary plastids (already known) in the ancestral percolozoan and within Euglenozoa. However, the suggestion [12] that euglenoid plastids were acquired even earlier, in the same event as chlorarachnean chloroplasts and nucleomorphs [18,19], is contradicted by cytoskeletal phylogeny [17]. Chlorarachnean chloroplast genome sequences should eventually confirm their independence.

Andersson and Roger [5] also noted highly divergent *gnd* genes in trypanosomes (Euglenozoa). It is unclear whether these came from the green algal symbiont or the host; the secondarily amitochondrial Archezoa also have comparably divergent *gnd* genes [5], conceivably related as Euglenozoa and Archezoa are both excavate protozoa [17]. Finding other analogous genetic relics of distant evolutionary events should provide an even clearer picture of the broad lines of early eukaryotic diversification.

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