Current Biology, Vol. 14, R514–R516, July 13, 2004, ©2004 Elsevier Ltd. All rights reserved. DOI 10.1016/j.cub.2004.06.041

# Evolution: Red Algal Genome Affirms a Common Origin of All Plastids

Dispatch

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**Photosynthetic organelles (plastids) come in many forms and were originally thought to have multiple origins. The complete genome of the thermophilic red alga *Cyanidioschizon merolae* provides further evidence that all plastids derive from a single endosymbiotic event more than 600 million years ago.**

The origin of photosynthetic plastids through engulfment and retention of a cyanobacteria-like cell by a eukaryote was a momentous event in eukaryotic evolution. This fusion of two cell lineages, which we refer to as the primary endosymbiotic origin of plastids, brought the power of autotrophy to eukaryotes, and descendants of this partnership have populated the oceans with algae and the land with plants, providing the world with most of its biomass. Much has been learned about the processes of endosymbiosis from the residue of the endosymbiont's genome that makes up modern plastid DNA [1]. Clearly, endosymbionts have lost most of their genetic blueprint and enormous numbers of these genes — typically more than 90% — are now housed in the host nucleus [1,2]. Completed host genome sequences are the latest resource for unravelling the mysteries of plastid origins, and the most recent to emerge is that of a red alga from the laboratory of Tsuneyoshi Kuroiwa [3].

One of the longest running debates in plastid endosymbiosis revolves around whether all plastids arose from a single endosymbiotic partnership between a cyanobacterium and eukaryotic host, or whether several independent partnerships — between different eukaryotes and different, possibly closely related, prokaryotes — spawned parallel lineages of plastid-containing organisms [4]. Modern plastids come in many different colours and functions, and some authors [5] postulate independent origins for these different plastids. Another school holds that all plastids share a common origin — that is, that they are monophyletic.

The key lines of evidence that plastids have a monophyletic origin are as follows. First, trees of both endosymbiont and host genes usually, though not always, unite the primary plastid containing organisms into one clade [6,7]. Secondly, shared architectural features of plastid DNAs, such as inverted rRNA repeats and plastid gene operons proposed to have been assembled post-endosymbiosis, are consistent with common descent [8]. Additionally, there are similarities in the residual complement of genes retained by plastids [1], though this could be convergence [5]. A further line of evidence concerns the protein that binds chlorophylls, the pigments that harvest light energy from the sun and direct it into photosynthesis. All photosynthetic plastids contain a nuclear-encoded chlorophyll binding protein with three transmembrane helices that bed the protein in the photosynthetic membranes [9]. Cyanobacteria have an obvious progenitor of this protein, but it has only a single transmembrane helix — this is thought to have undergone triplication only after the gene relocated to the nucleus [9]. If plastids have multiple origins, this seemingly complex triplication would have to have occurred convergently.

A crucial line of evidence supporting a singular origin of plastids is the apparent similarity in the mechanisms by which the products of genes transferred to nucleus are targeted back to the organelle. Plastid proteins encoded in the nucleus typically have an amino-terminal extension known as a transit peptide that directs attached proteins across the plastid membranes to their ultimate destination. Transit peptides from a diverse range of organisms are interchangeable [10–13], supporting the notion, first proposed by Cavalier-Smith [14], that invention of a mechanism to return gene products to the gene's compartment of origin was a key early step in the integration of an endosymbiont. Descendants of this 'protoalga' would then retain core components of this mechanism. Identifying components of the protein import machinery in the various plastid lineages could thus provide important clues about the evolution of plastids. As most of this machinery is nuclear-encoded, algal genomes might help identify it.

Plant biologists have identified a battery of proteins, located in and around the two membranes of plant chloroplasts (Figure 1), that escort expatriate proteins — several thousand [2] — from the plant cytosol back into the chloroplast [15]. How much of this apparatus can we find in non-plant chloroplasts? If core parts of the machinery are common to all plastids, it would provide solid evidence for a common origin of protein import and therefore plastid origin. Additionally, a comparison of the import apparatus with proteins from the cyanobacterium-like endosymbiont could reveal how much of the import machinery was co-opted from pre-existing proteins in the endosymbiont, and how much was invented by the host as part of its subjugation of the captive food factory.

As a bonus, algal genomes might also be the Rosetta Stone to help us understand the mysterious process of protein targeting in the so-called 'second-hand' plastids, which arose from eukaryote–eukaryote endosymbiosis events [4,16]. These organisms, which include the likes of diatoms, cryptomonads and the malaria parasite *Plasmodium*, typically have plastids with four bounding membranes. The inner two membranes are homologues of the plant chloroplast membranes, and the outer two membranes derive from the endosymbiont's plasma membrane and the host's endomembrane system, respectively [4,16]. What translocation machineries exist in the onion-like membranes of secondary plastids?

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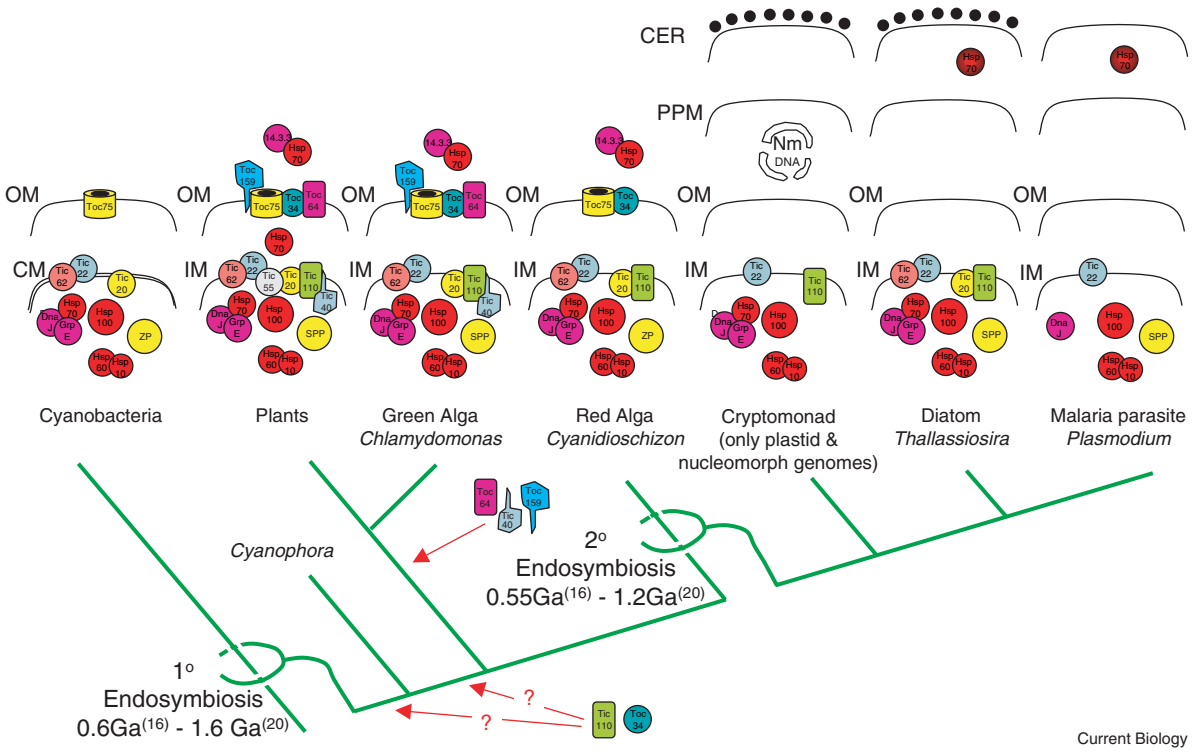


Figure 1. Gene-based models for the import apparatuses of various plastids and their homologues in cyanobacteria.

The tree shows the origin of green algal/plant chloroplasts and red algal plastids by a single primary endosymbiosis of a cyanobacteria-like endosymbiont. The subsequent secondary endosymbiotic origin of diatom, cryptomonad and malaria parasite plastids from a red alga is also shown. Much of the import machinery originates from cyanobacterial genes but novel additions, presumed to derive from host inventions, are apparent. One such invention, Tic110 — the putative pore in the inner plastid membrane [15] — occurs in all plastids (with the exception of the malaria parasite) and is a strong piece of evidence supporting a common origin of the import machinery and hence a common origin for all plastids. Experimental evidence for location and function of the depicted proteins is only available from plants and cyanobacteria [15], and roles for algal proteins are merely inferred from sequence similarity. Protein alignments are available on request. IM, inner membrane; OM outer membrane; PPM, periplastid membrane; CM, cell membrane; CER, chloroplast endoplasmic reticulum (with or without ribosomes); Nm, nucleomorph; SPP, stromal processing peptidase; ZP, zinc peptidase. *Chlamydomonas* and *Thalassiosira* genomes: www.jgi.doe.gov/genomes/

We searched the red algal genome [3], the *Plasmodium* parasite genome [17], cyanobacterial genomes and the almost complete genomes available for the diatom *Thalassiosira pseudonana* and the green alga *Chlamydomonas* for possible components of the plastid protein import apparatus (Figure 1). We also included the cryptomonad alga *Guillardia theta*, for which the nuclear genome is not available but several components of the import apparatus have already been identified from the plastid DNA and the relict nucleus of the secondary red algal endosymbiont [18]. Comparison of the plant chloroplast import and processing machinery with cyanobacterial proteins had already revealed endosymbiont ancestry for the outer membrane channel protein (Toc75) as well as the inner chloroplast membrane proteins Tic20, Tic22 and perhaps Tic55 [19]. Import-related components within the chloroplast stroma — including the molecular chaperones Hsp70 and Hsp100, their attendant factors GrpE and DnaJ, and the protein-folding chaperonins Hsp60/10 or GroEL/ES — are also derived from the endosymbiont (Figure 1).

All of these components — with the exception of Tic55 — are evident in the red algal genome (Figure 1) and likely have similar roles, as would the cytosolic components Hsp70 and 14-3-3. However, Toc159, the engine of outer membrane translocation in plants [15] and probably *Chlamydomonas* (Figure 1), cannot be identified in the red algal genome [3], indicating that this component evolved in the green algae/land plant lineage — perhaps from the related Toc34 — and begging the question of how proteins are driven through the putative outer membrane pore (Toc75) of red algal plastids.

These notable absences notwithstanding, the apparent conservation of a great deal of the import machinery in two plastids, red and green, suggests they share a common origin (Figure 1). The best form of evidence for a common ancestry of plastids, however, would come from novel components donated to the import process by the host. Tic110 is a central component of the inner membrane apparatus and possibly part of the pore through which proteins traverse the membrane [15]. Importantly, Tic110-like genes are identifiable in *Chlamydomonas*, red algae, cryptomonads and diatoms, but apparently absent from cyanobacteria (Figure 1). This distribution suggests that Tic110 is a shared derived component of all plastids and therefore

a very strong piece of evidence that these plastids have a common origin. Similarly, Toc34, which is believed to be a receptor for transit peptides [15] and a possible ancestor of Toc159, appears at the same time (Figure 1). Divergence of red algae and green algae/plants occurred at least 600 million years ago, and perhaps as much as 1,500 million years ago [20]. We propose that Tic 110 and Toc34 originated prior to this split (Figure 1).

Interestingly, no Toc components can be identified in the genomes of organisms with secondary plastids (Figure 1). Because the transit-peptide-like components of targeting motifs in secondary plastids function in plant chloroplasts as import signals [10,11], it was anticipated that the Toc machinery, which recognises transit peptides [15], would be present in the outer membrane homologue of these plastids [16]. Moreover, an extra set of Toc machinery is hypothesised to be present in the so-called periplastid membrane as a means of recruiting proteins from the endomembrane lumen into the endosymbiont [16]. Our inability to identify any Toc proteins based on sequence similarity in second-hand plastids (Figure 1) is a serious setback to these models, and some adroit cell biology may be required to work out how proteins traverse the periplastid membrane and two inner membranes of these plastids.

The red algal genome [3] allows us to infer models of plastid import that all but confirm a single origin for plastids. The genome will also address other contentious issues, such as the origin of secondary plastids [4] and mechanisms of endosymbiont division [3]. Next on our preferred genome wish list would be *Cyanophora*, a veritable coelocanth of the algal world. Of all plastids, *Cyanophora*'s most closely resembles its cyanobacterial ancestor — right down to retaining a bacterial-type peptidoglycan cell wall between its membranes and the blue-green pigments of cyanobacteria [13]. A peek at the import apparatus genes of *Cyanophora* could take us back to the very origin of plastids.

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