Minireview

Mergers and acquisitions: malaria and the great chloroplast heist

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Abstract

The origin of the relict chloroplast recently identified in malarial parasites has been mysterious. Several new papers suggest that the parasites obtained their chloroplasts in an ancient endosymbiotic event that also created some major algal groups.

The identification of a relict chloroplast (plastid) in human parasites caused quite a sensation [1-3]. How could organisms that live as obligate intracellular parasites in animals share a feature so definitive of algae and plants? The malaria parasite (*Plasmodium* spp), which invades red blood cells and eats the hemoglobin protein, can scarcely be any less plant-like. But for protistologists the revelation was not quite so strange. Molecular phylogenetics had clearly demonstrated that the malaria parasite, along with many other parasites such as *Toxoplasma* that together make up the phylum Apicomplexa, are the closest relatives of an algal group known as dinoflagellates [4]. And protistologists are becoming accustomed to strange bedfellows in their evolutionary trees. After all, *Euglena*'s closest cousins are the trypanosomes and leishmanias, and the golden flagellate algae (including the brown kelps and diatoms) are in the same group as *Phytophthora*, the fungal-like protist that caused the devastating Irish potato blight [4]. Close relationships between parasites and photosynthetic organisms seem unusual to us only because our early evolutionary schemes were based mainly on lifestyle, but technological advances such as electron microscopy, molecular biology and genomics are providing more accurate pictures of natural relationships.

So if malaria and dinoflagellates are close relatives, and both have plastids, what is all the excitement about their plastids? Well, in protist evolution the acquisition of plastids has been a complex tapestry of mergers and takeovers [5]. Indeed, the plastid story rivals the current shenanigans in the biotechnology sector. Just as covetousness for competitors' intellectual property is driving a virtual feeding frenzy of acquisitions and takeovers, a similar feeding frenzy has been playing itself out over hundreds of millions of years in the protist world [5]. But in this little drama, photosynthesis is the key piece of 'intellectual property'. And what better technology to have in-house than the ability to turn sunshine into food? The internecine struggle to control this resource is a bizarre tale of piracy, slavery, double crossing and microscopic treachery.

The origin of plant chloroplasts by endosymbiosis is now the stuff of textbooks. Microscopists recognized the gross similarities between chloroplasts and cyanobacteria and formulated the theory of endosymbiosis [6], which posits that chloroplasts were derived from cyanobacterium-like cells now living inside a eukaryotic host. Biochemistry and molecular biology have since proven beyond doubt that chloroplasts do indeed derive from endosymbiotic cyanobacteria [7]. But the story does not close there. The cyanobacterial endosymbiosis is the first chapter in a set of endosymbiotic events responsible for the acquisition of plastids in a range of eukaryotic lineages. This first chapter (cyanobacterium + eukaryotic host = photosynthetic eukaryote) is referred to as the primary endosymbiosis. A subsequent chapter in plastid acquisition is referred to as secondary endosymbiosis [5], and can be described by the equation 'photosynthetic eukaryote + eukaryotic host = different photosynthetic eukaryote'. In this chapter the product of the first equation (photosynthetic eukaryote) is the first component of the second equation. Although primary endosymbiosis happened (to the best of our knowledge) only once, secondary endosymbiosis occurred at least twice and, some would

argue, perhaps numerous times [5] (see Figure 1). Takeover of someone else's photosynthetic unit seems easier than developing one from a bacterial symbiont, and this is where we return to malaria and dinoflagellates.

Although dinoflagellates and Apicomplexa are close relatives [4], their plastids are of secondary origin [3], which leaves open the possibility that they have separate secondary acquisitions. How to address this? The standard approach has been to compare genes from the plastid genome of the secondary plastid with the candidates from the pool of primary plastid-containing organisms. For malaria parasites this was no problem since the entire plastid genome was already sequenced by Iain Wilson and colleagues [2], but, until recently, no plastid genes had been characterized from dinoflagellate plastids. Zhang *et al.* [8] now fill that gap by providing the sequence of rRNA genes from the plastids of several dinoflagellates. Dinoflagellate plastid DNA is unique in that each gene is carried on a single minicircle rather than the canonical circular chromosome with multiple genes in transcriptional arrays found in all other plastids [9-11]. Trees incorporating rRNA genes show dinoflagellate plastids to be most closely related to plastids of apicomplexan plastids (referred to as sporozoan plastids in [8]). At face value this relationship suggests that the plastids were acquired by a common secondary endosymbiotic event [8], but there could be a metaphorical serpent coiled in the branches of the evolutionary trees - a major bugbear of tree inference is rapidly evolving genes. Accumulating mutations at elevated rates, such sequences form long branches in trees. Tree-constructing algorithms tend to group long branches together - even branches that are not related [12]. The branches of dinoflagellate and apicomplexan plastids are extraordinarily long and their grouping must be regarded with abject caution. Moreover, nucleotide bias (a preference for one type of base pair in the genome) can wreak further havoc on tree construction [13] and dinoflagellate and apicomplexan genes are strongly biased to A/T pairs. This means that the sequence similarity could be convergent and might not reflect common ancestry.

A previous study of apicomplexan plastid genes concluded that the apicomplexan plastid derived from a secondary endosymbiotic green alga [3]. This made a common origin with plastids of dinoflagellates impossible, since dinoflagellates almost certainly contain a red algal endosymbiont [10,14]. Another factor is the inconsistency in membrane number; standard dinoflagellate plastids have three [5], whereas Apicomplexa are believed to have four bounding membranes [3,15], although the number of apicomplexan plastid membranes has been under debate [16], making it difficult to assess the relevance of this character. Another obstacle to common origin was the belief that two putatively early diverging Apicomplexa (*Perkinsus* and *Colpodella*) might

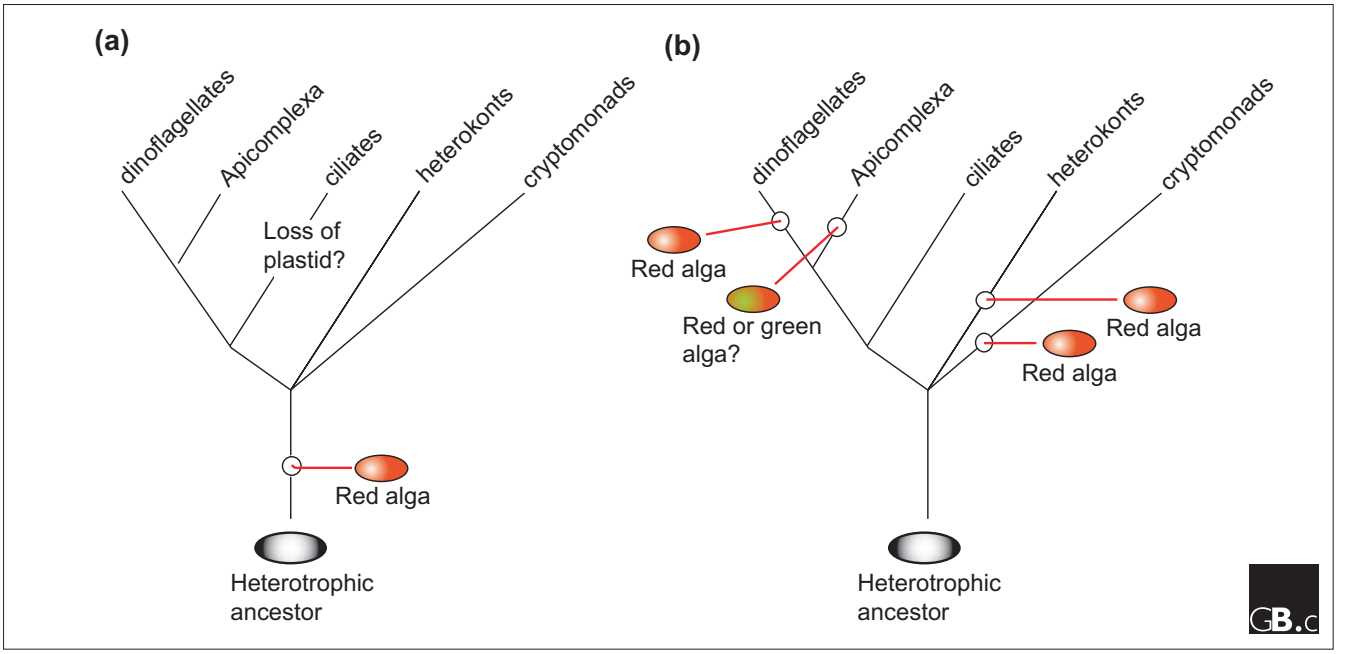

Figure 1
Schemes outlining two competing hypotheses for plastid origin(s) in algae containing secondary red algal symbionts. **(a)** New data from nucleus-encoded genes whose products are targeted to the plastid, and new data on dinoflagellate and *Perkinsus* plastids suggest that one secondary endosymbiotic origin spawned multiple groups, whereas **(b)** the canonical interpretation is that all these groups arose through independent secondary endosymbiotic events. Secondary endosymbioses are represented as introgressions of the red alga (or perhaps a green alga in the case of Apicomplexa) into circles within the lines of vertical descent. If scenario (a) is correct, ciliates have lost plastids secondarily, as have subgroups within the other lineages.

lack a plastid [17], suggesting that a plastid was acquired by Apicomplexa after they diverged from dinoflagellates [3].

Several new lines of evidence now lend support to Zhang *et al.* [8] for a common origin from a red algal endosymbiont, however. In contrast to the gene trees suggesting a green endosymbiont for Apicomplexa, apicomplexan plastid gene operon structure is much more congruent with a red algal endosymbiont [18-20]. In addition, new molecular evidence suggests that *Perkinsus* does have a plastid (X. Zhao and D.W. Duszinski, personal communication), removing one of the obstacles to a common origin. Thus, Zhang *et al.* [8] are out on a limb, but not entirely alone. The last, and potentially most telling, pieces of evidence for a common origin come not from the 'difficult-to-tree' plastid genes but from the nuclei of the hosts. Cells hosting secondary endosymbionts contain genes encoding plastid proteins in their nuclei [21]. The products of these genes are synthesized in the host cytoplasm and targeted into the plastid across the multiple membranes [21]. Typically these genes derive ultimately from the cyanobacterial endosymbiont and carry such a phylogenetic signal. Naomi Fast and colleagues (personal communication) are examining one such gene for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in Apicomplexa, dinoflagellates and other algae. As expected, the GAPDH genes of plants, green algae and red algae (all descendants of the primary endosymbiosis) have a cyanobacterial ancestry; they transferred to the eukaryotic host nucleus as part of the establishment of the primary endosymbiosis (N.M. Fast, J. Kissinger, D. Roos, P. Keeling, personal communication). In secondary endosymbiosis, the nuclear genes of the engulfed endosymbiont typically undergo yet another transfer into the new host nucleus [21,22]. Fast and colleagues, therefore, anticipated that the GAPDH genes in nuclei of secondary hosts should also show the cyanobacterial signature. Not so. In the Apicomplexa and dinoflagellates, as well as the heterokont and cryptomonad algae, the GAPDH targeted to the plastid is not cyanobacterial in origin but simply a duplicate of the secondary host nuclear gene for cytosolic GAPDH (N.M. Fast *et al.*, personal communication). Apparently evolution has taken a short cut. Rather than transferring the secondary endosymbiont's gene to the nucleus, these algae have copied their nuclear gene and appended a targeting leader to the amino terminus. Importantly, the structure of the GAPDH trees implies that this peculiar recruitment process happened just once (N.M. Fast *et al.*, personal communication). That means dinoflagellates, Apicomplexa, heterokonts, and perhaps even cryptomonads, could all have acquired their plastids in one common secondary endosymbiosis of a red alga (Figure 1), supporting the trees of Zhang *et al.* [8]. Moreover, recent phylogenetic analyses indicate that these organisms all belong in a previously unrecognised meta-assemblage dubbed the 'Chromalveolates' (Figure 1) [23] (S.L. Baldauf, A.J. Roger, I. Wenk-Siefert, W.F. Doolittle, personal communication).

Because the apicomplexan plastid is potentially an excellent target for anti-parasite drugs [15], it is important that we understand its evolutionary history. It seems likely that the GAPDH 'shortcut' will have been taken for other plastid-targeted proteins, so a confirmation could come from further study of nucleus-encoded plastid proteins. If a common origin for dinoflagellate and apicomplexan plastids is confirmed, we will have deepened our understanding of the origins of one of the world's worst disease-causing organisms. Dinoflagellates and Apicomplexa diverged at least 400 million years ago, but despite outward appearances they are not so fundamentally different. Both have the ability to associate closely with animals, dinoflagellates as endosymbionts of corals and other invertebrates and Apicomplexa as intracellular parasites [19]. An attractive scenario is that this ability to associate with animals goes back in time to their common ancestor and that one lineage (dinoflagellates) persisted with photosynthesis and commensal interactions, whereas another (Apicomplexa) abandoned photosynthesis, instead converting to parasitism to exploit the host [19]. This presumably happened quite early in animal evolution but the parasites are still with us. Why they keep a vestige of their plastid is the next burning question.

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