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The Origin of Primary Plastids: A Pas de Deux or a Ménage à Trois?

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The idea of an endosymbiotic origin of plastids has become incontrovertible, but many important aspects of plastid origins remain obscured in the mists of more than a billion years of evolutionary history. This commentary provides a critical summary of a recent proposal that primary plastid endosymbiosis was facilitated by the secretion into the host cytosol of effector proteins from intracellular Chlamydiales pathogens that allowed the host to utilize carbohydrates exported from the incipient plastid. Although not without flaws, the model provides an explanation for why primary plastids have evolved so rarely and why Archaeplastida, among all phagotrophic eukaryotes, succeeded in establishing primary plastids.

It was over a century ago in 1905 that Mereschkowsky proposed that plastids derived from engulfed cyanobacteria (Martin and Kowallik, 1999), and more than 40 years since this idea entered the biological mainstream, primarily through the work of Lynn Margulis (e.g., Sagan, 1967; Margulis, 1970, 1981). The basic idea of an endosymbiotic origin of plastids is supported by abundant lines of evidence, especially by phylogenetic analyses showing that plastid genomes represent a particular branch of the cyanobacteria. Nonetheless, many important aspects of plastid origins remain. Ball et al. (2013) have now contributed to our understanding of primary endosymbiosis, exploring the possible role of Chlamydiae, intracellular parasitic bacteria, in allowing for the integration of cyanobacterial and host carbohydrate metabolism.

Looking across the entire tree of life, we see many instances in which a lineage has made a remarkable transition in its way of life, often resulting in a major adaptive radiation. In seeking to explain these major transitions, evolutionary biologists generally start by identifying the traits needed for survival in the new adaptive zone and documenting the order in which they were acquired. However, a full evolutionary narrative should also explain why, if an evolutionary adaptive zone transition is so beneficial, did it not occur more frequently? And also why, among all of life's diversity, did one specific lineage make the leap? For example, the reason that among all the many lineages of multicellular algae it was just the

charophytes that succeeded in invading land (as land plants) has been suggested to be due to specific preadaptations of these algae, such as being specialized for freshwater (Graham, 1993; Becker and Marin, 2009) and having particular developmental (Graham et al., 2000) or biochemical characteristics (Sørensen et al., 2011). Ball et al. (2013) attempt to provide such a narrative to help explain why one particular clade of eukaryotes, the Archaeplastida, came to host the endosymbiotic cyanobacteria that evolved into plastids.

Phylogenies of plastid-encoded genes confirm that plastids form a single clade embedded within the Cyanobacteria, consistent with a single event of plastid origination. The data from the nuclear genome is also consistent with this inference: Viridiplantae (which includes green algae and land plants), rhodophytes, and glaucophytes, the three eukaryotic lineages that are inferred on anatomical grounds to have plastids that were derived directly from engulfed cyanobacteria (primary plastids), form a clade, Archaeplastida (Keeling, 2004, 2010; Price et al., 2012). Evolution of primary plastids has indeed been an evolutionary rarity of the highest degree.

The lack of additional origins of primary plastids is not simply because photoendosymbiosis is difficult. At least seven independent eukaryotic lineages have taken up single-celled eukaryotic algae as plastids, including ancestors of such successful groups as euglenoids, brown algae/diatoms, and dinoflagellates (Keeling, 2010). By contrast, there is only one other instance where a heterotrophic eukaryote

evolved a stable association with an endosymbiotic cyanobacterium, the cercozoan genus *Paulinella* (Marin et al., 2005; Nowack et al., 2008), and the status of this case as a true plastid has been subject to debate (Theissen and Martin, 2006). The obvious conclusion from the rarity of primary plastid origins is that it is more difficult to integrate a prokaryotic endosymbiont into the host's metabolism than another eukaryote. But what might the problem be, and how might the ancestor of Archaeplastida have overcome that impediment? Ball et al. (2013) argue that one of the main challenges during plastid origin is meshing host and plastid carbohydrate metabolism and that Archaeplastida were uniquely able to accommodate a cyanobacterial endosymbiont because they were subject to coinfections by chlamydial endoparasites.

The idea that Chlamydiae have been important in the evolutionary history of Archaeplastida was first hinted at by the discovery that a surprisingly large number of genes in the *Chlamydia trachomatis* genome have closely related genes in plants (Stephens et al., 1998). Subsequent analyses have suggested that this is the result of direct horizontal gene transfer from Chlamydiae into ancestral Archaeplastida (Greub and Raoult, 2003; Huang and Gogarten, 2007; Becker et al., 2008; Moustafa et al., 2008). Phylogenomic analyses have identified between 21 and 55 specific genes that appeared to have been acquired by plants from Chlamydiae (Huang and Gogarten, 2007; Becker et al., 2008; Moustafa et al., 2008). Ball et al. (2013) corroborate this conclusion with their own

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analysis of currently available sequence data. Even using rather stringent criteria, they show that there are many more cases of chlamydial genes being sister to archaeplastidial genes than is the case for other eukaryotic clades, even clades such as fungi and animals, which are overrepresented in sequence databases.

The conclusion that chlamydial genes' presence in plants is a result of direct horizontal gene transfer is not universally accepted. Some suspect that these genes are the result of more complex histories entailing gene transfers among different prokaryotic lineages, including the cyanobacterial ancestors of plastids, perhaps combined with gene transfers into Archaeplastida later in their evolution and/or transfers from Archaeplastida into Chlamydiae (Brinkman et al., 2002; Dagan and Martin, 2009; Martin et al., 2012). However, the relatively large number of chlamydial genes in plant genomes points to some history of stable association between Chlamydiae and Archaeplastida that allowed abundant gene transfer. This leaves open the possibility that Chlamydiae were present in the host cell at the time that primary plastids evolved (Huang and Gogarten, 2007). If that were so, might chlamydial genes have facilitated plastid evolution?

Following on from the group's prior work on carbohydrate metabolism in Archaeplastida (Deschamps et al., 2008), Ball et al. (2013) explore the possibility that key genes were acquired from Chlamydiae. They focused on genes that would have been needed for sugar nucleotides exported from the cyanobacterium to be converted into glycogen, the original storage polysaccharide, and those that would be needed to effectively use stored glycogen. Cyanobacteria are assumed to have exported ADP-Glc. This would have posed a problem, however, since eukaryotic glucan synthases use UDP-Glc as substrate, rather than ADP-Glc. Ball et al. (2013) argued that the archaeplastidial glucan synthases that are most likely to have had ADP-Glc activity in solution are members of the class III or class IV starch synthases. A phylogenetic analysis of glucan synthases identified a well-supported (98% bootstrap) clade composed only of archaeplastidial class III

and class IV sequences and a number of bacterial sequences (see Supplemental Figure 1 online in Ball et al., 2013). As shown in an unrooted version of this subtree (Figure 1A), the chlamydial sequences would end up sister to Archaeplastida under plausible rooting scenarios. Notably, this tree rules out a cyanobacterial origin of the archaeplastidial genes. Similarly, Ball et al. (2013) argued that effective use of glycogen stores required direct debranching activity, which is provided in modern Archaeplastida by isoamylase, which is closely related to chlamydial GlgX proteins (Huang and Gogarten, 2007). Phylogenetic analysis of GlgX/isoamylase sequences yields an unrooted tree on which plausible roots imply, once again, that archaeplastidial genes have chlamydial ancestry (Figure 1B).

However, the inference that genes needed for proper interactions between a eukaryotic host and a prokaryotic endosymbiont were

acquired from Chlamydiae poses a problem. It is unlikely that these genes entered the archaeplastidial genome before plastid endosymbiosis because they would have had no function, and genes do not persist in genomes for long if they lack a function. But equally, if these enzymes were critical for the establishment of the plastid, then they had to be present at the time of endosymbiosis. Ball et al. (2013) propose a resolution of this paradox: Suitable glucan synthases (GlgA) and direct glycogen debranching enzymes (GlgX) were present in the cytoplasm of the host cell because they were being secreted into the cytoplasm by coinfecting Chlamydiae. In support of this hypothesis, Ball et al. showed that many extant chlamydial proteins involved in carbohydrate metabolism, including GlgX, have sequences consistent with being effector proteins released into the host cell by a type III secretion system. Furthermore, an *in vivo*

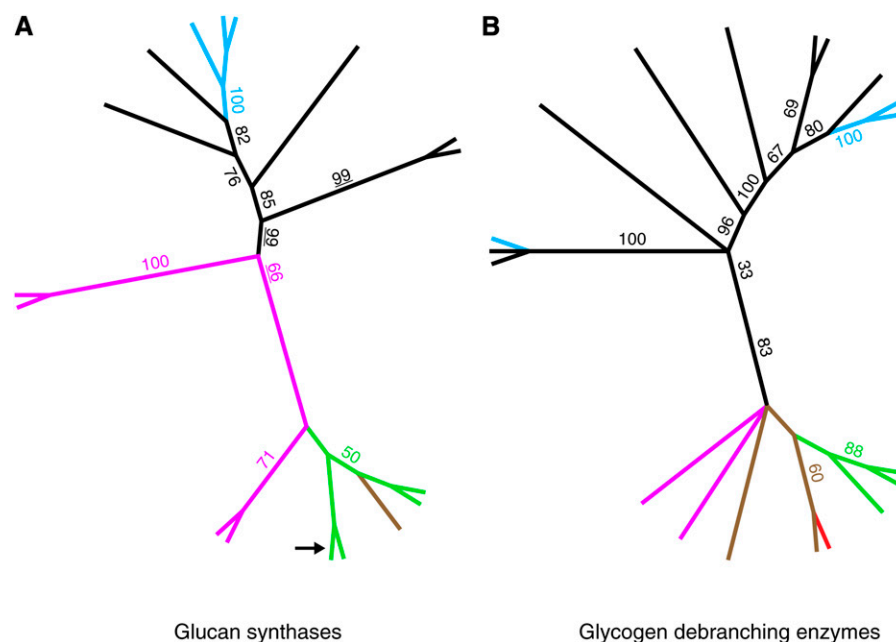


Figure 1. Unrooted Phylogenetic Trees for Gene Families Involved in Carbohydrate Metabolism.

Both trees are heavily pruned and have branches with bootstrap percentages below 50% collapsed. Branches are coded by taxon: green = Viridiplantae; red = red algae; brown = glaucophytes; pink = Chlamydiae; cyan = cyanobacteria; black = other bacteria.

(A) The starch synthase III-IV tree based on Supplemental Figure 1 from Ball et al. (2013). In the original analysis, this subtree was rooted on the branch marked with an arrow, but a more plausible rooting would be on one of the black (bacterial) branches.

(B) Glycogen direct debranching genes based on Figure 5 and Supplemental Figure 2 from Ball et al. (2013). This tree is unrooted, but the true root most likely resides on one of the black branches.

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assay using *Shigella flexneri* as a model confirmed that the N-terminal peptide of several of proteins, including GlgX and GlgA, allows secretion through the type III system, suggesting that they could act as effector proteins in Chlamydiae.

Putting the pieces together, Ball et al. (2013) propose a narrative in which the ancestral Archaeplastida were subject to infection by Chlamydiae, which injected a suite of proteins into host cells. These effector proteins served to manipulate carbohydrate metabolism to the parasite's advantage. The presence of these enzymes in the cytoplasm had the beneficial side effect of allowing the host and an incipient plastid to enter into a mutualistic association that would otherwise have been impossible because of the inability of the host to synthesize and use storage carbohydrates from ADP-Glc. Only later, after horizontal gene transfer introduced the enzyme-coding genes into the nucleus, could the mutualism persist without Chlamydiae. Only at that point would selection favor Archaeplastida that resisted chlamydial infection. Or, to put it simplistically, rather than viewing the endosymbiotic origin of plastids as a dance of two partners, Ball et al. suggest it was a more complex, ménage à trois.

The Ball et al. model has some compelling features. It provides an explanation for why primary plastids have evolved so rarely and why Archaeplastida, among all phagotrophic eukaryotes, succeeded in establishing primary plastids. However, for all its appeal, the hypothesis is not without its flaws. For example, many groups of eukaryotes are or have been subject to infection by Chlamydiae, so the model does not fully explain why there were no other origins of primary plastids. Also, there remains a possibility that the 50 or so genes transferred from Chlamydiae to Viridiplantae were acquired at different times in the course of simple parasitism, with the apparent enrichment of carbohydrate metabolism genes in the list being coincidental. Nonetheless, by combining new insights into the evolution of carbohydrate metabolism in Archaeplastida with phylogenomics and experimental

studies of potential metabolic effector proteins, Ball et al. (2013) have contributed to our understanding of one of the most important events in the origin of life on earth: the origin of plastids.

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