COMMENTARY

Hold the salt: Freshwater origin of primary plastids

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The evolution of oxygenic photosynthesis by cyanobacteria was arguably one of the most significant biological events in Earth's history, shaping the atmosphere and subsequently leading to diverse ecosystems (1). The permanent endosymbiotic merger between a cyanobacterium and a unicellular heterotrophic eukaryote in deep evolutionary time set the stage for the stunning diversity of photosynthetic eukaryotes and ecosystems seen today, giving rise to the supergroup Archaeplastida, the red, glaucophyte, and green algae and their descendent land plants. Later transfers of photosynthesis to other lineages of heterotrophic eukaryotes through eukaryote-eukaryote mergers (secondary and tertiary endosymbiosis) led to many near-shore and open-ocean species, including kelps, diatoms, coccolithophors, and dinoflagellates (2). The cyanobacterial ancestry of primary plastids is no longer debated, but the precise donor of primary plastids, the timing and ecological context of the merger, and modifications since the event have received much attention (3–6). In PNAS, Sánchez-Baracaldo et al. (7) examine the evolution of primary photosynthesis and its habitat of origin using the most comprehensive dataset thus far from photosynthetic cyanobacteria and eukaryotes.

Reconstructing the history of primary endosymbiosis is challenging due to its deep evolutionary time and long separation of descendent lineages. The oldest eukaryotic fossils (about 1.7 billion y ago) cannot be unequivocally assigned and most of the age constraints used for time-tree analyses are from Phanerozoic fossils. All three lineages of Archaeplastida possess primary plastids of cyanobacterial origin but, as seen from newly abundant plastid genomic data from diverse photosynthetic eukaryotes, each group has evolved distinct modifications of the inherited cyanobacterial genetic “toolkit” for plastid functions, with independent gene losses and transfers to the host nucleus of plastid targeted genes, thus solidifying the integration (3, 8, 9). Likewise, free-living cyanobacteria further diversified since their cousins participated in primary endosymbiosis. Alternative candidates for the sister group to Archaeplastida plastids range from among morphologically

Fig. 1. Schematic overview of major events in evolution of Archaeplastida primary plastids from freshwater cyanobacteria, and the later independent primary endosymbiosis in *Paulinella* (Rhizaria), from a derived group of marine cyanobacteria (after ref. 7). The cyanobacterium *Gloeomargarita* shares a most recent common ancestor with plastids of the eukaryotic red, green, and Glaucophyte algae, with an inferred freshwater ancestral habitat. Habitat transitions in red algae are from freshwater to marine in certain lineages. Habitat transitions in green algae are common and the early-diverging lineages of the two phyla of green algae have alternate habitats, so the ancestral form in green algae is uncertain (12).

simple, early-diverging (6) to more derived and morphologically complex cyanobacteria (5), to the possibility that primary plastids of Archaeplastida have multiple origins (10, 11).

Two exciting discoveries of novel and deeply diverging lineages of cyanobacteria and green algae, as well as growing availability of genomic data from diverse photosynthetic species, prompted a reinvestigation of these fundamental questions. The recent discovery of *Gloeomargarita lithophora*, a cyanobacterium found in microbialites of alkaline lakes in Mexico, made a splash because this species is among the early-diverging cyanobacterial lineages and is implicated as the closest relative to Archaeplastida (6). Second, an early-diverging lineage

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comprising the marine deep-water green algae *Palmophyllum* and *Verdiggella* was determined from analysis of plastid genome data (12).

Sánchez-Baracaldo et al. (7) analyzed multiple sources of existing plastid and bacterial genomic data, including that of the new species mentioned above, to address scenarios and timing for the evolution of primary endosymbiosis leading to the first photosynthetic eukaryotes and explicitly tested the freshwater origin of primary plastid lineages using ancestral state reconstruction of habitat data. Significantly, their study resolved the newly discovered cyanobacterium *Gloeomargarita* as the closest living relative to Archaeplastida and revealed a freshwater origin of primary endosymbiosis between cyanobacteria and eukaryotes (4, 6), with divergence of *Gloeomargarita* from the most recent ancestor of Archaeplastida at 2.1 billion y ago (Fig. 1). Thus, plastids evolved from among the early-diverging, small, unicellular cyanobacteria before the emergence of larger unicellular and filamentous forms that likely correspond to the first recognizable fossil cyanobacteria. Their analyses provided a mean age of diversification of green algae and red algae that is older than the fossil red *Bangiomorpha* (1.1 billion y ago). Given the freshwater ancestry of Archaeplastida, marine algae appear only in the late Proterozoic. Extant Archaeplastida groups include marine and freshwater species, but there is a growing appreciation for the prevalence and diversity of freshwater, terrestrial, and even aeroterrestrial taxa (13, 14), and 1-billion-y-old shales containing fossil eukaryotes have been interpreted as ancient freshwater lake beds (15). Sánchez-Baracaldo et al. (7) also reinforce the later (Neoproterozoic) timing of an independent primary endosymbiosis within the Rhizaria. The amoeba *Paulinella* has blue-green inclusions that were initially interpreted as intracellular symbiotic cyanobacteria but were later shown to be permanent plastids of cyanobacterial origin from among the alpha-cyanobacteria, a lineage distinct from the source of plastids in the Archaeplastida, the beta-cyanobacteria (16).

Although this is the most comprehensive analysis to date there is room for future work, especially in understanding the early branching of photosynthetic eukaryotes and their evolution of ecological preferences and other traits. We can start by collecting more information from known species. Existing data are limited to a single representative of the (albeit small) phylum Glaucophyta and this must be expanded to improve our understanding of the phylogeny and

physiology of this group, and environmental genomic data can be used to test the freshwater distribution of this group (17). Scoring the habitat preference of a given species does not necessarily reflect the habitat of an entire lineage, as habitat switching within lineages is well-documented. Also, some individual species have a remarkable capacity to tolerate a wide range of salinity and temperature (including early-diverging red algae, such as *Galdieria*). In green plants, Streptophyta is widely interpreted as a freshwater lineage (13, 18). Sánchez-Baracaldo et al. (7) place *Mesostigma* and *Chlorokybus* as the earliest-diverging lineage in green plants, at odds with analyses that reconstructed these taxa as the first diverging forms in Streptophyta (19). Despite this difference, sensitivity analysis indicated that the more widely accepted topology did not impact the dating of the green lineage or the interpretation of a freshwater origin for the green algae, although it may have impacted the dating of the two phyla. In Chlorophyta at least 10 distinct and early-diverging lineages of "prasinophyte" green algae, along with the recently discovered deep-water species, would imply either a marine origin of the phylum with later secondary transition(s) to freshwater in Ulvophyceae, Trebouxiophyceae, and Chlorophyceae or dozens of independent transitions to the marine habitat. However, critical portions of the Chlorophyta tree are so far poorly resolved. Thus, more information about the conditions tolerated by known species along with a detailed phylogeny based on a more comprehensive sample of species would enhance estimation of habitat transitions.

An exciting prospect is that we will uncover other novel and early-diverging lineages in the future, given that many species representing untapped diversity have been discovered in the last decade. Ultimately, as we refine our understanding of the sister lineage of primary plastids we can better test the hypothesis of monophyly of archaeplastids, the branching order of major archaeplastid lineages, and learn more about their ecologies. As we learn more about species such as *Gloeomargarita* we better understand the underlying biology and perhaps even the communities and conditions favoring permanent and long-lasting endosymbiotic mergers.

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