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Evolution of the 3-hydroxypropionate bicycle and recent transfer of anoxygenic photosynthesis into the Chloroflexi

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Various lines of evidence from both comparative biology and the geologic record make it clear that the biochemical machinery for anoxygenic photosynthesis was present on early Earth and provided the evolutionary stock from which oxygenic photosynthesis evolved *ca.* 2.3 billion years ago. However, the taxonomic identity of these early anoxygenic phototrophs is uncertain, including whether or not they remain extant. Several phototrophic bacterial clades are thought to have evolved before oxygenic photosynthesis emerged, including the Chloroflexi, a phylum common across a wide range of modern environments. Although Chloroflexi have traditionally been thought to be an ancient phototrophic lineage, genomics has revealed a much greater metabolic diversity than previously appreciated. Here, using a combination of comparative genomics and molecular clock analyses, we show that phototrophic members of the Chloroflexi phylum are not particularly ancient, having evolved well after the rise of oxygen (*ca.* 867 million years ago), and thus cannot be progenitors of oxygenic photosynthesis. Similarly, results show that the carbon fixation pathway that defines this clade—the 3-hydroxypropionate bicycle—evolved late in Earth history as a result of a series of horizontal gene transfer events, explaining the lack of geological evidence for this pathway based on the carbon isotope record. These results demonstrate the role of horizontal gene transfer in the recent metabolic innovations expressed within this phylum, including its importance in the development of a novel carbon fixation pathway.

carbon fixation | phototrophy | molecular clock | comparative genomics

From both biological and geological data, it is widely thought that anoxygenic photosynthesis preceded the development of oxygenic photosynthesis and the rise of atmospheric oxygen (1). Although it has been largely accepted that oxygenic photosynthesis evolved in ancient Cyanobacterial lineages (2), very little is known about the nature and evolutionary history of anoxygenic phototrophy, with most of our understanding stemming from assumptions and hypotheses based on the few extant bacterial taxa that host this metabolism. Given the significance of the accumulation of oxygen *ca.* ~2.3 billion years ago, it is of no surprise that the majority of efforts have focused on studying oxygenic photosynthesis. However, this has resulted in a paucity of studies critically examining basic and core questions on the origins of anoxygenic photosynthesis, such as what bacterial lineage developed this metabolism and when did it evolve? These questions are essential to our first-order knowledge of how early microbial metabolisms may have shaped the geochemical cycles of our planet.

Although chlorophyll-based photosynthesis can be found in seven known bacterial phyla (i.e., Cyanobacteria, Proteobacteria, Chlorobi, Firmicutes, Acidobacteria, Gemmatimonadetes, and Chloroflexi) (3), a number of studies have argued that one of the earliest forms of anoxygenic photosynthesis arose within the Chloroflexi phylum before the invention of oxygenic photosynthesis during the Archean Eon (4–7). These analyses are based on phylogenies generated from genes involved in photosynthesis. However, without the inclusion of valuable dating information to provide a hard geological constraint on these analyses, the timing of these evolutionary events remains relative, thus highlighting the uncertainty in our understanding of when and how anoxygenic photosynthesis may have originated.

A less recognized alternative is that anoxygenic photosynthesis might have been acquired in modern bacterial clades relatively recently. This possibility is supported by the observation that anoxygenic photosynthesis often sits within a derived position in the phyla in which it is found (3). Moreover, it is increasingly being recognized that horizontal gene transfer (HGT) has likely played a major role in the distribution of phototrophy (8–10). Together, these lines of evidence suggest that phototrophy may have first evolved in lineages that are either yet to be discovered or have gone extinct. Similarly, although a number of modern-day phototrophic members of the Chloroflexi have been identified, we cannot definitively draw the inference that their ancestors must also have had photosynthetic metabolisms. This reasoning highlights the nuances and challenges in interpreting the traits of ancient organisms, and the uncertainty inherent in attributing the evolution of photosynthesis to bacterial lineages that may have existed billions of years ago.

Reconstructing evolutionary histories over large geological timescales can be complicated, with issues stemming from HGT, deep coalescence, gene duplication, and extinction (11). Thus, older events are inherently more difficult to study. These hurdles make it challenging to piece together not only the evolution of phototrophy but also the origins of the six known carbon fixation pathways (12, 13), as they may have been some of the earliest metabolisms to evolve (14–18). To this end, the antiquity of these metabolisms has hampered efforts to retrace their origins and identify what pathway was used by the earliest photoautotrophs. For

Significance

Photosynthesis supports life on our planet; however, we know very little about the origins of this metabolism. Anoxygenic photosynthesis existed prior to the evolution of its more complex counterpart, oxygenic photosynthesis. It is not known which groups of microbes performed anoxygenic photosynthesis on early Earth; one idea has argued that anoxygenic photosynthesis evolved in the bacterial phylum Chloroflexi. We compared the genomes of different members of the Chloroflexi, finding that acquisition of photosynthesis and their unique carbon fixation metabolism evolved remarkably late in Earth history—a time long after the rise of oxygen in the atmosphere.

Author contributions: P.M.S., L.M.W., and W.W.F. designed research, performed research, analyzed data, and wrote the paper.

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example, the widespread diversity of bacteria containing the enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) (19) makes it difficult to definitively determine which clade first evolved this enzyme and the Calvin–Benson cycle.

The phylum Chloroflexi presents a unique opportunity to investigate a number of hypotheses central to our understanding of the early evolution of photosynthesis and carbon fixation. The first described Chloroflexi were discovered and characterized for their ability to perform both anoxygenic photosynthesis and a unique carbon fixation metabolism—the 3-hydroxypropionate (3HP) bicycle (20–22). Thus, these two metabolic traits have long been assumed to be tied to the origins of the evolution of the Chloroflexi phylum (4, 7). Importantly, a key piece of evolutionary insight can be obtained by better understanding the timing of these events in a geological context. Estimating the age and origin of photoautotrophic clades within the Chloroflexi phylum may provide key constraints to help resolve when photosynthesis and the 3HP bicycle evolved. However, this task is challenging, because, unlike other phyla, Chloroflexi lack specific diagnostic molecular biomarkers or microfossil morphologies to provide direct fossil evidence of their age (23, 24). Thus, with no direct geological and paleontological observations on which to rely, here we instead exploit advances in molecular clock analyses to estimate key divergences within the phylum. These estimates highlight the role that HGT has played in the transfer of anoxygenic photosynthesis and innovation of the 3HP bicycle in the Chloroflexi.

Results and Discussion

Cross-Calibrated Molecular Clock Analyses on the Chloroflexi Phylum. Phylogenies quantify the evolutionary relationships between different groups; however, these approaches typically provide little information on the absolute timing of evolutionary divergences. Molecular clocks provide a means to include time constraints into these analyses to test hypotheses for both the evolution and timing of specific events. We have previously demonstrated lower uncertainties and increased accuracy in dating deep evolutionary events using cross-calibrated molecular clock analyses (25), specifically by tying bacterial (Proteobacteria and Cyanobacteria) divergences to the eukaryotic plant and algal fossil records (via mitochrondria and plastids) and leveraging these calibrations across the tree. While estimated uncertainties for deep divergences remain large in comparison with geochronological constraints, this approach enables us to test hypotheses that place the evolutionary timing of phototrophy and the 3HP bicycle in the Chloroflexi before the rise of oxygen versus those that would place these evolutionary events after it.

To construct the molecular dataset, an alignment of concatenated conserved proteins found in bacteria, plastids, and mitochondria, as well as their 16S rDNA, was generated. With recent sequencing efforts expanding the genomic coverage across the Chloroflexi phylum, a wide diversity of taxa were chosen to include several order-level lineages: Chloroflexales, Herpetosiphonales, Anaerolineales, Caldilineales, Dehalococcoidetes, and Thermomicrobiales (26, 27). Although most widely known for

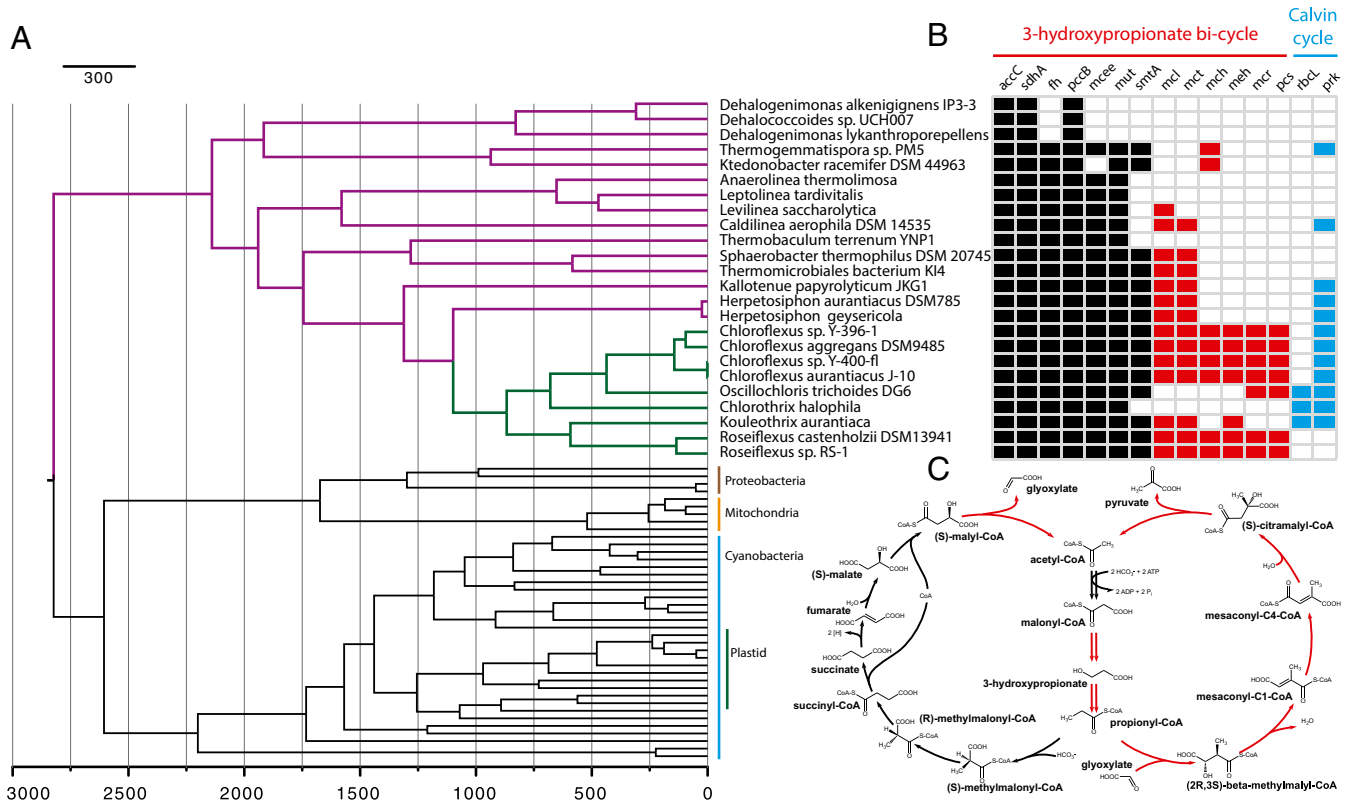

Fig. 1. Divergence time estimates for the Chloroflexi phylum. (*A*) Cross-calibrated Bayesian molecular clock analysis with a broad sampling across the Chloroflexi phylum (purple branches). The Chloroflexales clade (green branches) marks the members of the phylum that are phototrophic and use the 3HP bicycle. Although this clade was suggested to represent one of the oldest known phototrophic lineages, these analyses instead reveal a much more recent evolution of the Chloroflexales. (*B*) Phylogenetic distribution of genes that are involved in the 3HP bicycle demonstrates the stepwise acquisition of enzymes that enabled the last remaining enzymes (propionyl-CoA synthase and malyl-CoA lyase; see Fig. 2) to be horizontally acquired and complete a full 3HP bicycle within the Chloroflexales. The distribution of Calvin–Benson cycle enzymes illustrates that members of the Chloroflexales either have a Calvin–Benson cycle or a complete 3HP bicycle, and suggests that autotrophy via the Calvin–Benson cycle preceded the development of 3HP. (*C*) A metabolic schematic illustrating the complete 3HP bicycle, with enzymes specific to the pathway highlighted by red arrows.

members that perform anoxygenic photosynthesis, phototrophic taxa in the Chloroflexi are nearly all found in one derived monophyletic subclade within the order Chloroflexales, with the exception of one Anaerolinea genome reported to be a photoheterotroph (28). Of specific interest to this study, the Chloroflexales order is composed of the phototrophic genera Chloroflexus and Roseiflexus (highlighted in green in Fig. 1), but not Herpetosiphon. Although there is the possibility that phototrophy was lost in nearly all major groups of the Chloroflexi phylum, it is more parsimonious to explain the appearance of phototrophy in the derived Chloroflexales group via HGT. Results from the cross-calibrated molecular clock analyses reveal that the divergence of the Chloroflexales clade, and thus the acquisition of photosynthesis within the phylum, occurred remarkably late in Earth history, *ca.* 867 Ma, during the later part of Proterozoic time (Fig. 1, Table 1, and *SI Appendix*, Fig. S1).

Late Transfer of Anoxygenic Photosynthesis into the Chloroflexi Phylum. Although previous studies have postulated that anoxygenic photosynthesis originated in the Chloroflexi phylum (4–7), our molecular clock results are much too young to support this hypothesis, even by the limits of the uncertainties. These results imply that the Chloroflexi phylum gained the genes necessary for anoxygenic photosynthesis relatively late in Earth history, via HGT. This is not to say that phototrophy overall evolved much later than previously thought. As mentioned above, oxygenic photosynthesis evolved from some form of anoxygenic photosynthesis in some microbial group that must have been present before the rise of oxygen ∼2.3 Ga (29). Additionally, comparative structural relationships of the phototrophic reaction centers show deeply conserved homology, despite substantial evolutionary distances that places them into what is sometimes termed the "twilight zone" of sequence similarity (30). These observations illustrate that the type I and type II reaction centers diverged from a common homodimeric ancestor sufficiently long ago that little sequence identity unites them today. This inference can only be placed in relative time, but it is among the strongest pieces of evidence that illustrates that anoxygenic photosynthesis must be a very ancient metabolism (3). This does not indicate, however, that the taxa that do anoxygenic phototrophy today must be similarly ancient, due to the impact of HGT (8–10). It is also possible that anoxygenic photosynthesis evolved in stem group lineages that have gone extinct (3).

Interestingly, our molecular clock results show that phototrophy was acquired by Chloroflexi after the rise of oxygen, which is consistent with independent evidence that aerobic respiration predates photosynthesis in this phylum (*SI Appendix*, Fig. S2). Evolutionary relationships between the heme-copper oxygen reductase proteins (i.e., complex IV) indicate that aerobic respiration appears to be a synapomorphy of the Chloroflexia class, and was acquired before the divergence of the basal nonphototrophs Herpetosiphonales and Kallotenuales from the phototrophic Chloroflexales (*SI Appendix*, Fig. S3). This provides additional support for the idea that anoxygenic phototrophy can be relatively easily acquired by aerobic lineages, because of the overall similarity and modular nature of the high-potential electron transport chains involved in both aerobic respiration and phototrophy (3).

The 3HP Bicycle and the Carbon Isotope Record. A late origin of the Chloroflexales clade is also consistent with carbon isotope data from the geological record. If the pathway were a significant source of organic carbon, it should have left a noticeable signal in the carbon isotope record of organic matter in sedimentary rocks. Carbon fixation processes typically fractionate stable carbon isotopes from one another due to kinetic isotope effects associated with certain pathways, producing organic matter depleted in ^{13}C relative to dissolved inorganic carbon (DIC); each carbon fixation pathway tends to have a characteristic isotopic fractionation (13).

The 3HP bicycle produces biomass that is heavy compared with the Calvin–Benson cycle (fractionation between DIC and organic carbon of −13‰ versus ∼25‰) (12). If the 3HP bicycle provided a significant flux of organic carbon to the biosphere during early Earth history, it would be expected that organic matter in Archean-age rocks would display relatively heavy $\delta^{13}C$ values; rather, the opposite is true, with Archean rocks recording anomalously low $\delta^{13}C$ of organic carbon of −35 to −50‰, potentially recording carbon fixation via microorganisms using the reductive acetyl-CoA pathway (31). Formal carbon isotope mass balance analyses demonstrate that never in Earth history are sedimentary carbon isotope ratios consistent with a predominant 3HP source (*SI Appendix*, Fig. S4). This result makes sense, because our divergence time estimates imply that the evolution of the 3HP bicycle occurred after Cyanobacteria and even algae came to dominate primary productivity globally in most ecosystems (32). It additionally suggests that, although the 3HP bicycle is both energetically efficient and insensitive to dioxygen—features that make it unique from all other carbon fixation pathways—one of the reasons it was never used for oxygenic photosynthesis may have been because it wasn't present during the radiation of Cyanobacteria and the emergence of plastids. If correct, efforts to build a synthetic 3HP bicycle into oxygenic photosynthetic organisms might yield valuable solutions to the large carbon losses incurred by photorespiration (33).

Evolution of the 3HP Bicycle Enabled by Horizontal Gene Transfer. The presumed antiquity of all six known carbon fixation pathways has made it challenging to piece together the evolutionary events that enabled the de novo assembly of enzymes that constitute any of these metabolic pathways. Thus, the 3HP bicycle offers an intriguing opportunity to understand the process in which a given carbon fixation pathway may have evolved, as the 3HP bicycle has only evolved once across the Tree of Life within the small monophyletic clade of the Chloroflexales order. In contrast, other carbon fixation pathways can be found in a large diversity of distantly related phyla—possibly through HGT—thus confounding our ability to retrace their evolutionary origins. Moreover, our phylogenetic analyses suggest that the 3HP bicycle may be a relatively recent metabolic innovation, and thus may have a higher chance of reconstruction of its evolutionary

Table 1. Summary of key divergence events from cross-calibrated molecular clock analyses

Divergence event	Divergence date, Ma	Lower bound, Ma	Upper bound, Ma
Chloroflexales (phototrophy and 3HP bicycle)	867	617	1,140
Chloroflexales-Herpetosiphonales divergence	1,098	804	1,410
Chloroflexi phylum	2,139	1,693	2,679

The most recent common ancestor of the Chloroflexales clade—the group with known members possessing phototrophy and the 3HP bicycle—evolved during Late Proterozoic time. The maximum age estimate for the evolution of phototrophy and the 3HP bicycle in the Chloroflexales is 1,410 Ma, based on the upper bound 95% confidence interval of the divergence between Chloroflexales and their closest, nonphototrophic sister group, Herpetosiphonales. Lower and upper bounds are based on 95% confidence intervals. All ages shown are in millions of years before present (Ma).

history, compared with other carbon fixation pathways which may have evolved much earlier in the history of life.

Given our molecular clock results showing that the 3HP bicycle is a relatively recent innovation arising within the Chloroflexales order, we examined the evolutionary assembly of this novel metabolic pathway (Fig. 1). The 3HP bicycle uses many enzymes that are also involved in other pathways of central carbon metabolism, some of which are widely distributed within the Chloroflexi phylum. From the phylogenetic distribution of 3HP bicycle-involved genes, it appears that this metabolism was assembled gradually over the evolution of the Chloroflexi phylum. Basal lineages (i.e., Dehalococcoidetes members) have the smallest sets of genes; moving toward the tips of the species tree, however, lineages derived from the common ancestor with the Dehalococcoidetes begin acquiring key carbon metabolisms in a pattern best interpreted as an incremental gain of metabolic functions until the derived Chloroflexales clade acquired the last genes necessary for a complete 3HP bicycle. This stepwise acquisition of genes first starts with TCA genes (fumarate hydratase and succinate dehydrogenase) and propionate/odd-chain fatty acid metabolism (propinoyl-CoA carboxylase, methylmalonyl-CoA epimerase, and methylmalonyl-CoA mutase) in all non-Dehalococcoidetes. Next, members of the Thermomicrobiales and Herpetosiphonales gained homologs of (*S*)-malyl-CoA/β-methylmalyl-CoA/(*S*)-citramalyl-CoA lyase (MCL) and mesaconyl-CoA C1:C4 CoA transferase, ultimately enabling the last few enzymes necessary to complete the pathway to be acquired by stem group Chloroflexales.

A key question is whether or not this piecemeal evolution of the 3HP bicycle used proteins that originated within the Chloroflexi phylum, or if the last key enzymes were horizontally transferred into the Chloroflexales. If the Chloroflexales were the first to evolve these enzymes for the 3HP bicycle, one would expect most basal lineage in this clade to be of Chloroflexi origin. However, reconstruction of the malyl-CoA lyase and propionyl-CoA synthase phylogenies place Chloroflexi taxa on derived branches, with numerous basal clades, indicating that these enzymes were most likely horizontally transferred into the Chloroflexales (Fig. 2 and *SI Appendix*, Figs. S5 and S6). Thus, HGT appears to have been an important process enabling the final assembly of the 3HP bicycle *ca.* 867 Ma.

The photoheterotrophic lifestyle common to members of the Chloroflexales may have facilitated the evolutionary assembly of the 3HP bicycle. Many Chloroflexales species are found in microbial mat communities, where they consume a number of different organic compounds from the environment while performing phototrophy in a mixotrophic and photoheterotrophic manner (34). Correspondingly, the addition of organic acids has been shown to increase the growth rate of phototrophically growing cells (35). Interestingly, three lineages within the Chloroflexales (*Oscillochloris*, *Chlorothrix*, and *Kouleothrix*) have lost enzymes necessary for the 3HP bicycle, replacing it with the Calvin–Benson cycle via acquisition of phosphoribulokinase (PRK) and RuBisCO (*SI Appendix*, Figs. S7 and S8). By reconstructing phylogenies of these proteins, we discovered a clade of PRK that encompasses all known Chloroflexi as well as numerous cyanobacterial PRKs. The basal placement of Chloroflexi sequences within this PRK clade suggests that some Cyanobacteria may have horizontally acquired these Chloroflexi-type PRK. Surprisingly, a number of species outside of the Chloroflexales order also contain PRK, even though they are missing RuBisCO, and it is not clear how these species use PRK in their pentose phosphate metabolism in the absence of RuBisCO. The binary presence or absence of either of these two carbon fixation pathways may be indicative of the plasticity of central carbon metabolism in the Chloroflexales, where photoheterotrophy is constantly providing reducing equivalents and carbon for cells, and autotrophy can be plugged into cellular metabolism relatively easily, whether it be via the 3HP bicycle or the Calvin–Benson cycle.

Conclusions

Much uncertainty and ambiguity has surrounded our understanding of the origins of anoxygenic photosynthesis. Although numerous studies have suggested an early, Archean-age origin for anoxygenic photosynthesis within the Chloroflexi (4–7), we used a number of approaches to demonstrate that the phototrophic Chloroflexales clade evolved much later with the appearance of both anoxygenic photosynthesis and the 3HP bicycle within the phylum sometime near the end of Proterozoic time. Estimates on the timing of divergences within the Chloroflexi phylum provide a valuable reference point to mark when these metabolisms evolved. Moreover, it provides further insight into when key

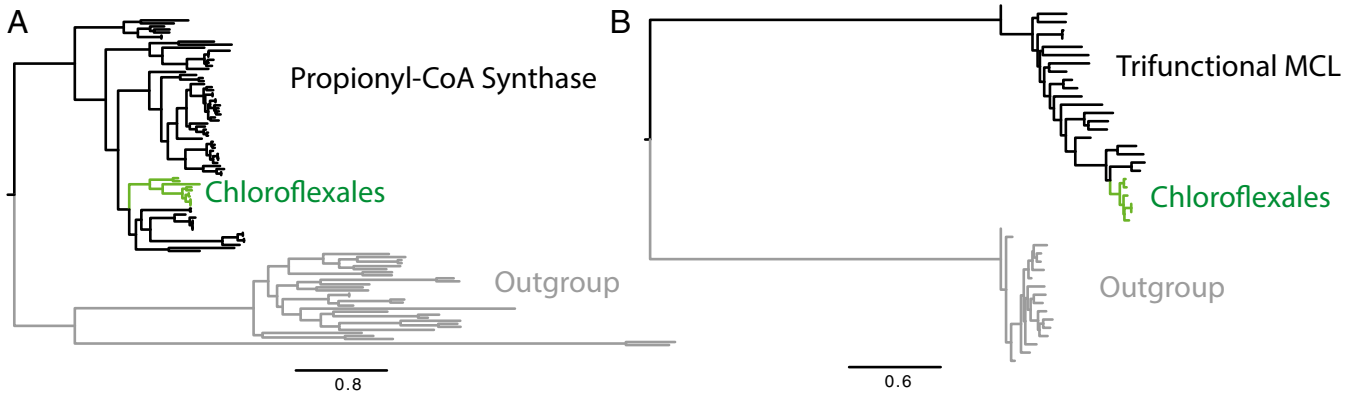

Fig. 2. Evolutionary relationships show that critical enzymes involved in the 3HP bicycle were horizontally transferred from other taxa into the phototrophic Chloroflexales order. (*A*) Phylogeny of the key 3HP enzyme, propionyl-CoA synthase, illustrating the derived placement of the Chloroflexales (green branches). The many basal taxa from non-Chloroflexi species indicate that the Chloroflexales appear to have acquired this gene via HGT, as it is not found in any other species within the Chloroflexi phylum. The acetyl-CoA synthetase family was used as the outgroup. (*B*) Phylogeny of malyl-CoA lyase (MCL) also shows a derived position and points to an HGT origin within the Chloroflexales. The 3HP bicycle requires a distinct trifunctional class of MCL that catalyzes three distinct enzymatic reactions [(*S*)-malyl-CoA/β-methylmalyl-CoA/(*S*)-citramalyl-CoA lyase activity]. This enzyme evolved from the bifunctional MCL family, which was used as the outgroup. Like in *A*, the placement of Chloroflexales taxa (green branches) with many non-Chloroflexi basal lineages suggests that this enzyme did not evolve within the Chloroflexi phylum, but rather was introduced to stem group members of the Chloroflexales via HGT. This supports the inference that evolution of the 3HP bicycle was a relatively recent metabolic innovation, one that occurred late in the radiation of the Chloroflexi.

genes were horizontally transferred into this phylum, enabling the de novo assembly and evolution of the full 3HP bicycle.

Time constraints add color to the frequently black-and-white interpretation of phylogenetic trees by anchoring evolutionary interpretations within a concrete geological context. Our findings demonstrate the utility of molecular estimates on the divergence times of bacterial phyla, because it is then possible—even with uncertainties measured in hundreds of millions of years—to test hypotheses that bear on the age and role of important microbial metabolisms. Specifically, understanding the timing of various phototrophic lineages may provide a key piece to identifying the original inventors of phototrophy. However, based on the Proterozoic ages of crown group lineages, there is a strong possibility that phototrophy may have originally evolved in an undiscovered or extinct stem group lineage. The paucity of constraints available for such ancient events highlights the importance of leveraging varying disciplines spanning phylogenetics, geological studies, genomics, and extant physiology to reconstruct these deep evolutionary histories.

Methods

Molecular Dataset. Sequences from subunits of ATP synthase, the ribosomal large subunit, the ribosomal small subunit, elongation factor Tu, and 16S rDNA were gathered (AtpB, AtpI, EfTu, Rpl2, Rpl16, Rps3, and 16S rDNA). Sequences and markers were chosen to provide a broad sampling of the Chloroflexi phylum, while including Archaeplastida, Cyanobacteria, Metazoan, and Proteobacteria taxa to enable cross-calibrations on molecular clock analyses. Plant mitochondrial genomes were used along with α-Proteobacteria to both serve as an appropriate outgroup and enable cross-calibration between the corresponding mitochondrial and plastid lineages. Alignments for each protein or nucleotide family were performed using the –maxiterate strategy in the alignment program MAFFT (36), and protein sequences were concatenated. The dataset was partitioned into two parts: concatenated protein and 16S nucleotide sequences, respectively.

Age Constraints. To avoid biasing analyses with controversial or equivocal microfossil occurrences and interpretations, we used geochronological constraints based on well-accepted divergence time estimates of plant fossils estimated by Smith et al. (37). Normal distributions at 217 ± 40, 327 ± 30, 432 ± 30, and 477 ± 70 Ma were used as divergence time calibration points for Angiospermae, Gymnospermatophyta, Tracheophyta, and land plants, respectively. The use of land plant divergence events enabled the cross-calibration between divergence events happening simultaneously in lineages that contain both plastids and mitochondria, as described by Shih and Matzke (25). The fossil *Bangiomorpha pubescens* was also used as a prior with a uniform distribution between 1,030 Ma and 1,200 Ma. A uniform prior between 2,400 and 3,800 Ma was used as a calibration for the last common ancestor of all taxa used in this study. The large range was used as an unbiased and largely unrestricted constraint, which formally assumes that oxygenic photosynthesis predated the rise of oxygen *ca*. 2.3 Ga. Moreover, these taxa could not have diverged before the Late Heavy Bombardment and some of the earliest potential evidence for life on Earth *ca*. 3,800 Ma (38).

Cross-Calibrated Molecular Clock Analysis. Molecular clock analyses were performed using the program BEAST (39) using the Cyberinfrastructure for Phylogenetic Research (CIPRES) Science Gateway v. 3.3 server (40). The CpREV model was chosen as the best-fitting amino acid substitution model, based on ProtTest analysis (41). The concatenated amino acid sequences under the CpREV model and the 16S rDNA sequences under the GTR+G model in accordance with the 16S rDNA molecular clock study using BEAST as previously reported (2). For all runs, we ran two Markov chain Monte Carlo chains for 50 million generations, sampling every 10,000th generation, and discarding the first 50% of generations as burn-in. Maximum clade credibility trees were generated using TreeAnnotator v1.7.5. All BEAST runs were inspected for convergence and adequate Estimated Sampling Size values using the program Tracer (42).

Comparative Genomics and Phylogenetic Distribution of Genes. The presence of genes was determined by using BLAST and using an e-value threshold of 1^{-10}. Sequences for individual gene trees were identified using BLAST (43) against GenBank. Sequences were aligned using the –maxiterate strategy in the alignment program MAFFT (36). Individual gene trees were generated in RAxML (44) on the CIPRES server (40).

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