REPORT

PHYLOGENETICS

On the origins of oxygenic photosynthesis and aerobic respiration in Cyanobacteria

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The origin of oxygenic photosynthesis in Cyanobacteria led to the rise of oxygen on Earth ~2.3 billion years ago, profoundly altering the course of evolution by facilitating the development of aerobic respiration and complex multicellular life. Here we report the genomes of 41 uncultured organisms related to the photosynthetic Cyanobacteria (class *Oxyphotobacteria*), including members of the class *Melainabacteria* and a new class of Cyanobacteria (class *Sericytochromatia*) that is basal to the *Melainabacteria* and *Oxyphotobacteria*. All members of the *Melainabacteria* and *Sericytochromatia* lack photosynthetic machinery, indicating that phototrophy was not an ancestral feature of the Cyanobacteria and that *Oxyphotobacteria* acquired the genes for photosynthesis relatively late in cyanobacterial evolution. We show that all three classes independently acquired aerobic respiratory complexes, supporting the hypothesis that aerobic respiration evolved after oxygenic photosynthesis.

The Cyanobacteria are one of the most important microbial groups on Earth; however, much remains to be learned about their diversity and evolution. Environmental 16*S* ribosomal RNA gene surveys suggest that there are at least three extant classes of Cyanobacteria: *Oxyphotobacteria*, *Melainabacteria*, and the basal branching *ML635J-21* clade (*1*, *2*). There are no published genomes available for class *ML635J-21*, and nothing is known about their metabolism. To address this shortcoming, we analyzed publicly available metagenome data sets for the presence of previously uncharacterized members of the *Melainabacteria* and *ML635J-21*. We assembled and binned three draft genomes belonging to class *ML635J-21*, for which we propose the name *Sericytochromatia* {Se.ri.cy.to.chro.ma'tia: Latin adv. *sero,* late or too late; New Latin n. cytochrome [from Greek n. *kutos*, a vessel or container (and in biology a cell); and Greek n. *khroma*, color]; suff. -ia, to denote a class; New Latin neuter pl. n. *Sericytochromatia*, intended to mean cytochromes that were acquired late or later in evolution}. *Sericytochromatia* genomes were recovered from both photic and aphotic environments: a coal bed methane well [CBMW_12 (*3*)], an algae-associated biofilm from a lab-scale bioreactor (LSPB_72; SRA073481), and subsurface groundwater [RAAC_196 (*4*)] (Fig. 1 and table S1). We also assembled and binned 28 *Melainabacteria* genomes from human gut, wastewater treatment, subsurface groundwater, and lake water metagenomes (table S1). These genomes greatly expand the coverage of the *Melainabacteria* (Fig. 1) and include the first genomes for the orders *SHAS531* and *V201-46 (1)*. Additionally, we discovered 10 previously misclassified genomes in public databases (*5*) that belong to the order *Gastranaerophilales* (*1*) in the *Melainabacteria* (table S1 and fig. S1).

These new genomes provide the opportunity to address fundamental issues concerning the evolution of oxygenic photosynthesis and aerobic respiration. None of the *Sericytochromatia* or *Melainabacteria* genomes contain genes for phototrophy or carbon fixation (Fig. 1). This strongly suggests that the last common ancestor of Cyanobacteria was nonphototrophic and that the *Oxyphotobacteria* gained the ability for photosynthesis through lateral gene transfer after their divergence from the *Melainabacteria*. This is consistent with fusion models for the evolution of photosynthesis in Cyanobacteria (*6*, *7*) but not with selective loss (*8*) or cyanobacterial origin (*9*, *10*) models.

The inference of a nonphotosynthetic cyanobacterial ancestor can be further tested by analyzing the evolutionary history of high-potential metabolism (photosynthesis and aerobic respiration). If photosynthesis and/or aerobic respiration were present in the ancestor of Cyanobacteria, it is expected that genes for complex III would be congruent within the Cyanobacteria phylum. However, if photosynthesis is a derived feature of *Oxyphotobacteria*, and aerobic respiration evolved after the rise of oxygen, then the Cyanobacteria classes would be expected to have acquired their high-potential electron transport chains (ETCs) independently. This predicts that members of the different Cyanobacteria classes would have distantly related complex IIIs and complex IVs.

There are two evolutionarily unrelated groups of complex IIIs: the cytochrome bc complexes (including the cytochrome bc_1 complex and cytochrome b_6f complex) and alternative complex III (ACIII) (*11*, *12*). The cytochrome bc complexes are widespread among the Bacteria and Archaea, with lateral gene transfer playing an important role in their distribution (*12*, *13*). The cytochrome b_6f complexes, which are only found in *Oxyphotobacteria*, contain two hemes (c_i and f) along with extra cofactors that are usually associated with photosynthesis (chlorophyll, β-carotene) (*14*). The ACIIIs have only been found in Bacteria, where they commonly occur in an operon with heme-copper oxygen reductases (HCOs). There are also two evolutionarily unrelated groups of complex IVs associated with aerobic respiration: the heme-copper oxygen reductases and the cytochrome bd oxidases. There are at least three major classes of HCOs—the A, B, and C families (*15*, *16*). The A family has a very broad taxonomic distribution and is adapted to high levels of oxygen. The B and C families are less common and have independently evolved to function under low oxygen levels (*17*). The bd oxidases appear to be widely distributed by lateral gene transfer and are also adapted to low oxygen levels (*18*).

Whereas only the *Oxyphotobacteria* can perform photosynthesis, there are members from all three cyanobacterial classes that are capable of aerobic respiration (Fig. 1 and Table 1). All *Oxyphotobacteria* share a common ETC consisting of a cytochrome b_6f complex, photosystem I (PSI), photosystem II (PSII), and an A-family oxygen reductase. In addition, some *Oxyphotobacteria* genomes encode bd oxidases and C-family oxygen reductases (*19*). Phylogenetic analyses of complex III and complex IV proteins show that the cytochrome b_6f complex and A-family oxygen reductase were present in the ancestor of *Oxyphotobacteria*, whereas the bd oxidases and C-family oxygen reductases were likely acquired later (Fig. 1 and Table 1).

The *Melainabacteria* exhibit more diversity in their ETCs. Four orders (*Vampirovibrionales*, *Obscuribacterales*, *SHAS531*, and *V201-46*) contain members capable of aerobic respiration. All aerobic *Melainabacteria* have a unique fused complex III–IV operon consisting of a C-family oxygen reductase and two cytochrome bc–related proteins (Fig. 1 and Tables 1 and 2). This operon appears to have been acquired early in *Melainabacteria* evolution because its phylogeny is congruent with genome trees. Members of the class lacking the operon (orders *Gastranaerophilales* and *Caenarcaniphilales*) likely lost the ability for aerobic respiration as they adapted to anoxic

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environments (Fig. 1). Additional aerobic respiratory components were acquired later within specific *Melainabacteria* groups. A second fused complex III–IV operon consisting of a cytochrome bc complex and a bd-like oxidase with a cytochrome c fused to the periplasmic side is found in the *Obscuribacterales* (*18*). *Vampirovibrio chlorellavorus* (order *Vampirovibrionales*) and SSGW_16 (order *V201-46*) both appear to have independently acquired bd oxidases later in evolution (Fig. 1 and Tables 1 and 2). Some members of the *Obscuribacterales* and *Caenarcaniphilales* also contain a cytochrome bc–related protein in an operon with nitrate reductase (fig. S2). The presence of only C-family oxygen reductases and bd oxidases in the *Melainabacteria* suggests that they are adapted to low-oxygen conditions.

Although currently represented by only three genomes, the *Sericytochromatia* have the greatest diversity of respiratory proteins of the three cyanobacterial classes, including cytochrome bc complexes, ACIII, and A- and C-family oxygen reductases (Fig. 1 and Table 2). CBMW_12 contains three complex IIIs and three complex IVs. It has a complex III–IV operon with a cytochrome bc complex and a highly modified A-family oxygen reductase that is missing its proton channels, suggesting that it is unable to pump protons (fig. S3). Similarly modified A-family oxygen reductases have been found in many other microorganisms (*16*). CBMW_12 also contains a second cytochrome bc complex, ACIII, and A- and C-family oxygen reductases. LSPB_72 has an ACIII as its sole complex III and A- and C-family oxygen reductases (Fig. 1 and Table 2). The third *Sericytochromatia* genome (RAAC_196) did not encode genes for high-potential metabolism. The aerobic members of the *Sericytochromatia* are predicted to respire under both high- and low-oxygen conditions because of the presence of A- and C-family oxygen reductases. The respiratory components of CBMW_12 and LSPB_72 are not closely related to each other, suggesting that extensive lineage-specific recruitment of aerobic respiratory genes may be common in the *Sericytochromatia*.

Comparison of high-potential metabolism within the Cyanobacteria shows that the three classes utilize very different sets of proteins to perform aerobic respiration (Fig. 1 and Table 2). Phylogenetic analysis of these proteins further indicates that homologs of cytochrome bc complexes, A- and C-family oxygen reductases, and bd oxidases are neither closely related between the classes nor phylogenetically congruent with cyanobacterial evolution (figs. S2 and S4 to S7). The most parsimonious inference from these data is that the last common ancestor of the Cyanobacteria did not use oxygen and that the three classes acquired aerobic respiration independently after their divergence. The absence of aerobic respiration in ancestral Cyanobacteria suggests that abiotic oxygen sources on early Earth were insufficient to allow for its evolution until after the appearance of oxygen produced by photosynthesis. If true, we expect that other phyla will exhibit the same

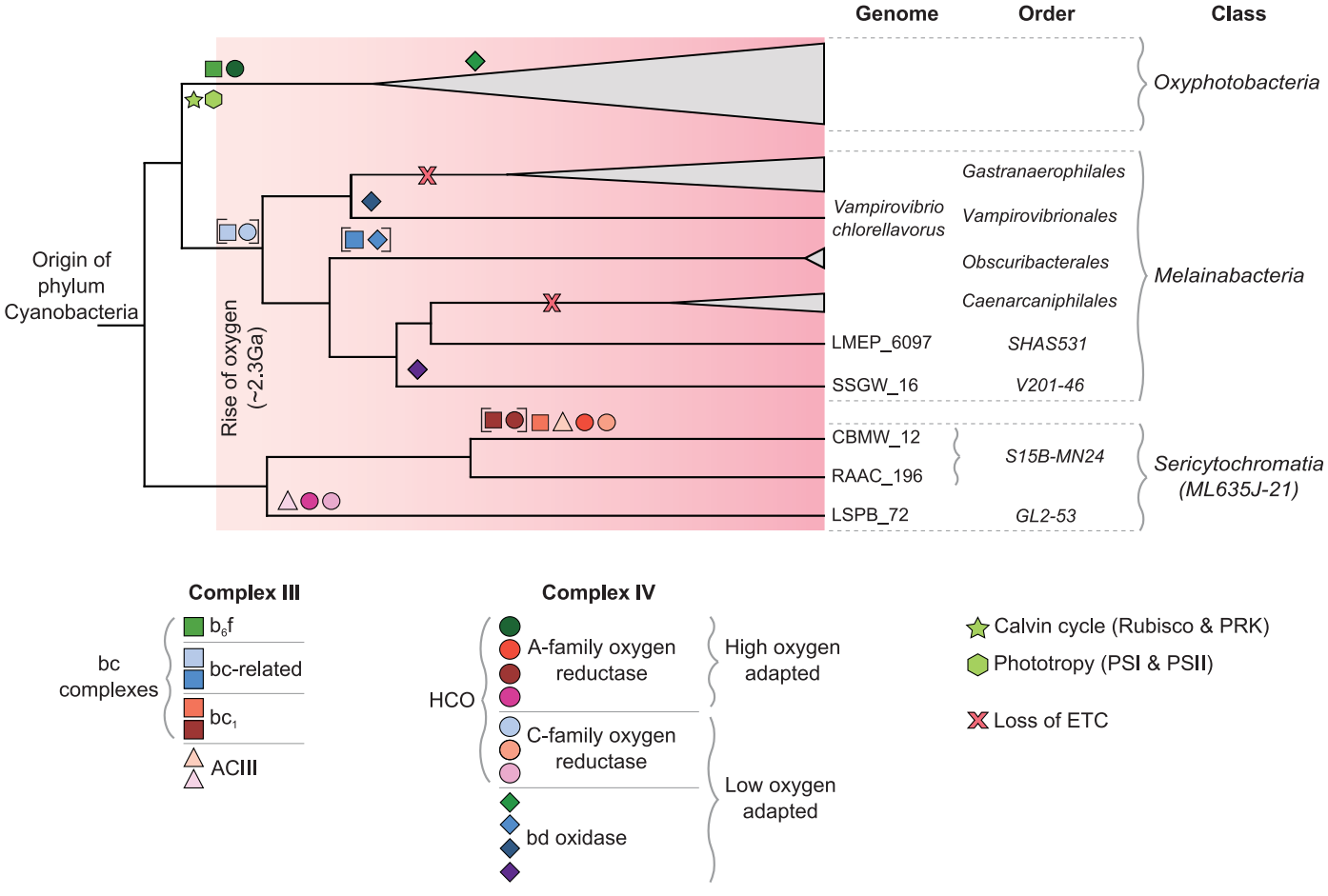
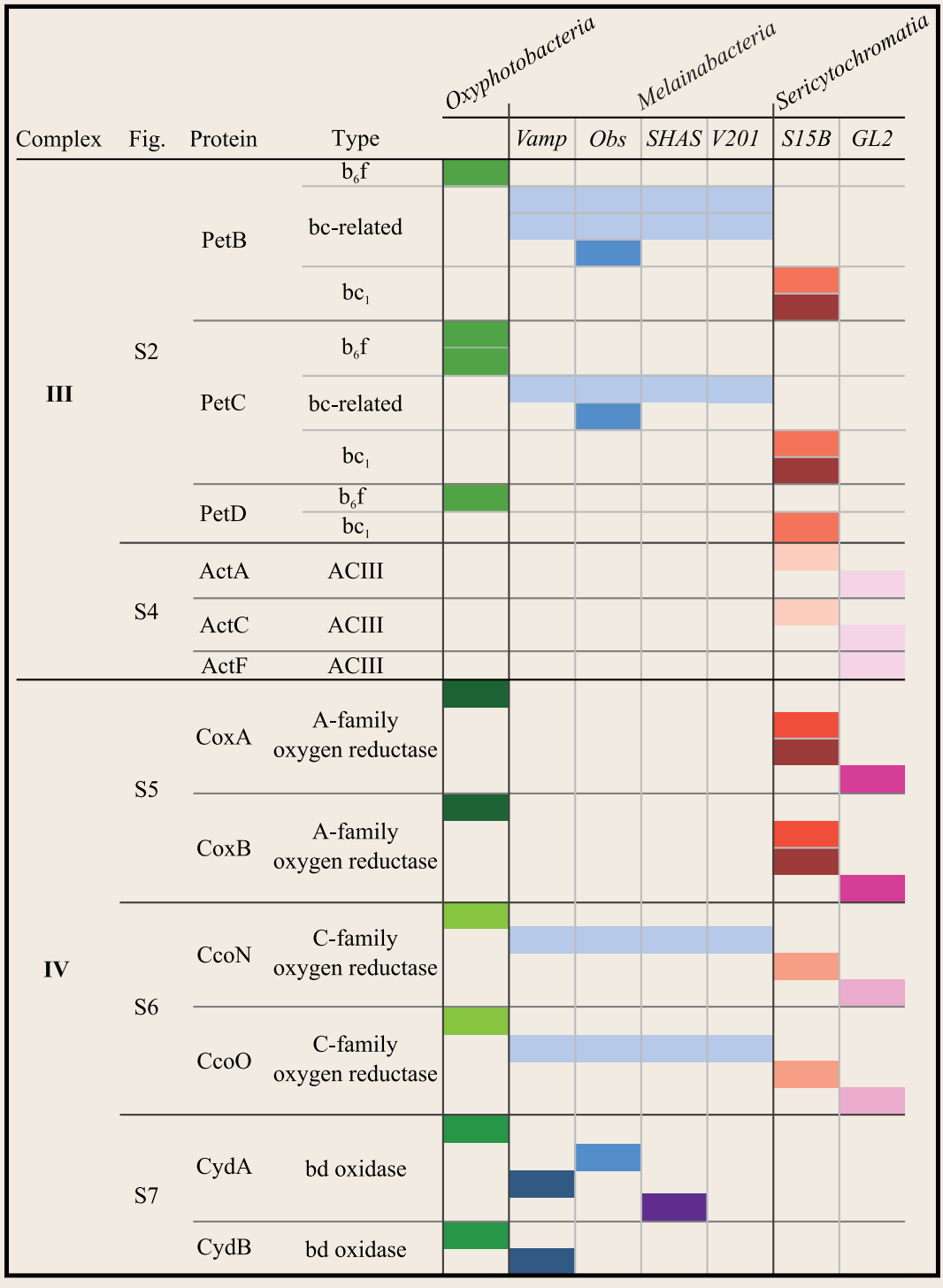


Fig. 1. Evolution of photosynthesis and aerobic respiration in Cyanobacteria. A cladogram based on the branching order of the concatenated gene tree (120 phylogenetically conserved proteins, table S2) shown in fig. S1. The Cyanobacteria are inferred to be ancestrally nonphototrophic and acquired the ability for photosynthesis (PSI and PSII) after the divergence of the *Oxyphotobacteria* from the *Melainabacteria*. The three Cyanobacteria classes likely acquired aerobic respiration independently after the rise of oxygen (atmospheric oxygen is represented by the red shading). Squares and triangles indicate acquisitions of complex III, whereas circles and diamonds indicate acquisitions of complex IV. *Oxyphotobacteria* acquisitions are shown in green (top), *Melainabacteria* acquisitions in blue and purple (middle), and *Sericytochromatia* acquisitions in red and pink (bottom). The different shades indicate phylogenetically distinct versions of a given complex. Square brackets denote operon fusions, and a red "X" indicates putative loss of ETCs. Orders with more than one representative have been collapsed (see table S1 for more detail) with the exception of *S15B-MN24*. Ga, billion years ago; PRK, phosphoribulokinase.

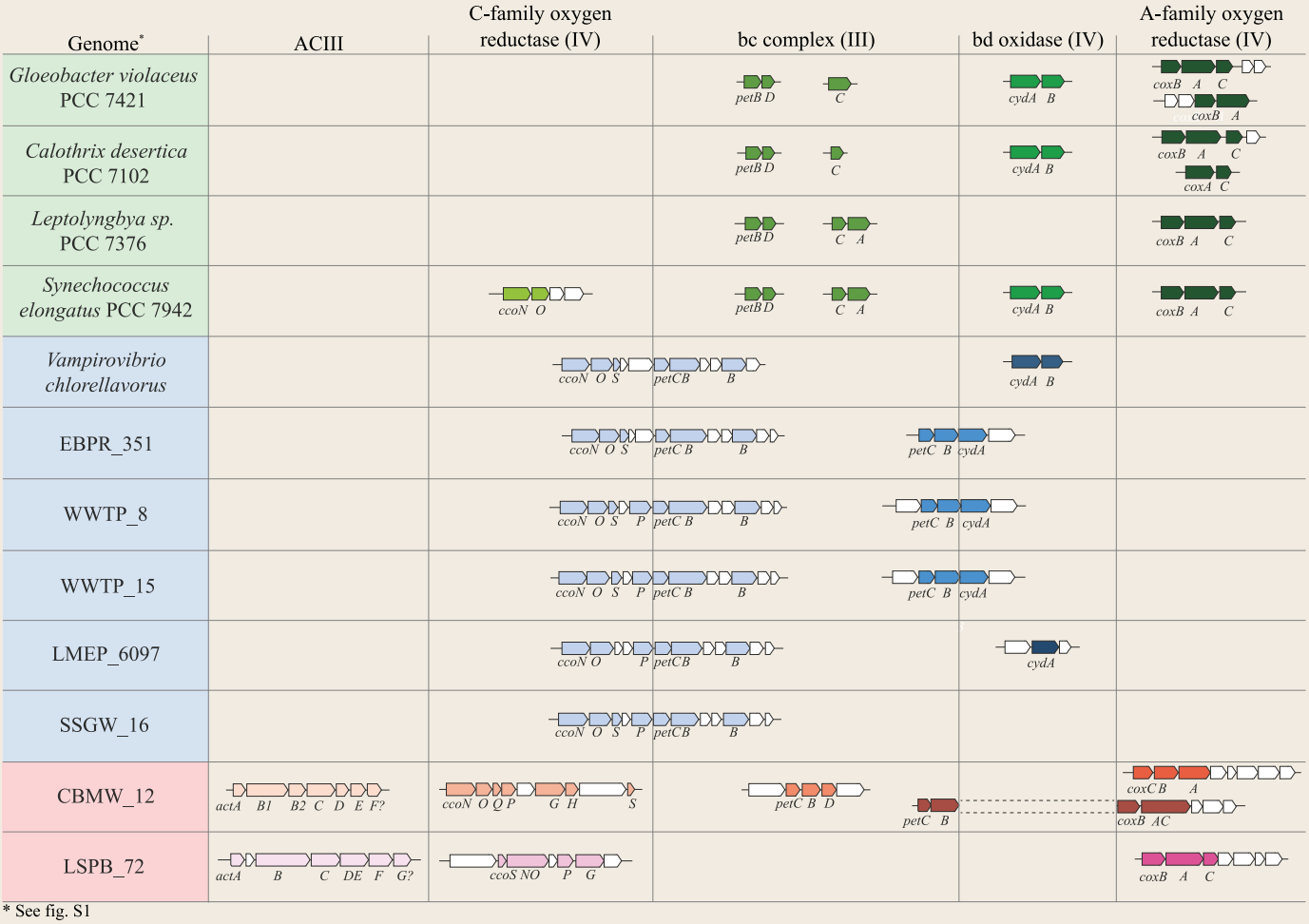
Table 1. Phylogenetic distribution of complex III and IV genes in Cyanobacteria containing aerobic respiratory chains. Each row indicates a phylogenetically distinct version of a given gene. Colored boxes indicate presence of an ortholog in a given lineage, according to the color scheme used in Fig. 1 and Table 2. Abbreviations: *Vamp, Vampirovibrionales; Obs, Obscuribacterales; SHAS, SHAS531; V201, V201-46; S15B, S15B-MN24; GL2, GL2-53.*



pattern for aerobic respiration as the Cyanobacteria—a lack of aerobic respiration in their last common ancestor, with a later acquisition involving lateral gene transfer.

There is a substantial and ongoing debate regarding the timing of both the origin of oxygenic photosynthesis and the appearance of oxygen on Earth, with different geological, geochemical, and paleontological data yielding interpretations that span 1.5 billion years of Earth's history (*6, 20*). The genomic data presented here only support the hypotheses in which oxygenic photosynthesis appears relatively late in bacterial evolution [e.g., (*6, 21*)] and are not consistent with inferences that place *Oxyphotobacteria* among the earliest taxa to appear in Earth's surface environments [e.g., (*22, 23*)]. Because oxygenic photosynthesis is a derived feature of the *Oxyphotobacteria*,

Table 2. Gene neighborhoods of aerobic respiratory complexes III and IV in the Cyanobacteria. Complex III and IV genes were identified in the *Melainabacteria* (*1*) and *Sericyotchromatia* genomes by using the U.S. Department of Energy–Joint Genome Institute (DOE-JGI) Microbial Genome Annotation Pipeline (MGAP v.4) (*25*). Different complex types are shown in columns such that operon fusions can be illustrated across column boundaries. Genes belonging to the classes *Oxyphotobacteria*, *Melainabacteria*, and *Sericytochromatia* are represented by the color scheme described in Fig. 1. White genes represent hypothetical proteins. Complex III orthologs inferred to be involved in anaerobic respiration (nitrate reduction) are not shown (see instead fig. S2).



* See fig. S1

its maximum age would be the divergence of the *Melainabacteria* and *Oxyphotobacteria*, recently estimated to be around 2.5 to 2.6 billion years ago by a cross-calibrated molecular-clock study (*2*). Geochemical evidence before the rise of oxygen for Mn-oxidizing phototrophy, the direct evolutionary precursor to oxygenic photosynthesis, is consistent with these dates (*24*). This suggests an origin of oxygenic photosynthesis close in time to the rise of oxygen and strengthens the possibility that the rise of oxygen ~2.3 billion years ago was directly caused by the evolution of oxygenic photosynthesis.

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SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/355/6332/1436/suppl/DC1
Materials and Methods
Figs. S1 to S7
Tables S1 and S2
References (*26*–*37*)

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Photosynthesis evolution in Cyanobacteria

How and when Cyanobacteria evolved the ability to produce oxygen through photosynthesis is poorly understood. Soo *et al.* examined the genomes of Cyanobacteria and other related bacterial lineages. The phylogenetic relationships of these prokaryotes suggest that the evolution of aerobic respiration likely occurred multiple times. This, along with evidence that the modern photosynthetic system apparently arose through the lateral gene transfer and fusion of two photosynthetic systems, supports a relatively late origin of photosynthesis in evolutionary history.

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