Three classes of oxygen-dependent cyclase involved in chlorophyll and bacteriochlorophyll biosynthesis

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The biosynthesis of (bacterio)chlorophyll pigments is among the most productive biological pathways on Earth. Photosynthesis relies on these modified tetrapyrroles for the capture of solar radiation and its conversion to chemical energy. (Bacterio)chlorophylls have an isocyclic fifth ring, the formation of which has remained enigmatic for more than 60 y. This reaction is catalyzed by two unrelated cyclase enzymes using different chemistries. The majority of anoxygenic phototrophic bacteria use BchE, an O_2-sensitive [4Fe-4S] cluster protein, whereas plants, cyanobacteria, and some phototrophic bacteria possess an O_2-dependent enzyme, the major catalytic component of which is a diiron protein, AcsF. Plant and cyanobacterial mutants in *ycf54* display impaired function of the O_2-dependent enzyme, accumulating the reaction substrate. Swapping cyclases between cyanobacteria and purple phototrophic bacteria reveals three classes of the O_2-dependent enzyme. AcsF from the purple betaproteobacterium *Rubrivivax* (*Rvi.*) *gelatinosus* rescues the loss not only of its cyanobacterial ortholog, *cycI*, in *Synechocystis* sp. PCC 6803, but also of *ycf54*; conversely, coexpression of cyanobacterial *cycI* and *ycf54* is required to complement the loss of *acsF* in *Rvi. gelatinosus*. These results indicate that Ycf54 is a cyclase subunit in oxygenic phototrophs, and that different classes of the enzyme exist based on their requirement for an additional subunit. AcsF is the cyclase in *Rvi. gelatinosus*, whereas alphaproteobacterial cyclases require a newly discovered protein that we term BciE, encoded by a gene conserved in these organisms. These data delineate three classes of O_2-dependent cyclase in chlorophototrophic organisms from higher plants to bacteria, and their evolution is discussed herein.

photosynthesis | chlorophyll | bacteriochlorophyll | cyclase

The (bacterio)chlorophylls, or (B)Chls, are ubiquitous pigments used by chlorophototrophic organisms for light harvesting and photochemistry, so elucidation of their biosynthetic pathways is of great importance. The least well-characterized step in the common pathway for all (B)Chls is formation of the isocyclic E ring, occurring via oxidation and cyclization of the C13 propionate group of magnesium protoporphyrin IX monomethyl ester (MgPME), producing 8-vinyl protochlorophyllide (8V Pchlide) (Fig. 1). The reaction is catalyzed by two distinct enzymes using different chemistries: an O_2-sensitive protein containing [4Fe-4S] and cobalamin prosthetic groups (1) that derives oxygen from water (2), and an oxidative diiron enzyme that requires molecular oxygen (3). The O_2-independent MgPME cyclase [EC:1.21.98.3] is believed to be encoded by a single gene, *bchE* (4), that is essential for BChl biosynthesis in bacterial phototrophs inhabiting anoxic environments. The O_2-dependent MgPME cyclase [EC:1.14.13.81] catalyzes this reaction in plants and cyanobacteria (5, 6), and is believed to be composed of multiple subunits (7).

The first subunit assigned to the O_2-dependent reaction was identified in the purple betaproteobacterium *R b a* (*R .*) *ge a* and was named AcsF (aerobic cyclization system Fe-containing subunit) (8). Subsequently, it was demonstrated that *R . ge a* contains both BchE and AcsF cyclases, conferring the ability to synthesize BChl under varying O_2 regimes (9). Orthologs of *ac F* are widely distributed in phototrophs and have been studied in higher plants (10, 11), algae (12) and cyanobacteria (13), the green nonsulfur bacterium *Ch f e a a ac* (14), and the purple alphaproteobacterium *Rh d bac e* (*Rba*.) *hae de* (15) (Fig. S1).

Two isoforms of AcsF in the unicellular alga *Ch a d a e ha d* , CRD1 and CTH1, catalyze E ring formation under copper-deficient and -replete conditions, respectively (12, 16). These proteins are localized to both the thylakoid membrane and chloroplast envelope (17), a pattern shared with the single AcsF in *A ab d ha a a*, CHL27 (10, 18). The cyanobacterium *S ech c* sp. PCC 6803 (hereinafter *S ech c*) also contains two isoforms of AcsF, designated CycI and CycII (19). Constitutively expressed *c cI* encodes the sole AcsF protein responsible for cyclase activity under oxic conditions, with *c cII* expressed only under microoxic conditions (13); overexpression of

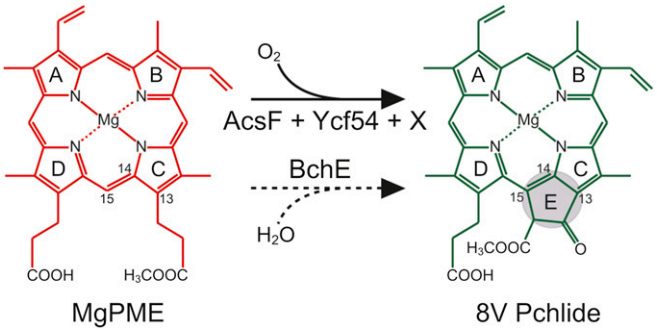

Fig. 1. Cyclization reactions involved in (B)Chl biosynthesis. Shown is isocyclic ring formation via O_2-dependent and -independent routes, catalyzed by AcsF and Ycf54 (solid arrow) and BchE (dashed arrow), respectively. Here x denotes the as-yet unassigned subunit required for the O_2-dependent reaction. International Union of Pure and Applied Chemistry numbering of the relevant macrocycle carbons is indicated, and formation of the ring E is highlighted. The oxygen sources for the O_2-dependent and -independent enzymes are molecular oxygen and water, respectively.

component of the O_2-dependent cyclase. The subunit requirement for this enzyme across phototrophic organisms has not been resolved, however.

In the present study, we demonstrate that *acsF* from *Rhodobacter gelatinosus* corrects the loss of both *cycI* and *ycf54* in *Synechocystis*, suggesting that this AcsF protein does not require a Ycf54 component. Reciprocally, CycI substitutes for AcsF in *Rhodobacter gelatinosus* only in the presence of Ycf54, providing validation of this protein as a subunit of the O_2-dependent cyclase. Furthermore, we identify BciE as a cyclase subunit conserved among AcsF-containing Alphaproteobacteria. This work delineates three distinct classes of O_2-dependent cyclase; phylogenetic analysis identifies defined clades, the evolution of which is discussed.

Results

***Rvi. gelatinosus acsF* Complements the Loss of *cycI* in *Synechocystis*, Regardless of the Presence of *ycf54*.** The apparent absence of *ycf54* orthologs in phototrophic bacteria containing orthologs of *acsF* suggests that the Ycf54 component of the O_2-dependent cyclase either is not required for function of the bacterial enzyme or that an unrelated protein performs the same function in its place. To determine which of these possibilities is the case, we integrated *acsF* from *Rhodobacter gelatinosus* into the genome of the *ycf54*-containing model cyanobacterium *Synechocystis* in place of the nonessential, light-responsive *bAII* as described previously (24) (Fig. 2*A*). Deletion of the native CycI-encoding gene (sll1214) was attempted in this *acsF*$^{Rg+}$ background; a previous attempt to delete *cycI* in the wild type (WT) under oxic conditions proved unsuccessful (19). Full segregation of Δ*cycI* in *acsF*$^{Rg+}$ was achieved (Fig. 2*B*), indicating that *acsF* complements the loss of *cycI* in *Synechocystis*. Subsequently, deletion of *ycf54* (slr1780) in *acsF*$^{Rg+}$ Δ*cycI* was achieved by replacement of the native gene with a zeocin resistance cassette as described previously (22), yielding *acsF*$^{Rg+}$ Δ*cycI*Δ *ycf54* (Fig. 2*C*).

We performed phenotypic analyses of the *acsF*$^{Rg+}$ strains lacking *cycI* and both *cycI* and *ycf54*, along with WT and Δ *ycf54* controls. Liquid cultures were grown photomixotrophically under low light to an OD_{750} of ∼0.4. Absorption spectra of these suspensions indicate that deletion of *ycf54* almost abolishes the assembly of Chl-containing photosystems, as judged by the near absence of a peak at ∼680 nm (Fig. 2*D*). The restoration of a 680-nm absorption band by the introduction of *acsF*^{Rg} into strains lacking *cycI*, irrespective of the presence of *ycf54*, shows that *acsF*^{Rg} is necessary and sufficient for Chl *a* biosynthesis in *Synechocystis*. This conclusion is further reinforced by the Chl content of these strains grown under moderate light, calculated when all apart from Δ *ycf54* were grown without glucose ($mg \cdot L^{-1} \cdot OD_{750}^{-1}$, % relative to WT): WT, 3.22 ± 0.05, 100%; Δ *ycf54*, 0.24 ± 0.01, 7.5%; *acsF*$^{Rg+}$Δ*cycI*, 3.08 ± 0.07, 96%; *acsF*$^{Rg+}$ Δ*cycI*Δ *ycf54*, 3.08 ± 0.01, 96%. These data indicate that the O_2-dependent cyclase of *Rhodobacter gelatinosus* integrates into a

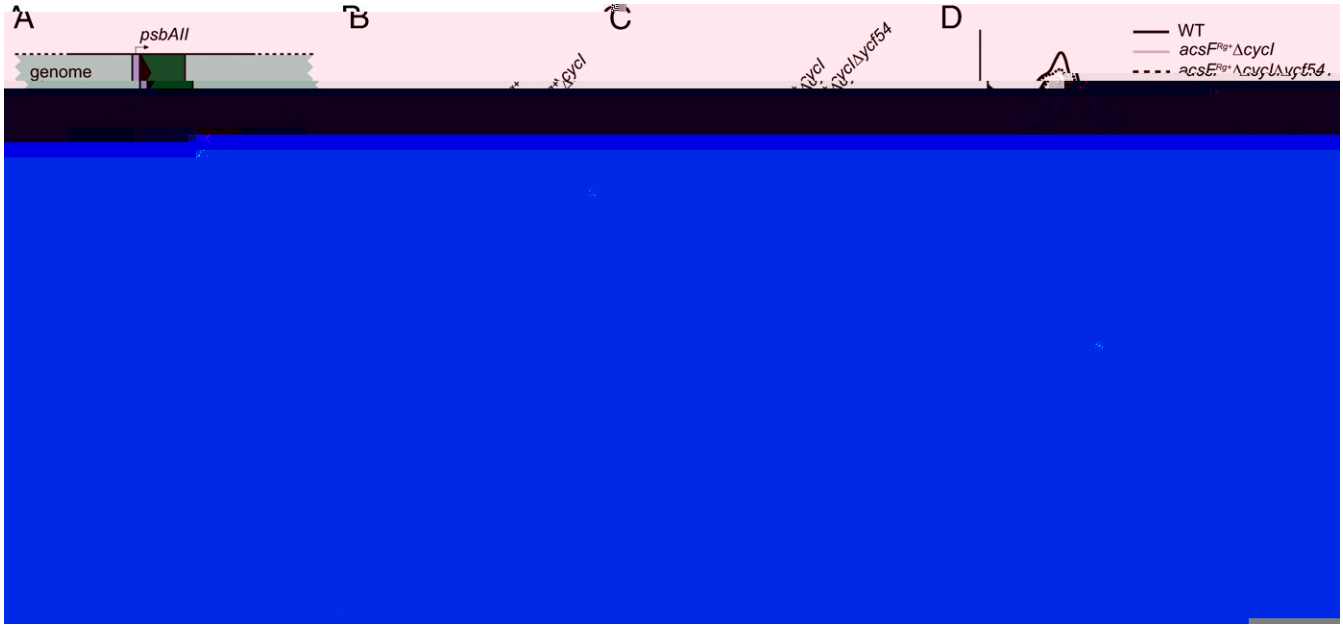

Fig. 2. Construction and phenotypic analyses of *Synechocystis* cyclase mutants. (*A*) Diagram depicting replacement of the *psbAII* gene with *acsF*^{Rg} via pPD[*acsF*^{Rg}] (*Upper*), and construction of the fully segregated strain confirmed by colony PCR (*Lower*). (*B* and *C*) Inactivation of *cycI* (*B*) and *ycf54* (*C*) genes via replacement with chloramphenicol and zeocin resistance cassettes, respectively, confirmed by colony PCR. (*D*) Whole-cell absorption spectra of strains grown mixotrophically under low light conditions. The peaks for Chl-containing complexes are marked with a green shadow. (*E*) Drop growth assays of strains on solid agar, supplemented with or lacking glucose. Photographs were taken after incubation for 12 d.

cyanobacterial Chl pathway and dispenses with the requirement for Ycf54 normally exhibited by its native partner, CycI.

In addition, we performed drop growth assays on solid agar with and without 5 mM glucose (Fig. 2*E*). As expected, supplementation with glucose resulted in improved growth at identical dilutions for each strain. Strains containing *ac F*^{Rg} showed the same pattern as seen in WT and grew under photoautotrophic conditions; they also were able to grow at higher dilutions than the Δ *cf54* mutant under photomixotrophic conditions. These data suggest that AcsF^{Rg} restores Chl biosynthesis and photoautotrophic growth to *S ech c* in the absence of Ycf54.

Ycf54 Is a Catalytic Component of the O_2-Dependent Cyclase Enzyme in Oxygenic Phototrophs. Previous work has shown that *S ech c* Δ *cf54* makes a small amount of Chl, ∼13% of WT levels (21, 22); thus, Ycf54 appears to be important, but not essential, for cyclase activity. To assess the contribution of Ycf54 more precisely, we developed a reciprocal system for the heterologous expression of *S ech c* genes in *R . ge a* , which synthesizes BChl *a* under conditions ranging from oxic to anoxic using O_2-dependent and -independent cyclase enzymes, respectively (8, 9). Genes encoding the known components of these enzymes were removed using an in-frame, markerless deletion method to avoid the polar effects often encountered with resistance cassette-mediated gene disruption. The O_2-independent cyclase was inactivated by deletion of *bchE* (Fig. S2*A*), and BChl biosynthesis was completely inactivated by the subsequent deletion of *ac F* (Fig. S2*B*). This Δ*bchE*Δ*ac F* strain, which accumulates the cyclase substrate MgPME, provides a background for testing components of the O_2-dependent enzyme. The *c cI* gene from *S ech c* was integrated at the original *ac F* locus both alone and in combination with *cf54* from the same organism encoded downstream of *c cI* (Fig. S2*B*). A third complemented strain used *ac F* from *Rba. hae de* , which was recently shown to be essential for O_2-dependent cyclase activity in this model anoxygenic phototroph (15) (Fig. S2*B*).

The three resulting strains, Δ*bchE*Δ*ac F*::*c cI*, Δ*bchE*Δ*ac F*::*c cI- cf54*, and Δ*bchE*Δ*ac F*::*ac F*^{R} , along with positive and negative control strains Δ*bchE* and Δ*bchE*Δ*ac F*, respectively, were cultured under oxic conditions in the dark in liquid medium, standardized by OD_{680}, and pelleted. The pigments accumulated by these strains were extracted, and BChl *a* content was analyzed by HPLC (Fig. 3). As expected, BChl *a* accumulated to a high level in Δ*bchE* (Fig. 3*A*), but Δ*bchE*Δ*ac F* was unable to synthesize BChl (Fig. 3*B*). The presence of *c cI* in this background did not restore BChl biosynthesis (Fig. 3*C*), whereas BChl was detected in the strain complemented with both *c cI* and *cf54* (Fig. 3*D*). These data confirm that Ycf54 is essential for activity of the the O_2-dependent cyclase from oxygenic phototrophs. Surprisingly, although a cyanobacterial cyclase was functional, *ac F* from the more closely related *Rba. hae de* was unable to restore BChl biosynthesis to this strain (Fig. 3*E*).

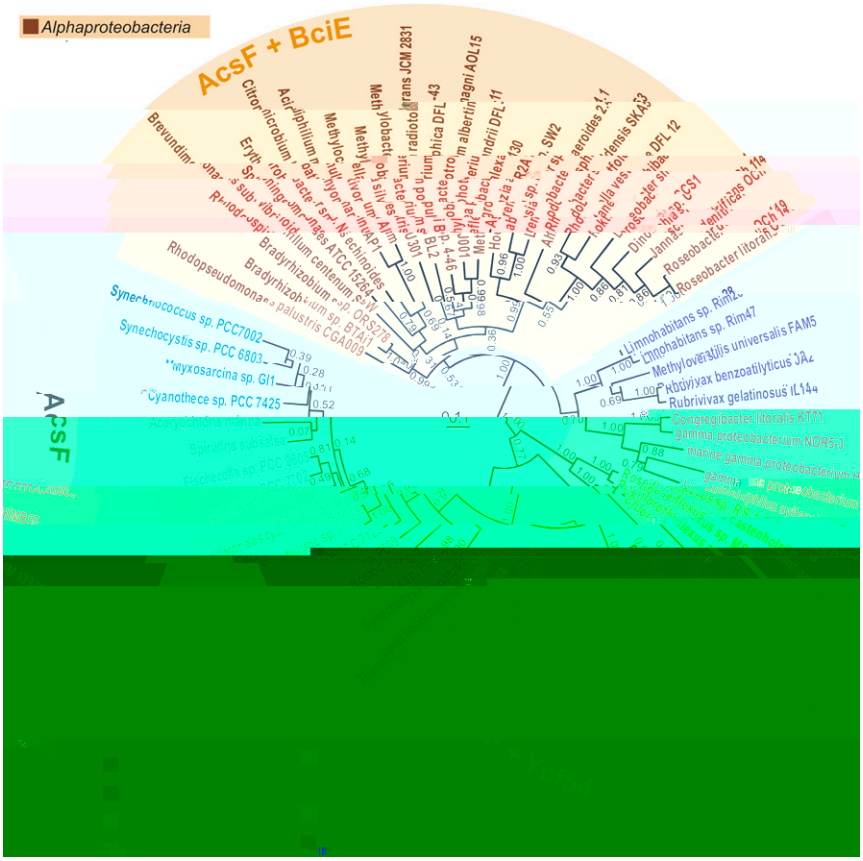

Fig. 5. Phylogenetic analysis of AcsF proteins. Evolutionary analysis via a phylogenetic tree was conducted in MEGA6 using the maximum likelihood method based on the JTT matrix-based model. The analysis involved 69 protein sequences. The tree with the highest log-likelihood (−17,513.1099) is shown. Numbers next to each node indicate bootstrap values (1,000 replicates) as percentages. Phyla are distinguished by color of species name. The length of each branch represents the number of amino acid substitutions per site in proportion to the scale bar at the center of the tree. The presence/absence of BciE/Ycf54 is indicated by shading over the species names: gray, no BciE or Ycf54; orange, BciE present; green, Ycf54 present. Note that orthologs of both *bciE* and *ycf54* are not found together in the genome of any organism sequenced to date.

Rsp_6110 to function. Therefore, we propose, according to the Demerec nomenclature, that rsp_6110 be renamed *bc E*.

To determine whether heterologous expression of *bc E* from *Rba. hae de* , along with *ac F* from the same organism in *R . ge a* Δ*bchE*Δ*ac F*, is able to restore BChl biosynthesis, where *ac F*^{R} alone is not, we amplified the overlapping *bc E* and *ac F* genes directly from the genome of *Rba. hae de* and integrated at the *ac F* locus of *R . ge a* , as described earlier (Fig. S4*A*). This strain, Δ*bchE*Δ*ac F*::*bc E-ac F*^{R}, was grown, and its pigments were extracted and analyzed as described above. Coexpression of *bc E* and *ac F*^{R} resulted in accumulation of BChl (Fig. S4*B*).

Phylogenetic Analysis of AcsF Proteins. To investigate the evolutionary history of AcsF orthologs, we conducted phylogenetic analysis with example protein sequences from plants, algae, and *ac F*-containing phyla of phototrophic (cyano)bacteria, as described in *Ma e a a d Me h d* (Fig. 5). AcsF proteins from species belonging to the same group cluster in the same clade and the topology of the tree correspond closely with the evolutionary relationships among the species being analyzed (28) (Fig. 5). In addition, we checked for the presence or absence of BciE and Ycf54 orthologs in the 69 studied species by performing DELTA-BLAST searches using either *Rba. hae de* BciE (WP_002720458) or *S ech c* Ycf54 (P72777) as a query. Distribution patterns of BciE and Ycf54 orthologs are seemingly related to the phylogeny of AcsF proteins.

Discussion

The O_2-dependent cyclase is the "missing link" in (B)Chl biosynthesis, and it has remained enigmatic for more than 60 y. Before the present study, only AcsF had been identified as a bona fide cyclase subunit (8, 13). Ycf54 and its ortholog LCAA were subsequently discovered to be required for the normal activity of cyanobacterial and plant enzymes, respectively (21, 22), but the absence of genes encoding Ycf54 from *ac F*-containing anoxygenic bacterial phototrophs suggested that it might not be a catalytic subunit. Our demonstration that only the Ycf54-CycI combination replaces the native cyclase function of AcsF in *R . ge a* shows that, irrespective of the other roles of Ycf54 in *S ech c* , it can be considered a subunit of this enzyme. In the case of *S ech c* Δ *cf54*, it may be that the small amount of Chl *a* produced by this strain is due to the presence of three orthologs of *bchE* in the genome of this organism. It once was believed that proteins encoded by these genes did not contribute to Chl biosynthesis (13); however, the recent finding that cyanobacterial *bchE* orthologs from two strains of *C a hece* restore BChl *a* biosynthesis in a *bchE* mutant of *Rba. ca a* demonstrates the activity of O_2-independent BchE orthologs from oxygenic phototrophs (29).

In the other half of the reciprocal experiment, expression of *ac F*^{Rg} in *S ech c* was found to complement the loss of either the native *c cI* or *cf54*. The step occurring after the formation of ring E in (B)Chl biosynthesis, conversion of Pchlide to chlorophyllide, can be catalyzed by two unrelated enzymes, light-activated Pchlide oxidoreductase (POR) and dark-operative POR (DPOR), both of which are present in *S ech c* . The light-activated POR is dominant under the conditions that we used for culturing the complemented strains of *S ech c* (30). This enzyme is absent from all anoxygenic bacterial phototrophs, so the recombinant bacterial AcsF is able to functionally integrate into the Chl pathway in an oxygenic phototroph; this tallies with observations that other recombinant (B)Chl enzymes expressed in cyanobacterial or purple phototrophic hosts can function in non-native pathways (31–33). These results suggest that there is much promise for combining pigment biosynthetic pathways with the aim of producing novel (B)Chls with unique spectral properties.

The existence of cyclase enzymes that require an extra subunit in plants and cyanobacteria is reminiscent of the role played by Gun4 in the activity of magnesium chelatase (MgCH), the

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enzyme catalyzing the first committed step in (B)Chl biosynthesis. Plant and cyanobacterial mutants in *g 4* display reduced Chl content and impaired growth, and they accumulate the substrate for MgCH, whereas phototrophic bacteria lack orthologs of *g 4* (34). Gun4 was found to stimulate cyanobacterial MgCH activity in vitro (35) and to be involved in increasing flux into the Chl biosynthesis pathway in vivo (36). Similarly, it is conceivable that Ycf54 may play a role in substrate channeling; pull-down experiments identified protein–protein interactions between Ycf54 and the cyanobacterial AcsF, CycI (21), and CycI with other Chl biosynthesis enzymes (22). These interactions are abrogated in the absence of *cf54*, and the level of CycI is reduced, suggesting that Ycf54 may stabilize the CycI protein (22).

We have identified and validated a subunit of the O_2-dependent cyclase in Alphaproteobacteria, which we have named BciE. The ORF encoding this protein is found directly upstream of *ac F* in this bacterial class. Deletion in *Rba. hae de* led to the abolition of O_2-dependent cyclase activity, reinstatement of *bc E a* restored activity, and $AcsF^R$ was found to require BciE to function in the heterologous *R . ge a* system. No conserved domain can be identified in BciE based on the National Center for Biotechnology Information's Conserved Domain Database (37). Thus, BciE may represent a novel protein family; its precise role in the alphaproteobacterial enzyme is unclear, but it may play a role similar to that of Ycf54 or Gun4 in stabilization of the major subunit and/or stimulation of the forward reaction.

A cyanobacterial progenitor of algal and plant chloroplasts (38) is also the likely origin of the *ac F* and *cf54* genes common to all oxygenic phototrophs. Our phylogenetic analysis is consistent with this theory, with Ycf54-requiring AcsF sequences clustering in a well-defined clade. In addition, it is believed that *ac F* was horizontally transferred from cyanobacteria to Proteobacteria before the divergence of Alphaproteobacterial, Betaproteobacterial, and Gammaproteobacterial lineages, because cyanobacteria were initially oxygenating the atmosphere, conferring an advantage to anoxygenic phototrophs previously reliant solely on O_2-sensitive BchE (26). A newly discovered bacterial phototroph belonging to the phylum Gemmatimonadetes is believed to have acquired an *ac F*-containing purple bacterial PGC via horizontal gene transfer (39), and thus is more recent than the acquisition of *ac F* by the Proteobacteria. The *ac F*

trees for the heuristic search were obtained by applying the neighbor-joining method to a matrix of pairwise distances estimated using the Jones–Taylor–Thornton (JTT) amino acid substitution model (46). Evolutionary history was inferred by using the maximum likelihood method with the JTT model and was tested by the bootstrap method with 1,000 replicates. The tree with the highest log-likelihood (−17,513.1099) was adopted and visualized using Interactive Tree of Life v2 (47).

ACKNOWLEDGMENTS. We thank Dr. Sakiko Nagashima (Kanagawa University) for supplying the *Rvi. gelatinosus* IL144. G.E.C. was supported by a University of Sheffield doctoral studentship. D.P.C. and C.N.H. were supported by a grant from the Biotechnology and Biological Sciences Research Council (BB/M000265/1). C.N.H. is supported by the European Research Council (Advanced Award 338895). D.P.C. is supported by the European Commission (Marie Skłodowska-Curie Global Fellowship 660652).

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Supporting Information

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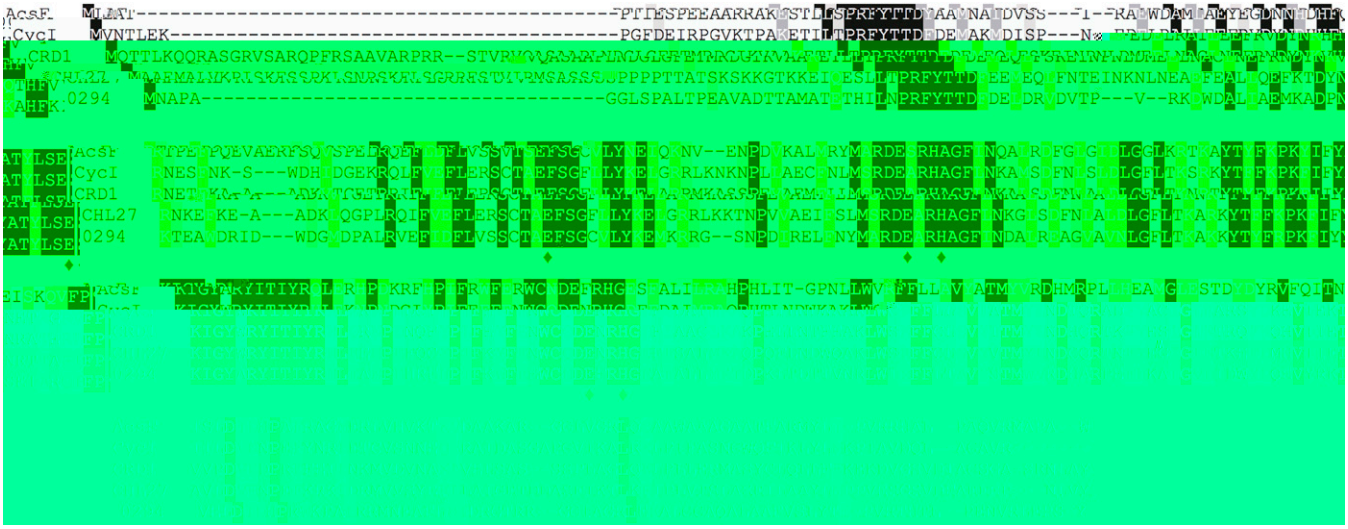

Fig. S1. Amino acid sequence alignments of known AcsF proteins. Sequences are those from *Rvi. gelatinosus* (AcsF), *Synechocystis* (CycI), *C. reinhardtii* (CRD1), *A. thaliana* (CHL27), and *Rba. sphaeroides* (Rsp_0294; abbreviated as 0294). Conserved, highly similar, and similar residues are highlighted in black, dark gray, and light gray, respectively. The putative diiron center ligands are marked by red diamonds.

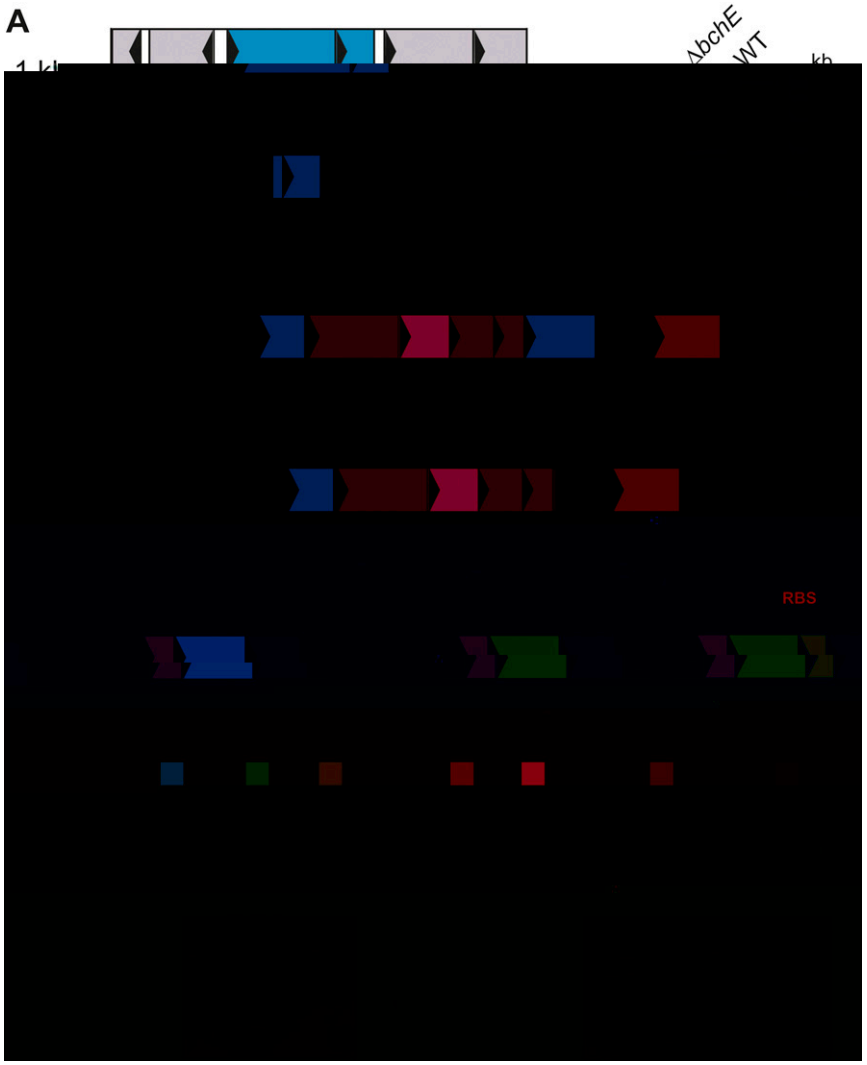

Fig. S2. Genetic knockouts and replacements in *Rvi. gelatinosus.* (*A*) Depiction of the deletion of *bchE* (*Left*), confirmed by colony PCR (*Right*). (*B*) Depiction of deletion of *acsF*, and subsequent integration of foreign genes at the *acsF* locus, under control of the native promoter (*Upper*), confirmed by colony PCR (*Lower*). The regions subjected to genetic manipulation are depicted in proportion to the scale bar. ORFs are represented as colored filled rectangles, within which the arrow indicates the direction of transcription. Crt, carotenoid biosynthesis; RC&LHC, reaction center and light-harvesting complexes.

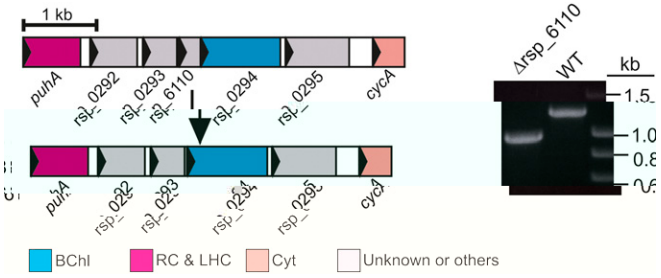

Fig. S3. Deletion of rsp_6110 in *Rba. sphaeroides.* Diagram depicting deletion of rsp_6110 (*Left*), and confirmation by colony PCR (*Right*).

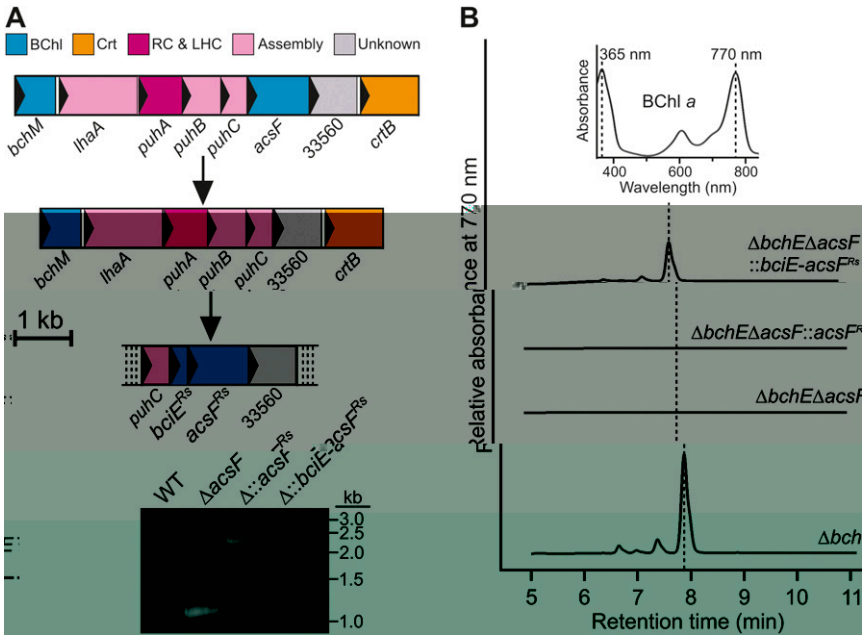

Fig. S4. Construction and phenotypic analysis of *Rvi. gelatinosus* mutant expressing *bciE* and *acsF* from *Rba. sphaeroides*. (*A*) Diagram depicting integration of *bciE* and *acsF* from *Rba. sphaeroides* in place of the native *acsF* in *Rvi. gelatinosus* (*Upper*), and confirmation by colony PCR (*Lower*). (*B*) HPLC analysis of pigments extracted from *Rvi. gelatinosus* strains, extracted from the same number of cells of each strain except for the Δ*bchE* strain, which had a much greater BChl *a* content compared with the other strains. (*Inset*) Retention times and Soret/Q_y maxima of peaks were used to identify BChl *a*.klj.

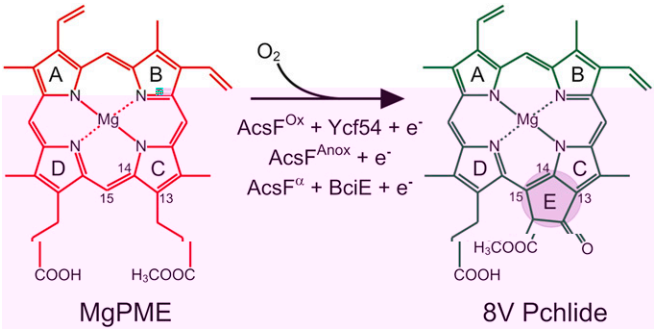

Fig. S5. Current status of known components of the oxygen-dependent cyclase. $AcsF^{\alpha}$, $AcsF^{Anox}$, and $AcsF^{Ox}$ represent AcsF proteins from Alphaproteobacteria, anoxygenic phototrophs other than the Alphaproteobacteria, and oxygenic phototrophs, respectively. e^- denotes the electron donor to the diiron center of AcsF.

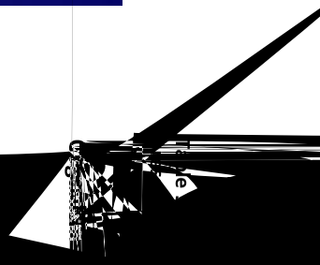

Table S2. Strains and plasmids described in this study

Strain/plasmid	Genotype/characteristics	Source
E. coli		
JM109	Cloning strain for plasmid constructs	Promega
S17-1	Conjugation strain for pK18*mobsacB* constructs	(48)
Rvi. gelatinosus		
WT	IL144	S. Nagashima*
Δ*bchE*	Unmarked deletion mutant of *bchE* in WT	This study
Δ*bchE*Δ*acsF*	Unmarked deletion mutant of *acsF* in Δ*bchE*	This study
Δ*bchE*Δ*acsF*::$acsF^{Rs}$	$acsF^{Rs}$ replacement of *acsF* in Δ*bchE*	This study
Δ*bchE*Δ*acsF*::*bciE-*$acsF^{Rs}$	*acsF* replaced with rsp_6110-$acsF^{Rs}$ in Δ*bchE*	This study
Δ*bchE*Δ*acsF*::*cycI*	*cycI* replacement of *acsF* in Δ*bchE*	This study
Δ*bchE*Δ*acsF*::*cycI-ycf54*	*cycI-ycf54* replacement of *acsF* in Δ*bchE*	This study
Synechocystis		
WT	sp. PCC6803	R. Sobotka†
$acsF^{Rg+}$	$acsF^{Rg}$ and Km^R replacement of *psbAII* in WT	This study
$acsF^{Rg+}$ Δ*cycI*	Cm^R replacement of *cycI* in $acsF^{Rg+}$	This study
$acsF^{Rg+}$ Δ*cycI*Δ*ycf54*	Zeo^R replacement of central portion of *ycf54* in $acsF^{Rg+}$Δ*cycI*	This study
Δ*ycf54*	Zeo^R replacement of central portion of *ycf54* in WT	(22)
Rba. sphaeroides		
WT	2.4.1	S. Kaplan‡
Δ*bchE*Δ*ccoP*	Unmarked deletion mutant of *bchE* and *ccoP* in WT	(15)
Δ*bchE*Δ*ccoP*Δ*acsF*	Unmarked deletion mutant of *acsF* in Δ*bchE*Δ*ccoP*	(15)
Δ*bchE*Δ*ccoP*Δ6110	Unmarked deletion mutant of rsp_6110 in Δ*bchE*Δ*ccoP*	This study
Plasmids		
pK18*mobsacB*	Allelic exchange vector, Km^R	J. Armitage§
pK18Δ$bchE^{Rg}$	Upstream-*NdeI*-downstream of $bchE^{Rg}$ cloned into *Bam*HI/*Hin*dIII sites of pK18*mobsacB*	This study
pK18Δ$acsF^{Rg}$	Upstream-*NdeI*-downstream of $acsF^{Rg}$ cloned into *Bam*HI/*Hin*dIII sites of pK18*mobsacB*	This study
pK18Δ6110	Upstream-downstream of rsp_6110 cloned into *XbaI*/*Hin*dIII sites of pK18*mobsacB*	This study
pK18[$acsF^{Rs}$]	$acsF^{Rs}$ cloned into the *NdeI* site of pK18Δ$acsF^{Rg}$	This study
pK18[6110-$acsF^{Rs}$]	rsp_6110-$acsF^{Rs}$ cloned into the *NdeI* site of pK18Δ$acsF^{Rg}$	This study
pK18[*cycI*]	*cycI* cloned into the *NdeI* site of pK18Δ$acsF^{Rg}$	This study
pK18[*cycI-ycf54*]	*cycI-ycf54* cloned into the *NdeI* site of pK18Δ$acsF^{Rg}$	This study
pPD-FLAG	Cloning site, Km^R, flanked by *psbAII* upstream and downstream regions, Amp^R	(21)
pPD[$acsF^{Rg}$]	$acsF^{Rg}$ cloned into *NdeI*/*BglII* sites of pPD-FLAG	This study
pBBRBB-$Ppuf_{843–1200}$	Expression vector carrying the 843–1,200 region of *puf* promoter of *Rba. sphaeroides*, Km^R	(27)
pBB[6110]	rsp_6110 cloned into the *BglII*/*NotI* sites of pBBRBB-$Ppuf_{843–1200}$	This study

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Table S3. Primers used in this study

Primer	Sequence (5′-3′)
6110UpF	GCTCTAGAGGAGCTGATCCCGCCCTTCC
6110UpR	GGAGAGCCCTCCGGCCGGCGCGTTCATGGGGGTTCCCTTCTCTTGG
6110DownF	CCAAGAGAAGGGAACCCCCATGAACGCGCCGGCCGGAGGGCTCTCC
6110DownR	GCAAGCTTCCCAGGTTCACCGCCACGCC
6110CheckF	GCCCCGGAGCGACAAGGAC
6110CheckR	GTATTTCTTGGCCTTGGTCAGG
6110F_NdeI	GAGTCTCATATGGGTCTGTTCACGAAACAAGCG
6110F_BglII	GGCAGATCTATGGGTCTGTTCACGAAACAAGCGGAA
6110R_NotI	TCTGCGGCCGCTCACAGCGTCACCTGCTCGGAGAA
0294F_NdeI	CCAGTACATATGTGAACGCGCCGGCCGGAGG
0294R_NdeI	CCAGTACATATGTCAATAGCTCGGCTCCAGTCGG
45840UpF	CTAGGTCAAGTAGGATCCTCATGCCGGCGGCGATCATG
45840UpR	CTAGGTCAAGTACATATGGGAAACGGCTCCTCGCGATTC
45840DownF	CTAGGTCAAGTACATATGCGACGGCTGGGTCACGATGC
45840DownR	CTAGGTCAAGTAAAGCTTTGCCGGTGTAGAAGTCGCACGC
45840CheckF	TAGCCGCCGACCATGCCGA
45840CheckR	GCGGTGCACCAGCACCGTGA
33550UpF	GAGTCTGGATCCCTGCATGAGCGACAACGCGTC
33550UpR	GAGTCTCATATGGAGGGTCTCCGTGGTGTGTCA
33550DownF	GAGTCTCATATGAAGCGAGGACAGGATGCTGAGC
33550DownR	GAGTCTAAGCTTGGAACTCCTCGCTCAGGTTGCG
33550CheckF	GAACGTTTGCCGGACACGGT
33550CheckR	ACGAGGTACTTCAGGTGCTCC
33550F_NdeI	GAGTCTCATATGCTCGCGACCCCGACGATCG
33550R_BamHI	GAGTCTGGATCCTCACCATGCCGGGGCCATG
1214UpF	GCCGATCCGGTTAACCTAGGCA
1214UpR	ATATCCAGTGATTTTTTTCTCCATAGAGTTGTTTAAAATAGTTTCC
1214UpCmF	GGAAACTATTTTAAACAACTCTATGGAGAAAAAAATCACTGGATAT
1214DownCmR	GGTGATCCAGCGGAAGACAACCTTACGCCCCGCCCTGC
1214DownF	GCAGGGCGGGGCGTAAGGTTGTCTTCCGCTGGATCACC
1214DownR	TGGAGTTGTTGGGAGAGTTCGGTC
1214F_NdeI	GGAATTCCATATGGTTAATACCCTCGAAAAGCCCG
1214R_NdeI	GGAATTCCATATGTTAGCGCACAGCTCCAGCCA
1214RBS1780F	GTTGGCTGGAGCTGTGCGCTAATATAGGAGCTTGGATTGTGGAAAGTTGGGCATTGACGA
1214RBS1780R	TCGTCAATGCCCAACTTTCCACAATCCAAGCTCCTATATTAGCGCACAGCTCCAGCCAAC
1780F	GTGGAAAGTTGGGCATTGACG
1780R	CTAATCCAGGGATGCAAGGGG
1780R_NdeI	GAGTCTCATATGCTAATCCAGGGATGCAAGGGG