Supporting Information

Chen et al. 10.1073/pnas.1701687114

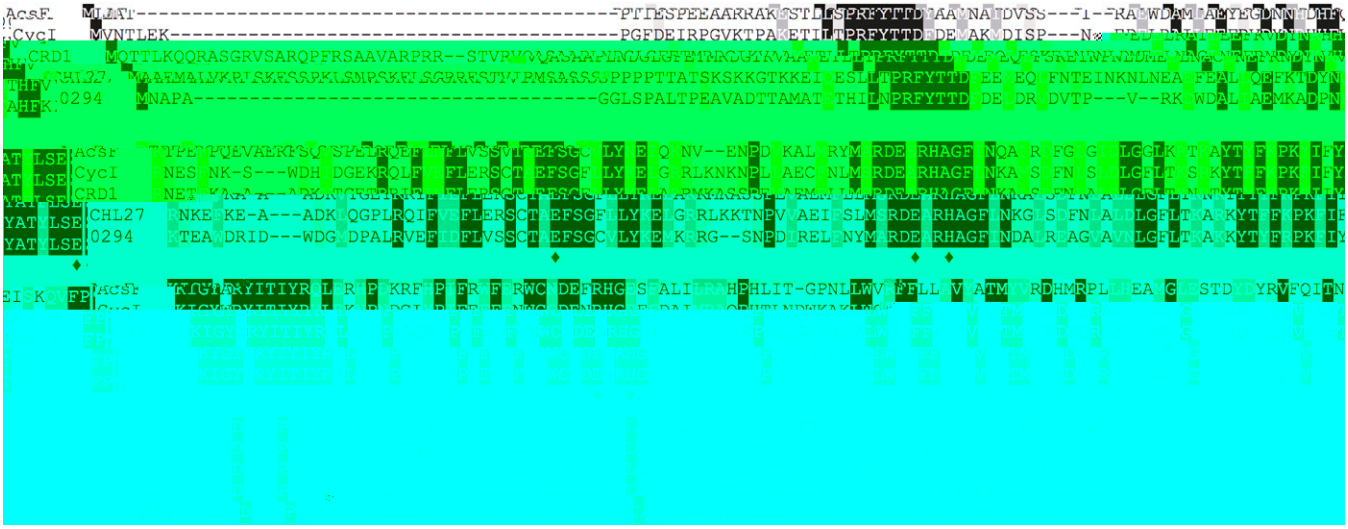

Fig. S1. Amino acid sequence alignments of known AcsF proteins. Sequences are those from *Rvi. gelatinosus* (AcsF), *Synechocystis* (CycI), *C. reinhardtii* (CRD1), *A. thaliana* (CHL27), and *Rba. sphaeroides* (Rsp_0294; abbreviated as 0294). Conserved, highly similar, and similar residues are highlighted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

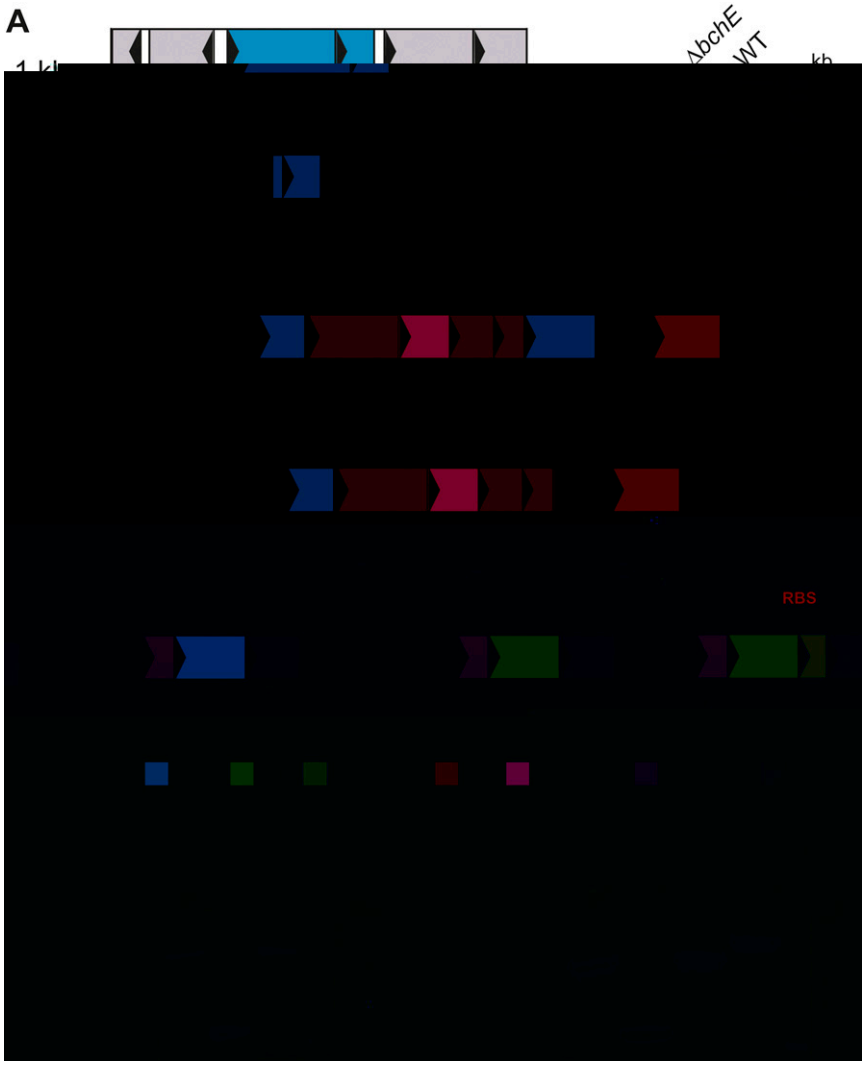

Fig. S2. Genetic knockouts and replacements in *Rvi. gelatinosus.* (*A*) Depiction of the deletion of *bchE* (*Left*), confirmed by colony PCR (*Right*). (*B*) Depiction of deletion of *acsF*, and subsequent integration of foreign genes at the *acsF* locus, under control of the native promoter (*Upper*), 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.

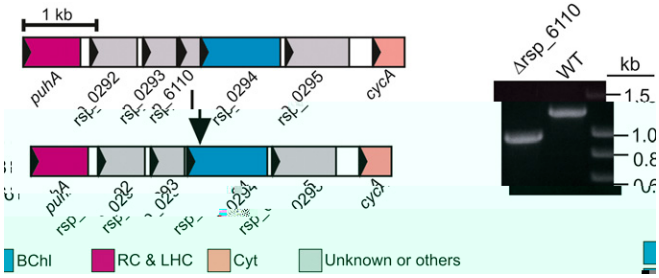

Fig. S3. Deletion of rsp_6110 in *Rba. sphaeroides.* Diagram depicting deletion of rsp_6110 (*Left*), and confirmation by colony PCR (*Right*).

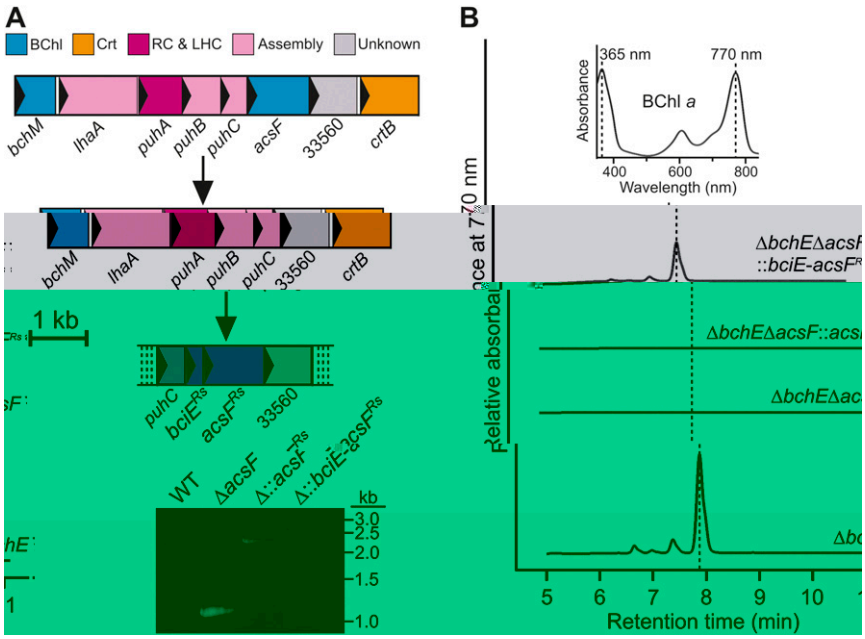

Fig. S4. Construction and phenotypic analysis of *Rvi. gelatinosus* mutant expressing *bciE* and *acsF* from *Rba. sphaeroides*. (*A*) Diagram depicting integration of *bciE* and *acsF* from *Rba. sphaeroides* in place of the native *acsF* in *Rvi. gelatinosus* (*Upper*), and confirmation by colony PCR (*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.

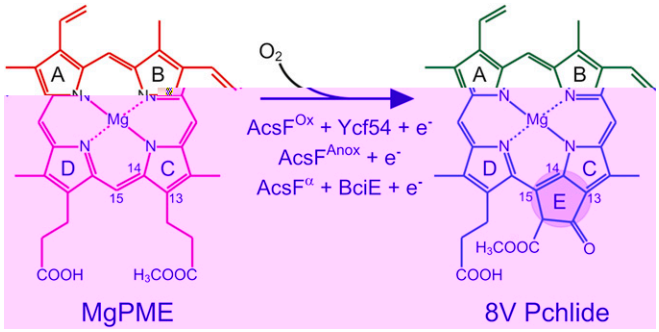

Fig. S5. Current status of known components of the oxygen-dependent cyclase. $AcsF^{\alpha}$, $AcsF^{Anox}$, and $AcsF^{Ox}$ represent AcsF proteins from Alphaproteobacteria, anoxygenic phototrophs other than the Alphaproteobacteria, and oxygenic phototrophs, respectively. e^- denotes the electron donor 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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§Department of Biochemistry, University of Oxford, Oxford, United Kingdom.

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