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This is a postprint of an article that originally appeared in Science on September 12, 2014. http://www.sciencemag.org/


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Extensive remodeling of a cyanobacterial photosynthetic apparatus in far-red light

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Abstract
Cyanobacteria are unique among bacteria in performing oxygenic photosynthesis, often together with nitrogen fixation and, thus, are major primary producers in many ecosystems. The cyanobacterium, Leptolyngbya sp. strain JSC-1, exhibits an extensive photoacclimative response to growth in far-red light that includes the synthesis of chlorophyll d and f. During far-red acclimation, transcript levels increase ≥2-fold for ~900 genes and decrease ≥2-fold for ~2000 genes. Core subunits of photosystem I, photosystem II, and phycobilisomes are replaced by proteins encoded in a 21-gene cluster that includes a knotless red/far-red phytochrome and two response regulators. This acclimative response enhances light harvesting for wavelengths complementary to the growth light (λ = 700 to 750 nm) and enhances oxygen evolution in far-red light.

Cyanobacteria are unique among bacteria in performing oxygenic photosynthesis and are ecologically important primary producers (1). Marine cyanobacteria, mostly Prochlorococcus, Synechococcus, and Trichodesmium species, account for >25% of the net primary productivity in oceans, and terrestrial cyanobacteria also contribute significantly to global photosynthesis (2, 3). The estimated cyanobacterial biomass in terrestrial, endolithic, and freshwater lake, ponds, and 1100 nm), the capacity to use far-red light to perform oxygenic photosynthesis in terrestrial niches could thus have important consequences in natural and engineered systems.

Cyanobacteria mainly use three large, multisubunit complexes to harvest and convert light into stable, energy-rich compounds: Photosystem I (PS) I, Photosystem II (PS) II, and phycobilisomes (PBS) (1, 4–7). Cyanobacteria have evolved many mechanisms to maximize their photosynthetic efficiency in response to the incident irradiation. Examples include adjusting the total cellular chlorophyll (Chl) content and the ratio of PS II to PS I (“intensity adaptation”) (8), state transitions that redistribute light energy transfer from PBS to PS I and PS II (9, 10), and non-photochemical quenching by orange carotenoid-binding protein (11). Although cyanobacteria were once thought to have simple pigmentation (Chl a, b-carotene, and phycobiliproteins), it is now recognized that some cyanobacteria synthesize Chls b, d or f (12), many functionally distinct carotenoids (13), and a spectroscopically diverse phycobiliproteins (7). Limitation for iron and other nutrients causes changes in light-harvesting proteins (14–16).

One of the best-characterized acclimative responses in cyanobacteria, Complementary Chromatic Acclimation (CCA; formerly “Adaptation”), was discovered more than 100 years ago. Gaidukov (17, 18) observed that Oscillatoria sancta is reddish brown when grown in green light but blue-green when grown in red light, and he correctly surmised that the color differences were due to altered pigment synthesis (fig. S1). CCA results from compositional remodeling of the peripheral rods of PBS (19, 20) and occurs through transcriptional and post-transcriptional regulation of specific phycobiliprotein genes. Genes encoding phycoerythrin and its associated bilin reductases, bilin lyases, and linker proteins are expressed in cells grown in green light, while those for “inducible” phycocyanin and associated proteins are expressed in cells grown in red light (19–22). Central regulatory elements controlling CCA include two response regulators (RcaF and RcaC) and a phytochrome-related sensor histidine kinase, RcaE (19, 20, 23). Because PBS are primarily but not exclusively asso-ciated with energy transfer to PS II (9, 10), CCA enhances the overall rate of photosynthesis when the incident irradiation is complementary to cell coloration.

Using a systems biology approach, including genome sequencing, comparative genomics, transcription profiling (RNA-Seq), biochemical and spectroscopic analyses, and proteomics, we show that Leptolyngbya sp. strain JSC-1 (hereafter JSC-1) (24) remodels its photosynthetic apparatus in response to far-red illumination. These changes are probably controlled by a red/far-red responsive phytochrome, JSC-1 specifically synthesizes both Chl d and Chl f, in addition to Chl a, when cells are grown with light wavelengths ≥700 nm. Halomicronema hongdechloris, a cyanobacterium isolated from a stromatolite from Shark Bay, Austral-ia, also synthesizes Chl f when cells are grown in far-red light (25, 26). However, JSC-1 additionally undergoes an extensive acclimative re-sponse, in which the expression of ≥40% of the genome changes more than two-fold after a shift from white light to far-red light. The resulting changes in gene expression lead to the replacement of most of the core proteins of PS I and PS II and to structural remodeling of PBS core sub-structures. We named this global acclimation response “Far-Red Light Photo-acclimation” (FaRLIP), and we show here that FaRLIP significantly improves photosynthetic performance in far-red light.

The JSC-1 genome has a 21-gene cluster that encodes paralogs of most of the core subunits of PS I, PS II, and PBS (fig. S2). Similar clusters occur in 12 other cyanobacteria, including members of all five sections of the taxon Cyanobacteria (27) (fig. S3). All but one of these clusters contain six genes encoding subunits of PS I: psaA2, psaB2, psaL2, psaF2, and psaJ2 (in the remaining strain, psaF2-psaJ2 are adjacent and probably cotranscribed with psaB3). The psaA2 and psaB2 genes are significantly diverged from the psaA1 (~77% identity, 85% similarity), psaB1 (85% identity, 92% similarity) and psaB3 (82% identity, 90% similarity) genes, respectively. The other psa genes in this cluster (psaL2, psaL2, psaF2, and psaJ2) are similarly distantly related to paralogous psa genes (psaL1, psaL3, psaL1, psaF1, psaJ1) encoded elsewhere in the genome (see table S1 for a list of genes for subunits of PS I, PS II, and PBS and related proteins). Transcriptional analyses (ta-bles S1 and S2) showed that the psaA2B2L2L2F2J2 genes are not expressed in cells grown under standard growth conditions for many
cyanobacteria (28). Interestingly, this locus also includes genes for a
knotless phytochrome (rfpA), a DNA-binding response regulator (rfpB)
due to two CheY receiver domains and a winged-helix DNA binding
domain, and a cheY-like gene (rfpC), which form an apparent operon up-
stream from psbA4 (fig. S2; rfp = Regulator of Far-red Photoacclimation).
Phytochromes are widespread red/far-red photore-
ceptors (29), but orthologs of rfpA only occur in 12 other cyanobacteria,
within photosynthetic gene clusters similar to that in JSC-1 (fig. S3).
Based upon phylogenetic analyses and conserved domain architecture,
these photoreceptors form a distinctive subfamily among knotless phyto-
chromes (Fig. 1). On the basis of these observations and biochemical
studies described below, we hypothesize that the RfpA photoreceptor
controls gene expression from this cluster.

Expression of rfpA or its GAF domain in an engineered, phycoec-
yanobilin-producing strain of *Escherichia coli* (28, 30) yielded red/far-red
reversible proteins with nearly identical photochemical properties (figs.
S5 and S6A). Like the knotless phytochrome NpR4776 from *Nostoc punctiforme*,
recombinant RfpA-GAF holoprotein photoconverts be-
tween red-absorbing (P r) and far-red-absorbing (P fr) species under re-
spective far-red or red illumination (fig. S6A). The P r form of
cyanobacterial phytochromes is initially synthesized in vivo (31, 32).
RfpA is converted to the P fr form under a broad range of light wave-
lengths, but only far-red light (λ ≥ 700 nm) specifically regenerates the
P r form (fig. S6B). Because genetic tools are not available to produce an
*rfpA* mutant in *JSC-1*, the postulated role of RfpA in sensing far-red light
and controlling expression of the 21-gene photosynthesis gene cluster
cannot be verified by reverse genetics. However, RfpA specifically sens-
es far-red light, exhibits higher transcript abundance in cells grown in
far-red light, and is uniquely co-localized with genes that are only ex-
pressed in far-red light (figs. S2, S3, S5, and S6 and table S1). The JSC-
1 genome contains other phytochromes and related photosensors, but
transcript levels only increase in far-red light for the knotted phyto-
chrome CYJSC1_DRAFT_40400 (Fig. 1 and table S2). *Calothrix* sp.
PCC 7507 and *Synechococcus* sp. PCC 7335 have RfpA orthologs (fig.
S3) but lack Cph1 orthologs. Thus, the distribution and photochemical
properties of RfpA strongly support our proposal that RfpA controls the
expression of the 21-gene cluster.

to examine the photobiology more closely, JSC-1 cells were grown
under six light conditions (28): white fluorescent light (WL), green-
filtered fluorescent light (GL), red-filtered fluorescent light (RL), 645-
nm or 710-nm light provided by LEDs, and far-red light (FR) produced
after filtered tungsten light (λ ≈ 700 nm) (see fig. S7). The absorption
spectra of cells grown in 710-nm light and FR (Fig. 2) showed that they
had gained absorption at ~700–750 nm (λmax = 706 nm) that was not
present in cells grown in WL, GL, or 645-nm light. Low-temperature
fluorescence emission spectra at 77 K (fig. S8) of cells grown under the
latter three conditions had emission maxima at 683 nm and 695 nm for
PS II and a strong emission maximum at 725 nm from PS I. These spectra
are typical of those for cells synthesizing Chl a and having a relatively
high PS LPS II ratio (24); JSC-1 cells grown in 710-nm light or FR have
weak fluorescence emission at 683, 695 and 717 nm and strong emission
at 745 nm, resembling cells synthesizing Chl f (25, 26).

Pigments extracted from these cells were subjected to reversed-phase
HPLC chromatography. Only Chl a (figs. S9 and S10, peak 3) and carot-
enoids were observed in pigment extracts from cells grown in WL or
645-nm light. Cells grown in FR or 710-nm light still produced Chl a as
the major Chl, but they also synthesized a pigment with an absorption
spectrum identical to that of Chl f (figs. S9 and S10, peak 2) (33, 34).
This pigment was confirmed to be Chl f by converting it to the corre-
sponding phophytin by removing the Mg2+ ion (figs. S11 and S12) and
subsequent mass spectrometry (figs. S13 and S14). Another Chl, which
was slightly more hydrophilic than Chl f (figs. S9 and S10, peak 1), was
also detected. The absorption properties (figs. S10 and S12) of this Chl,
and MS and MS-MS analyses (figs. S15 and S16) of the corresponding
phophytin, showed that it was Chl d (33, 34). Therefore, JSC-1 synthe-
izes three Chls: Chl a, Chl d, and Chl f.

Preliminary RT-PCR experiments confirmed that genes of the *psa2*
operon (fig. S2) are transcribed in cells grown in FR (table S2). Cells
were therefore grown in WL, transferred to FR for 24 hours, and tran-
scription profiling (RNA-Seq) was performed (28). Transcript levels for
the photosynthesis-related genes in the 21-gene cluster (fig. S2) in-
creased from 3–fold to 278–fold in cells grown in FR (Fig. 3 and table
S1), and transcript levels increased ≥2-fold for ~900 genes (table S2).
Transcript levels decreased ≥2-fold for ~2000 genes in FR (table S2),
including most of the paralogous genes encoding core subunits of pho-
tosynthetic complexes (Fig. 3 and table S1). The transcriptional changes
during acclimation to FR are surprisingly extensive and exceed those
for heterocyst (1036 genes) or hormogonia (1762 genes) differentiation in
*Nostoc punctiforme* (35).

The transcription profiling data indicated that most core polypeptides
of PS I, PS II, and PBS should be replaced by products of the 21-gene
cluster when cells are grown in FR. To verify that this was the case, we
analyzed the proteins of photosynthetic complexes isolated from JSC-1
cells grown in WL, FR, 645-nm light, and 710-nm light. Fractions en-
riched in PS I and/or PS II were isolated on sucrose gradients after solu-
bilization of thylakoid membranes with n-dodecyl-β-D-maltoside (Fig.
4A). For JSC-1 cells grown in WL and 645-nm light, two green fractions
were observed: an upper green fraction containing PS I monomers and
PS II dimers, and a lower green fraction containing PS I trimers (Fig.
4A). Gradients prepared with solubilized membranes from JSC-1 cells
grown in FR and 710-nm light differed dramatically. No PS I trimers
were observed, and only a single fraction containing PS I monomers and
PS II dimers was observed (Fig. 4A). The complexes derived from cells
grown in WL or 645-nm light had absorption (Fig. 4B) and 77K fluores-
cence emission maxima (Fig. 4C) expected for PS I (725 nm) and PS II
(685 and 695 nm) complexes containing Chl a. However, complexes
isolated from cells grown in FR and 710-nm light had additional absorp-
tion features at ~720 nm (Fig. 4B) and had fluorescence emission maxi-
ma at 745 nm (Fig. 4C). These spectra demonstrate that both PS I and PS
II complexes of cells grown in FR and 710-nm light contain Chl f.

Proteins associated with isolated photosynthetic complexes were an-
alyzed by trypsin digestion and mass spectrometry of the resulting pep-
tides (table S1). This analysis demonstrated that the core polypeptides
comprising complexes from cells grown in FR and 710-nm light were
the products of those genes whose transcript levels strongly increased in
cells grown under FR (e.g., *psaA2*, *psaB2*, *psaL2*, *psaF2*, *psbB2*, *psbC3*,
*psbD3*, *psaA3*, *psbA4*, *psbH2*) (table S1). In contrast, the core proteins
in complexes from cells grown in WL or 645-nm light instead contained
products of paralogous genes with lowered transcript levels under FR
(e.g., *psaA1*, *psaB1*, *psbB1*, *apcE1*) (Fig. 3, table S1). Thus, the proteins
comprising PS I and PS II differed in response to the growth light condi-
tions.

PBS were isolated from cells grown in 645-nm and 710-nm light to
assess whether absorption differences accompanied polypeptide re-
placement in FR (Fig. 5A). PBS isolated from cells grown in 710-nm
light have an absorbance feature at 708 nm that is correlated with a low-
temperature fluorescence emission peak at 723 nm, but PBS isolated
from cells grown in 645-nm light lack these features (Fig. 5B). The 708-
mm absorption band is red-shifted nearly 40 nm compared to allophy-
cocyanin-β (λmax = 670 nm), previously the most red-shifted absorption
for characterized native phycobiliproteins (36). After denaturation of
PBS proteins in 8.0 M urea at pH 3 (fig. S17), only phycocyanobilin
and phycocerythrin were detected (37), suggesting that the long-
wavelength absorption arises from phycocyanobilin and not from a new
bilin chromophore. Strikingly, even PBS ultrastructure changes in cells
grown in FR. The *ApcE1* core linker phycobiliprotein expressed in WL


and 645-nm light has four linker-repeat (REP) domains and thus is predicted to assemble PBS with “pentacylindrical” core substructures (7, 38) (Fig. 6A, top). ApcE1 has a highly conserved cysteine residue at position 197 that ligates a phycocyanobilin chromophore via a thioether linkage (7). ApcE2, the core linker phycobiliprotein synthesized in FR, has only two REP domains and thus is predicted to produce PBS with only two core cylinders (7, 38) (Fig. 6A, bottom). ApcE2 has no cysteine residues in its N-terminal phycobiliprotein domain; thus, ApcE2 should bind phycocyanobilin noncovalently. Noncovalently bound phycocyanobilin would have one additional conjugated double bond, which should shift the absorption and fluorescence emission of ApcE2 to longer wavelength, as shown by site-directed mutagenesis of apecE in Synchococcus sp. PCC 7002 (39). Such changes could significantly modify the energy transfer pathway(s) and efficiency in the cores of PBS in cells grown in FR. These observations raise the possibility that other core PBS subunits (ApcA2, ApcB2, ApcD3, ApcD4) could also bind phycocyanobilin noncovalently to increase absorption of FR light (λ > 700 nm).

JSC-1 thus remodels its photosynthetic apparatus during FaRLiP by synthesizing Chl d and f, replacing the core subunits of PS I and PS II, and modifying the proteins of the PBS core as summarized in Fig. 6. Analogously to CCA, these changes produce photosynthetic complexes with absorption that is complementary to the incident irradiation between 700 and 750 nm. Additionally, JSC-1 alters relative transcript levels for >40% of the JSC-1 genome, leading to extensive modification of cellular metabolism (table S2). The transcription changes and global replacement of core components of PS I, PS II, and PBS during FaRLiP are distinct from the incorporation of PsbA variants in PS II complexes of Synechocystis sp. PC 6803 and other cyanobacteria in response to high light intensity or anoxic conditions (40) and from reported transcription changes for psbD3 and psbE2 of Acaryochloris marina cells grown in FR or WL at very low irradiance (41).

There is no overlap between the structural remodeling of photosynthetic complexes that occurs during CCA and FaRLiP, and interestingly, JSC-1 performs both acclimation responses (see fig. S1) (24). We assume that PS I, PS II, and PBS subunits produced during FaRLiP have adapted through evolution to perform photosynthesis more efficiently when some Chl a molecules are replaced by Chl f (and Chl d) in cells growing in FR. Indeed, JSC-1 cells that have acclimated to 710-nm light have 40% greater oxygen evolution with far-red actinic light than cells acclimated to 645-nm light, although the two types of cells have identical light saturation behavior when the actinic light is WL (fig. S18). This enhanced photosynthetic performance in FR would be ecologically significant whenGumber cells grow in light that is strongly filtered by Chl a absorbance—for example, in mats, stromatolites, cyanobacterial blooms, or in the shade of plants. FaRLiP should also benefit organisms living in sandy soils, because far-red light penetrates deeper than visible wave-lengths (42). The 730 nm: 650 nm ratio shifts from ~0.84 at the soil surface to 2.8 at a depth of 6 mm (43), and this could be further enhanced by Chl a absorption filtering. Thus, FaRLiP could have a significant impact on cyanobacterial photosynthesis in soil crusts (two organisms in fig. S3 are soil isolates).

Our results show that it is possible for cyanobacteria to retain paralogous copies of genes for functionally specialized photochemical reaction centers. This observation has important implications for the evolution of type-1 and type-2 reaction centers during the evolution of photosynthesis as well as for the extension of Chl biosynthetic pathways (44). If type-1 reaction centers evolved first, a likely mechanism for the origin of type-2 reaction centers was gene duplication and functional divergence (44–46). As shown in this study, functionally distinctive and divergent PS I and PS II reaction centers are formed in strain JSC-1 during growth in FR. Finally, our findings could have important implications for introducing the capacity to utilize FR into plants.

References and Notes
22. D. A. Bryant, The photoregulated expression of multiple phycocyanin species:


28. Materials and methods are available as supplementary materials on Science Online.


**ACKNOWLEDGMENTS**

This study was funded by grant MCB-1021725 from the National Science Foundation to D.A.B. The genome sequence of *Leptolyngbya* sp. strain JSC-1 was determined under the auspices of the U.S. Department of Energy's Office of Science, Biological and Environmental Research Program, and by the University of California, Lawrence Berkeley National Laboratory under contract no. DE-AC02-05CH11231, Lawrence Livermore National Laboratory under contract no. DE-AC52-07NA27344, and Los Alamos National Laboratory under contract no. DE-AC02-06NA25396. Spectroscopic characterization of RfpA and NpR4776-PCM was funded by a grant from the Chemical Sciences, Geosciences, and Biosciences Division, Office of Basic Energy Sciences, Office of Science, U.S. Department of Energy (DOE DE-FG02-09ER16117 to J.C.L.) The authors thank James R. Miller for assistance in performing mass analyses on Chls *d* and *f*; Bruce Stanley and Anne Stanley for advice and technical assistance in performing the tryptic peptide mass fingerprinting; and Craig Praul and Candace Price for assistance in performing RNA-Seq profiling. The authors thank Juliette Lecomte, Wendy Schluchter, John Golbeck, and Alexander Glazer for reading the manuscript and helpful suggestions. This Whole-Genome Shotgun project for *Leptolyngbya* sp. strain JSC-1 (alternative name, *Marsacia ferruginosa*; IMG taxon ID 202287900; GOLD ID = Gi02032) has been deposited at DDBJ/EMBL/GenBank under the accession JMKF0000000; the version described in this paper is version JMKF01000000. RNA-Seq data were deposited in the NCBI Sequence Read Archive (SRA) under accession number SRP041154.

**SUPPLEMENTARY MATERIALS**

[www.sciencemag.org/cgi/content/full/science.1256963/DC1](http://www.sciencemag.org/cgi/content/full/science.1256963/DC1)

Materials and Methods
- Figs. S1 to S18
- Tables S1 and S2
- References (49–60)
Fig. 1. RfpA forms a distinctive lineage of knotless phytochromes. The GAF-PHY domains of selected knotless phytochromes, including the products of ten rfpA genes (fig. S3), were aligned with *Synechocystis* sp. PCC 6803 Cph1 and two closely related, knotted phytochromes from *Leptolyngbya* sp. JSC-1 as an outgroup (fig. S4). The resulting alignment was used to produce a maximum likelihood phylogenetic tree; open and closed circles at nodes show the approximate statistical support for the branching pattern as indicated. The colors of the sequence names reflect seven domain architectures for knotless phytochromes (lower left), with phytochrome and related photosensory GAF domains highlighted in red and blue, respectively. RfpA orthologs have a conserved domain architecture (type IV) and form a distinct subfamily of knotless phytochromes, providing two independent lines of evidence that RfpA orthologs form a distinct lineage associated with FarLiP gene clusters (fig. S3).
Fig. 2. JSC-1 cells have enhanced absorption at 700 to 750 nm when grown in far-red light. (A) Appearance of cells grown in WL, GL, 645-nm light and 710-nm light. (B) Absorption spectra of strain JSC-1 cells grown in WL (black line), GL (green line), 645-nm light (red line), FR (solid blue line) and 710-nm light (dotted blue line).
Fig. 3. Transcription of the 21-gene cluster in JSC-1 in far-red light is part of an extensive acclimative response. Scatter plot showing relative transcript levels for JSC-1 cells 24 hours after a shift from WL to FR. The circled stars indicate the relative transcript levels for the genes in the 21-gene cluster shown in fig. S2. The magenta, pale green and aqua triangles indicate relative transcript levels for paralogous genes encoding subunits of PS I, PS II, and PBS, respectively, that are preferentially expressed in WL. The parallel lines indicate a 2-fold increase or decrease in transcript abundance.
Fig. 4. PS I and PS II complexes are remodeled under far-red light. (A) Sucrose density gradient centrifugation for isolation of Chl-containing complexes from thylakoid membranes of JSC-1 cells grown in WL, 645-nm light, 710-nm light, and FR after solubilization with β-D-dodecylmaltoside. (B) Absorption spectra of gradient fractions containing PS I trimers (WL, solid black line; 645-nm light, solid red line) and a mixture of PS I monomers and PS II from WL (dashed black line); 645-nm light (dashed red line); FR (solid blue line); and 710-nm light (dashed blue line). (C). Low-temperature fluorescence emission spectra for the same fractions as in (B).
Fig. 5. Phycobilisomes isolated from cells grown in far-red light have enhanced absorption from 700 to 750 nm. (A) Sucrose gradients showing phycobilisomes isolated from cells grown in 645-nm light and 710-nm light. (B) Absorption spectra (solid lines) and fluorescence emission spectra (dashed lines) for PBS fractions from cells grown in 645-nm light (red lines) and 710-nm light (blue lines).
Fig. 6. Diagrams summarizing the remodeling of components of PBS, PS I, and PS II, during FaRLIP. (A) Remodeling of PBS cores in FR. Except ApCF and ApCC (see table S1), all allophycocyanin-related components (shown in aqua) comprising the PBS core substructure are replaced in FR. ApCE1 has four REP (linker) domains and should assemble a pentacylindrical substructure in cells grown in WL, GL, or RL (upper model). ApCE2 has only two REP (linker) domains and should assemble a bicylindrical core substructure in FR (7, 38) (lower model). PBS assembled in FR contain some phycoerythrin (table S1; shown as red disks; phycocyanin is shown in blue). This may occur because FR cannot efficiently photoconvert the CCA photoreceptor into its green-absorbing (Pz form) (23). Remodeling of PS I (B) and PS II (C) illustrated using the X-ray structures of PS I (PDB = 1JB0) (47) and PS II (PDB = 3BZ2) (48) from Thermosynechococcus elongatus. The view of PS I is from the luminal side, so Psac, Psad, and Psae are not visible. The view of PS II is from the cytoplasm-facing side. Each subunit is shown in a different color in the upper structures, and subunits replaced in FR are shown in red in the lower structures. Subunit Ycf12 of PS II is not encoded in the draft genome of JSC-1.