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Genome-Wide Analysis of Light Sensing in *Prochlorococcus*▽†

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***Prochlorococcus* MED4 has, with a total of only 1,716 annotated protein-coding genes, the most compact genome of a free-living photoautotroph. Although light quality and quantity play an important role in regulating the growth rate of this organism in its natural habitat, the majority of known light-sensing proteins are absent from its genome. To explore the potential for light sensing in this phototroph, we measured its global gene expression pattern in response to different light qualities and quantities by using high-density Affymetrix microarrays. Though seven different conditions were tested, only blue light elicited a strong response. In addition, hierarchical clustering revealed that the responses to high white light and blue light were very similar and different from that of the lower-intensity white light, suggesting that the actual sensing of high light is mediated via a blue-light receptor. Bacterial cryptochromes seem to be good candidates for the blue-light sensors. The existence of a signaling pathway for the redox state of the photosynthetic electron transport chain was suggested by the presence of genes that responded similarly to red and blue light as well as genes that responded to the addition of DCMU [3-(3,4-dichlorophenyl)-1,1-*N*-*N*′-dimethylurea], a specific inhibitor of photosystem II-mediated electron transport.**

The acclimation of plants and phototrophic microorganisms to the ambient light climate is crucial for survival. All photosynthetic organisms respond to changes in light, including changes in light quantity and quality, i.e., wavelength, by regulating their gene expression. Adaptations to light color detection involve red-light (RL)-, green-light (GL)-, and blue-light (BL)-absorbing receptors, such as phytochromes, cryptochromes, rhodopsins, and xanthopsins (16). Photoreceptors are common to both eukaryotes and prokaryotes, playing important roles in processes such as phototaxis, circadian regulation, and the regulation of flavonoid and alkaloid synthesis (13, 17, 50).

Prochlorococcus is a marine oxyphotobacterium that thrives in the vast oligotrophic gyres of the open ocean between 40°N and 40°S. In these areas, it numerically dominates the phytoplankton, accounting for up to 50% of the total chlorophyll (40, 51). Light appears to have been one of the most important environmental factors driving the speciation and adaptation of *Prochlorococcus* (36). In the natural environment, *Prochlorococcus* cells occur from the water surface down to about 200 m (39), experiencing differences in light intensity of over 4 orders of magnitude and a strong spectral shift from white light (WL) to blue light with increasing depth. The ability to grow over this broad range of light intensities is attributed, in part, to the existence of distinct "ecotypes" that have different light optima for growth and different relative abundances with depth in the ocean (1, 9, 24, 53, 56) and constitute distinct phylogenetic clades (24, 35, 38, 44, 52).

Little is known about photoreceptor-mediated light regulation in *Prochlorococcus*, but several things indicate that studies of light adaptation and acclimation in this organism may yield some interesting insights. First, the MED4 strain has the smallest genome of a free-living photoautotroph (1,716 genes), a third of which have not been functionally characterized (45). It has a limited number of regulatory systems, e.g., 4 histidine kinases and 6 two-component response regulators, compared to 43 histidine kinases and 38 response regulators found in the related cyanobacterium *Synechocystis* PCC 6803 (33, 45). Except for sequence-based evidence for a photolyase gene (45) and two related genes (10) that might code for cryptochromes, typical photoreceptors have not been identified to date in this group, raising the question: has the reduction in genome size reduced the ability for light sensing, or does it have photoreceptors not previously identified in other organisms?

In contrast to other cyanobacteria, which use phycobilisomes for light capturing, the Pcb antenna system of *Prochlorococcus* is based on two major pigments, divinyl-chlorophylls *a* and *b*. Thus, *Prochlorococcus* differs fundamentally not only from other cyanobacteria but also from plants and algae in its light-harvesting apparatus, and this results in a unique light absorption spectrum (35, 41).

Genome analyses of the low-light-adapted *Prochlorococcus* isolates MIT9313, NATL2A, and SS120 and of the high-light-adapted strains MED4 and MIT9312 (7) have shown that these genomes differ by hundreds of genes, likely facilitating their niche partitioning in the oceans (1, 10, 24, 45, 56). MED4, for example, has a disproportionate number of high-light-inducible genes (30), likely an adaptation to the relatively high-light environment in which it and its close relatives (the eMED4 clade, sensu reference 1) are most numerous. Although members of this clade and the other high-light-adapted clade,

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eMIT9312, have relatively high light optima for growth and usually dominate the surface waters, they are often abundant down to 100 m and their distribution extends as deep as 200 m (24, 56). Thus, they experience significant changes in the quality and quantity of light in the oceanic environment.

In clear ocean water, visible light decreases approximately 10-fold for every 75 m. Between about 40 and 60 m, UV, red, orange, and yellow light becomes undetectable, whereas light from the green and blue parts of the spectrum penetrates as deep as 200 m (26). The optical properties in clear ocean water are very different from those in the vast majority of coastal and freshwater ecosystems, in which the blue part of the spectrum becomes more rapidly filtered out than the red one due to light scattering and absorbance by organic matter. Thus, one might expect *Prochlorococcus* to be different from "model" cyanobacteria, which evolved in freshwater, have much more complex genomes, and use an extensive variety of phytochromes and other photoreceptors. To better understand the mechanisms through which marine cyanobacteria acclimate to alterations in light variability in the marine habitat, we studied changes in global gene expression in *Prochlorococcus* MED4 in response to short-term exposure to a spectrum of light qualities (white, blue, red, and green) and quantities (white-light photon flux) by using high-density Affymetrix microarrays.

MATERIALS AND METHODS

Culture and experimental growth conditions. *Prochlorococcus* MED4 was grown at 21°C in Sargasso seawater-based Pro99 medium (37) under 30 μmol quanta m^{-2} s^{-1} continuous cool white light. Under these conditions, its growth rate was 0.28 day^{-1}. Triplicate 2.4-liter cultures were concentrated by centrifugation (10,000 × *g*, 10 min, 20°C) and divided into seven 20-ml subcultures. After a darkness adaptation for 5 h (a sufficient time to reacclimatize cells from centrifugation stress and to reset mRNA level to darkness conditions), cultures were shifted to the respective experimental conditions for 45 min. The conditions of light quantity were WL (13 μmol photon m^{-2} s^{-1}), with and without 4 μM 3-(3,4-dichlorophenyl)-1,1-*N*-*N*′-dimethylurea (DCMU), and "high" white light (HL; 55 μmol photon m^{-2} s^{-1}). Note that although this does not represent an extremely high light intensity for this organism, it is the highest light intensity that the cells can tolerate in a step function increase from darkness; abrupt extreme increases in light intensity can cause cell death in *Prochlorococcus*. The conditions of light quality were BL (13 μmol photon m^{-2} s^{-1}; dark blue, no. 119; Lee Filters [spectra for filters can be found at http://www.leefilters.com/home.asp]), RL (13 μmol photon m^{-2} s^{-1}; primary red, no. 106; Lee Filters), and GL 13 μmol photon m^{-2} s^{-1}; primary green, no. 139; Lee Filters). Control cultures were kept in darkness. Cells were harvested under the respective light conditions by rapid filtration on Supor-450 membranes. Filters were immersed in 2 ml RNA resuspension buffer (10 mM sodium acetate [pH 5.2], 200 mM sucrose, 5 mM EDTA), snap frozen in liquid nitrogen, and subsequently stored at −80°C. The entire harvesting process took less than 2 min.

RNA isolation. Total RNA was extracted from cells on filters by using a hot phenol method described previously (29). Briefly, the samples were extracted after a 15-min incubation with 1/2 volume of phenol (pH 4.5 to 5) preheated to 60°C and the subsequent addition of 1/2 volume chloroform-isoamyl alcohol (24:1, vol/vol). All following extractions in the aqueous phase were carried out with chloroform-isoamyl alcohol (24:1, vol/vol). RNA was precipitated with 1 volume of isopropanol and, after a wash step with 70% ethanol, resuspended in 200 μl H_2O. Total nucleic acids were quantitated based on the absorption at 260 nm. Integrity of quantified RNA was verified by electrophoresis. Twelve micrograms of total nucleic acids was treated with 6 U DNase (DNA free; Ambion, Austin, TX) for 60 min at 37°C. RNA was precipitated with 1/10 volume 3 M sodium acetate (pH 5.2), 3 volumes ethanol and resuspended in H_2O at a concentration of approximately 1 μg/μl RNA.

Real-time PCR. RNA samples used in real-time PCR were DNase treated as described above, with the following modifications. Approximately 3 μg of RNA was incubated with 6 U DNase for 30 min at 37°C. About 0.5 μg RNA was reverse transcribed using Marligen reverse transcriptase (BioCat, Heidelberg, Germany). Each 20-μl reaction mixture contained 1× cDNA synthesis mixture (buffer, primers, and deoxynucleoside triphosphates [dNTPs]) and 50 U reverse transcriptase (amended with RNase H^+). Reaction mixtures were incubated at 22°C for 5 min and then at 42°C for 30 min. The enzyme was inactivated at 85°C for 5 min.

TABLE 1. Primers used for qPCR analysis[a]

Oligonucleotide name	Oligonucleotide sequence (5′–3′)	cDNA dilution
RnpBFW	TTG AGG AAA GTC CGG GCT C	1:200
RnpBRV	GCG GTA TGT TTC TGT GGC ACT	
pmm1671FW	GAG GTC ATT ACA AGA TTC TGC TC	1:20
pmm1671RV	AAC GCA GAC GCT TCA GAA TCC	
pmm1672FW	GCA TCA CCA CTC TTT CTC AGA C	1:20
pmm1672RV	TGC CTC AAC ATC TTT AAA CAT CCA A	
PgkFW	GTA AGT AGC AAA ATT GGA GTT TTG G	1:200
PgkRV	ACT CTT CCC AAC ATC TAA TCC TC	
PsaKFW	GCA AGA GCC ACA ATA AGA AAA CC	1:200
PsaKRV	ATA TGT CCT AGG CAA TTA GCT CC	
hli01FW	AGA TTT GGT TTT GTC AAC TTT GCA G	1:200
hli01RV	TTT GTC CTG TAA TCA ATT CTG TAC C	
hli03FW	AAA GAA AAC TAC CTC GTT ACG GG	1:200
hli03RV	TAA GTG CTA TAA AAC CAA TCA TCG C	
hli08FW	CTC TAA CTG CTT TAC TCG TTG C	1:200
hli08RV	AGT CCG ATC ATT GCA AAT CTA CC	

[a] The applied cDNA dilution for qPCR analysis is given in the last column. All oligonucleotides were optimized for an annealing temperature of 59°C.

Quantitative PCR was performed with a PE Biosystems GeneAmp 5700 sequence detection system using the PE SYBR green PCR core reagents. Each 25-μl reaction mixture contained 1× buffer, 1 mM of each dNTP, 3 mM $MgCl_2$, 0.625 U of polymerase, 10 μl of the reverse transcription reaction in various dilutions, and 10 pmol of each primer (Table 1). The reaction mixtures were incubated for 2 min at 50°C and then 10 min at 95°C, followed by 40 cycles of 15 s at 95°C, 30 s at 59°C, and 30 s at 72°C. After the last cycle, the PCR products were subjected to heat denaturation over a temperature gradient from 60°C to 95°C at 0.03°C s^{-1}. All reactions were performed in triplicate for two biological replicates. All samples were tested for the presence of residual DNA during quantitative real-time PCR (qPCR) with a reverse transcriptase-free control.

The real-time PCR data were analyzed using PE GeneAmp 5700 sequence detection system software version 1.3. Data were plotted as normalized reporter signals, representing the levels of fluorescence detected during the PCR process after subtraction of background noise versus cycle number. A threshold was set manually in the middle of the linear phase of the amplification curve. The cycle threshold (C_T) value is defined as the cycle in which an increase in the reporter signal (fluorescence) crosses the threshold. The average of the C_T values for the triplicate PCRs is ΔC_T. The change (*n*-fold) in gene *x* cDNA relative to that of the endogenous standard (RNase P RNA, *rnpB*) was determined by $2^{-[\Delta C_T(\text{gene } x) - \Delta C_T(rnpB)]}$, summarized as $2^{-\Delta\Delta C_T}$.

cDNA synthesis, labeling, and microarray hybridization. Labeling, hybridization, staining, and scanning were carried out according to Affymetrix protocols for *Escherichia coli* (http://www.affymetrix.com/support/technical/manual/expression_manual.affx), with minor changes. Specifically, 2.5 μg of total RNA was annealed to random hexamer primers (25 ng/μl) for 10 min at 25°C after denaturation for 10 min at 70°C. Reverse transcription of RNA was performed using 25 U/μl Superscript II (Invitrogen Life Technologies, Carlsbad, CA) in 50 mM Tris (pH 8.3), 75 mM KCl, 3 mM $MgCl_2$, 10 mM dithiothreitol, 0.5 mM dNTPs, and 1 U/μl RNase Out RNase inhibitor (Invitrogen Life Technologies, Carlsbad, CA). The mixture was first incubated for 10 min at 25°C, followed by 60-min incubations at 37°C and 42°C. Subsequently, Superscript II was inactivated for 10 min at 70°C. RNA was removed by incubating the reaction mixture for 30 min at 65°C with 0.25 N NaOH and then neutralized with HCl, followed by a purification step for the resultant cDNA with MinElute PCR purification columns (QIAGEN, Hilden, Germany). cDNA was digested for 10 min at 37°C with DNase I (0.6 U per μg cDNA), yielding fragments of 50 to 200 bp in length. The enzyme was inactivated for 10 min at 98°C. End labeling of fragmented cDNA with biotin was performed at 37°C for 60 min by using a BioArray terminal labeling kit (Enzo Life Sciences, Farmingdale, NY). The reaction was stopped by freezing at −20°C, and the mixture was stored overnight at this temperature. Quality of end labeling was verified by gel shift assays with NeutrAvidin (Pierce Chemical, Rockford, IL) on 1% TBE (Tris-borate-EDTA) agarose gels.

Hybridization of biotin-labeled cDNA in the MD4-9313 custom Affymetrix

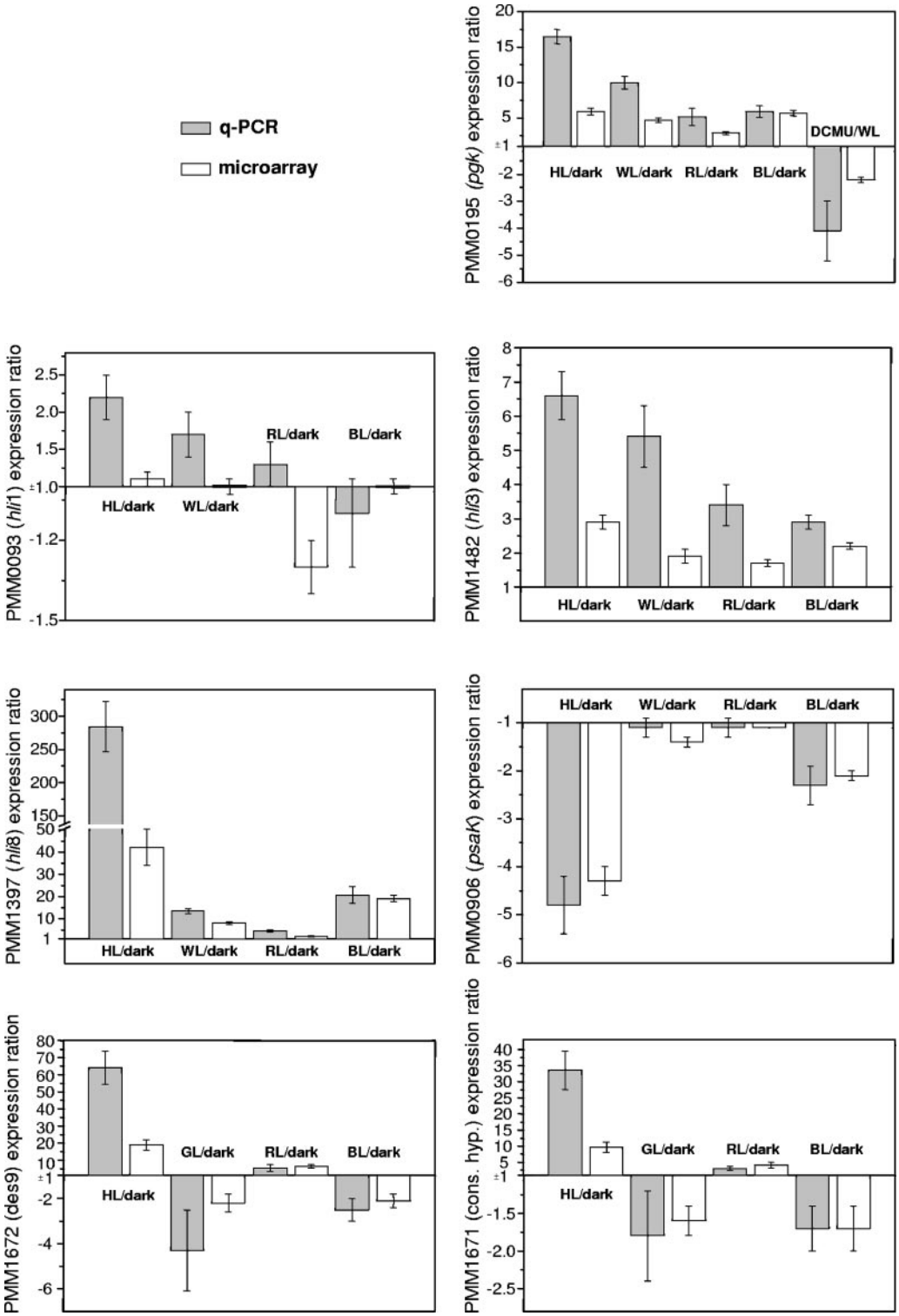

FIG. 1. Verification of microarray data by qPCR. Expression ratios correspond to values determined by microarray analysis and qPCR employing the identical RNA samples also used in the array hybridizations. Microarray and qPCR ratios are average values ± standard errors for three microarrays and four independent PCR runs, each in triplicate ($n = 12$). Tested conditions were HL, WL, RL, BL, and GL with respect to darkness and DCMU with respect to WL.

array was done at 45°C for 16 h in an aqueous hybridization solution (100 mM MES [morpholineethanesulfonic acid], 1 M NaCl, 20 mM EDTA, 0.01% Tween 20) and in the presence of 0.1 mg/ml herring sperm DNA, 0.5 mg/ml bovine serum albumin, 7.8% dimethyl sulfoxide, and 3 nM of prelabeled Affymetrix hybridization B2 oligonucleotide control probe mixture in a GeneChip 320 hybridization oven at 60 rpm. Washes and stains were carried out on a GeneChip 450 fluidics station (Affymetrix, Santa Clara, CA), following the ProkGE_WS2v3 Affymetrix protocol. The first wash with wash buffer A (6× SSPE [1× SSPE is

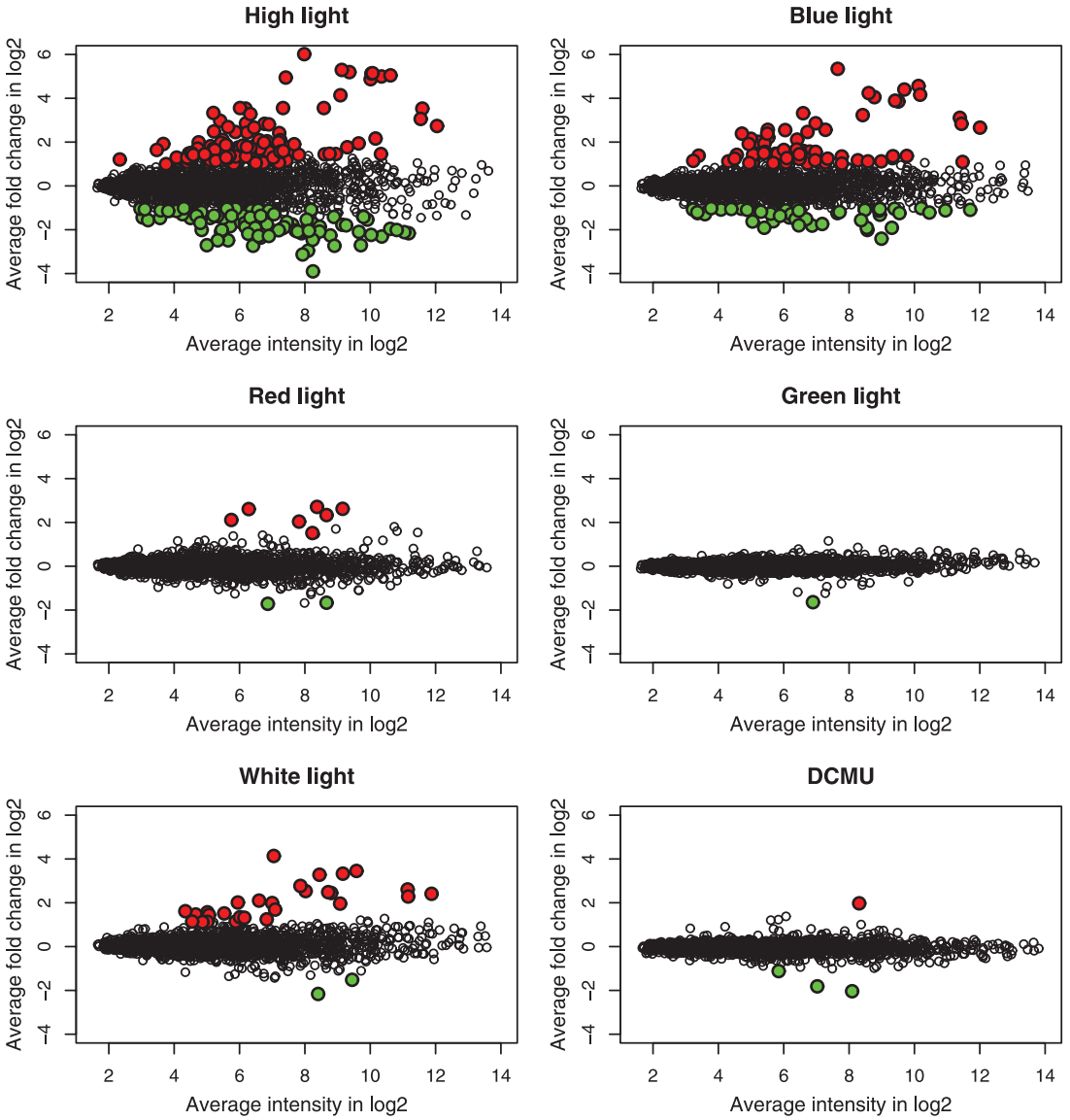

FIG. 2. Synopsis of the array results, revealing genes that were differentially upregulated (red) and downregulated (green) in response to different treatments: HL, WL, BL, RL, and GL treatment with respect to treatment in darkness and DCMU with respect to white light. The average logged changes (*n*-fold) are plotted against the average logged array signal intensity levels. Each dot corresponds to a probe set on the Affymetrix chip. Significant differences in expression were defined by changes (*n*-fold) larger than 2 and FDRs of ≤0.05 (see Materials and Methods).

0.18 M NaCl, 10 mM NaH_2PO_4, and 1 mM EDTA {pH 7.7}] and 0.01% Tween 20), for 10 cycles (2 mixes/cycle) at 25°C, was followed by a second, more stringent wash in wash buffer B (100 mM MES, 0.1 M NaCl, and 0.01% Tween 20) for 4 cycles (15 mixes/cycle) at 45°C. Staining was performed with 10 μg/ml streptavidin (Pierce Chemical, Rockford, IL) for 10 min at 25°C in stain buffer (100 mM MES, 1 M NaCl, 0.05% Tween 20, containing 2 mg/ml bovine serum albumin), followed by a 10-cycle wash (4 mixes/cycle) in wash buffer A at 30°C. For antibody binding, 5 μg/ml biotinylated anti-streptavidin goat antibody (Vector Laboratories, Burlingame, CA) was incubated for 10 min at 25°C in stain buffer in the presence of 0.1 mg/ml goat immunoglobulin G (Sigma-Aldrich, St. Louis, MO) and then stained with 10 μg/ml streptavidin-phycoerythrin conjugate (Invitrogen Molecular Probes, Carlsbad, CA) for 10 min at 25°C in stain buffer. After a final wash in wash buffer A for 15 cycles (4 mixes/cycle) at 30°C, the array was scanned with a GeneChip Scanner (Affymetrix, Santa Clara, CA) using factory settings, with excitation set at 570 nm and a 2.5-μm resolution.

Normalization and LPE test. Expression summaries for each gene were computed from the probe intensities in Affymetrix CEL files by robust multiarray average normalization using S-Plus software with an implemented ArrayAnalyzer module (version 2.0.1, 2004; Insightful Corporation, Seattle, WA). For each sample (including the reference sample), one microarray was hybridized and subsequent changes (*n*-fold) estimated by dividing the expression value for the tested condition by the expression value for that of the reference sample (in most cases treatment in darkness). The statistical significance of the differential expression levels was assessed by applying the local-pooled-error (LPE) method (22). The LPE approach aims at reducing the within-gene variance of expression by pooling error estimates within regions of similar expression intensity. The significance is subsequently derived from the *z* score calculated on the basis of the observed average change (*n*-fold) and the estimated error. Since multiple tests were performed, statistical significance was adjusted based on the Benjamini and Hochberg algorithm (2). Following this approach, each gene is assigned a false-discovery rate (FDR), which indicates the percentage of false-positive outcomes among all positive test outcomes. Only genes with FDRs of ≤0.05 and changes (*n*-fold) equal to or above 2 were considered differentially expressed (see Table S1 in the supplemental material).

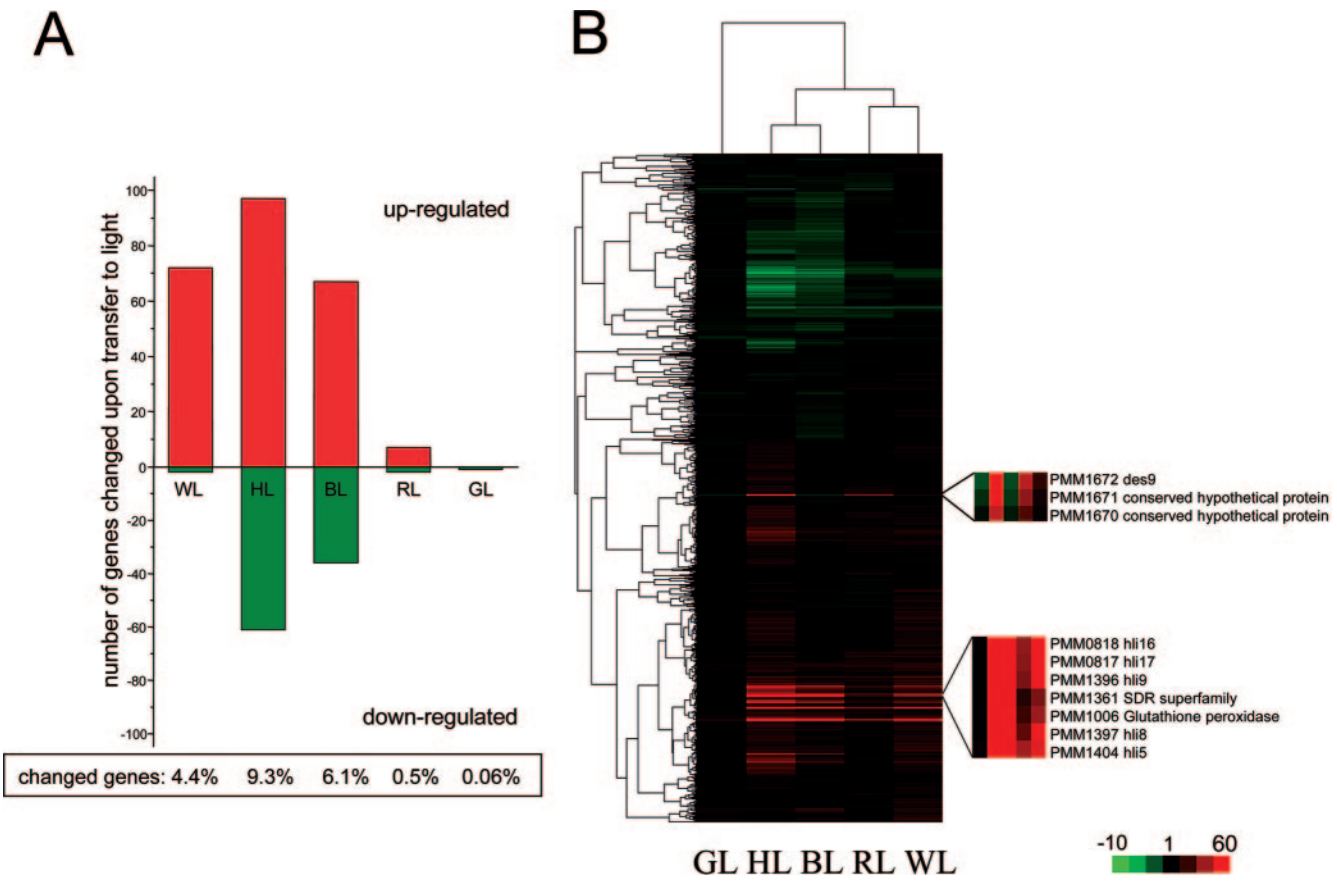

FIG. 3. Summary of genes that were significantly upregulated (red) or downregulated (green) when cells were shifted from darkness to WL, HL, BL, RL, or GL. (A) Numbers of genes that were at least twofold down- or upregulated under each condition. (B) Hierarchical clustering tree of dark/light expression ratios for each condition. Clusters on top show the relationships of the different light treatments, and clusters on the left represent gene clusters. Green and red indicate down- and upregulated genes, respectively.

Reliability of the microarray analysis. We used the Affymetrix high-density array MD4-9313, made for *Prochlorococcus* MED4, which covers all gene coding regions, with a probe pair (perfect match and mismatch) covering approximately every 80 bases spread across the gene and every 45 bases in the intergenic region in both sense and antisense orientations. (This array also includes another *Prochlorococcus* strain, MIT9313, as well as two *Prochlorococcus*-infecting cyanophages [49], P-SSP7 and P-SSM4). Probes are made up of 25-base oligomers identical to those in the target sequence for a perfect match and a 1-base difference in the center of the sequence for the mismatch probe.

Technical replicate arrays revealed a high reproducibility, with correlation values between 0.96 and 0.99. Therefore, we worked with biological triplicates, omitting further technical replicates. Estimates for the variances of biological replicate arrays (based on within-group comparisons) yielded maximal changes (n-fold) of ± 1.4 to 1.6 for the 95% confidence intervals. Since these differences derive mainly from experimental variability, such deviations indicate upper thresholds for nondifferential expression.

The differential regulation of seven genes was independently validated by quantitative real-time PCR employing the identical RNA samples also used in the array hybridizations (Fig. 1). It was particularly important to verify gene expression for some members of the *hli* gene family due to the potential for cross-hybridization. Quantitative real-time PCR data for *hli8* are representative for 8 out of the 22 *hli*s since *hli8* is arranged in an operon with *hli6*, *hli7*, and *hli9*, which was duplicated, yielding *hli16* to *hli19*. The results (Fig. 1) showed verification of the qualitative data, whereas the quantitative changes in gene expression tended to be higher in the real-time PCR experiment than in the array. The single gene for which correlation between microarray and qPCR results was lacking (*hli1*) showed a slight change in expression by qPCR, while the less sensitive microarray analysis did not. This is consistent with the notion that microarray data underestimate the relative changes in mRNA expression between experimental and control samples (55) and may not adequately detect low-level differential expression for some genes that can be detected by more-sensitive methods, such as qPCR.

Wavelength-dependent gene expression. Interference filters with maximal transmission wavelengths at 478 nm, 623 nm, 657 nm, 699 nm, and 737 nm were used to investigate wavelength-dependent gene expression. Two 400-ml *Prochlorococcus* MED4 cultures were grown as described above. Cells were divided into seven 40-ml subcultures and transferred to darkness for 5 h. Cultures were then transferred to 13 μmol photon m^{-2} s^{-1} of specific light by using interference filters (for transmission spectra, see Fig. 5) or kept in darkness as a control. Cell harvesting, RNA isolation, and quantitative real-time PCR were performed as described above.

RESULTS AND DISCUSSION

Genome-wide response to a shift from darkness to light. Approximately 10% and 5% of all genes displayed significant differential expression levels of at least twofold or more (with an FDR of less than 0.05) when shifted from darkness to high white light and white light, respectively (Fig. 2 and 3A; also see Table S1 in the supplemental material). Hierarchical clustering revealed that the responses to high white light and blue light were very similar and different from that to the lower-intensity white light (Fig. 3B), suggesting that the actual sensing of high light is mediated via a blue-light receptor. Red and green light with photon fluxes similar to that of white light had little overall effect on expression; a few genes responded specifically

TABLE 2. Expression data for photosystem genes under different light exposures[a]

Subunit	Ratio for indicated treatments				
	HL/dark	WL/dark	BL/dark	RL/dark	GL/dark
PS I					
psaA	−2.5	1.0	−1.4	1.0	1.1
psaB	−2.5	1.1	−1.4	1.0	1.2
psaC	−2.5	−1.1	−1.7	−1.1	1.0
psaD	**−4.3**	1.0	−1.7	−1.7	1.0
psaE	**−3.7**	1.1	−2	−1.4	1.0
psaF	**−5.3**	−1.1	−2.5	−1.7	−1.3
psaI	**−4.0**	−1.3	−1.7	−1.4	1.1
psaJ	**−4.4**	−1.1	−2	−1.1	1.0
psaK	**−4.3**	−1.4	−2	−1.1	1.0
psaL	**−4.2**	−1.3	−1.7	−1.3	1.1
psaM	**−3.8**	−1.3	−1.4	−1.1	1.1
PS II					
psbA	2.3	1.9	**2.0**	1.6	1.5
psbB	−1.7	1.2	−1.1	1.1	1.3
psbC	−1.3	1.1	1.1	−1.1	1.2
psbD	1.1	1.4	1.3	1.0	1.3
psbE	−1.7	−1.1	−1.3	−1.1	1.0
psbF	−1.7	1.0	−1.3	−1.1	1.1
psbH	−2.5	−1.3	−1.7	−1.1	1.0
psbI	−1.3	1.1	−1.1	1.2	1.1
psbJ	−1.4	1.1	−1.1	1.0	1.1
psbK	−2.5	−1.1	−1.4	1.2	1.1
psbL	−1.7	1.0	−1.3	−1.1	1.1
psbM	−1.4	−1.3	−1.4	−1.1	−1.1
psbN (*psbTc*)	**4.9**	2.1	**2.8**	1.5	−1.2
psbO	−1.7	1.5	−1.1	1.2	1.3
psbP	1.1	1.4	1.2	−1.3	1.1
psbT	−2	1.2	−1.1	1.3	1.2
psbZ	−2	1.0	−1.4	1.3	1.1
psb27	−1.1	1.3	1.2	1.0	1.2
psb28	2.7	1.6	1.7	1.2	1.0

[a] Significant changes (≥2-fold, with FDRs of ≤0.05) are shown in bold numbers. Positive values indicate induction and negative values reduction of gene expression upon transfer to the respective light treatments.

to red light, whereas for green light, significant changes were detectable only for one gene (Fig. 2 and 3). Generally, expression changes were higher for upregulated than downregulated genes (Fig. 3B).

Sigma factors play a key role in the initiation of transcription. We found two group II sigma factors (PMM1629 and PMM1697) that were antagonistically regulated under our light shift conditions. PMM1629 was upregulated in all light treatments, whereas PMM1697 was downregulated in high white and blue light and to some extent in white and red light (see Table S1 in the supplemental material). These group II sigma factors could be the major factors for the fine-tuning of transcriptional expression in response to light, as is the case of group II sigma factors in *Synechocystis* PCC 6803 (20).

PS genes. Light is one of the crucial factors for the modulation of photosynthesis gene expression (18, 19, 42), and indeed, our data may indicate a reorganization of the photosystem I (PSI) and PSII complexes upon shifting to high-light conditions. PSI genes, for example, were differentially expressed only under the high-white-light condition (Table 2), suggesting that a certain threshold of photon flux must be exceeded to trigger a response (Table 2). The inner core subunits of PSI, *psaA*, *psaB*, and *psaC*, were downregulated 2.5-fold upon transfer from darkness to high light, whereas many external subunits of PSI (*psaF*, *psaI*, *psaJ*, *psaK*, *psaL*, and *psaM*) declined in gene expression four- to fivefold (Table 2). Interestingly, *psaD*, a PSI core subunit also declined 4.3-fold, similar to the external subunits. One could speculate that the greater downregulation of the transcript levels of the PSI subunits involved in antenna binding (*psaJ*, *psaF*, and *psaK*) (23, 25) and trimerization (*psaI*, *psaL*, and *psaM*) (6, 28) might be caused by a reduction in the number of PSI complexes and light-harvesting antennae and their reorganization under high-light conditions. PSII gene expression of *psbA*, *psbN* (new designation *psbTc*), and *psb28* was induced upon high-light exposure (Table 2). D1, the gene product of *psbA*, is the most rapidly turned over component of the thylakoid membrane (31). Its continuous recycling is critical for maintaining PSII activity (43), and thus, the protein needs to be synthesized even when the de novo synthesis rate of PSII is declining. *psbTc* is dispensable for photoautotrophic growth but necessary for the dimerization of PSII subunits in the thermophilic cyanobacterium *Thermosynechococcus elongatus* (21). The mechanism of dimerization and the physiological role of the PSII dimer are not clear (21). Psb28 is a peripheral subunit of PSII, which might link PSII dimers in rows to minimize the contact of PSII and PSI and prevent a spillover of excess light energy (C. W. Mullineaux, personal communication).

Thus, the changes in PS gene expression in response to a shift to higher light intensities suggest that acclimation to elevated light intensities involves a structural reorganization of both PSI and PSII in *Prochlorococcus*. While genes involved in PSI showed the most pronounced response, up-scaling of PSII D1 synthesis capacity was evident, as was putative Psb28-mediated spatial modification. A clear picture of the light-dependent structural rearrangements of the photosynthesis systems in *Prochlorococcus* awaits the characterization of protein complex changes using biochemical and functional approaches.

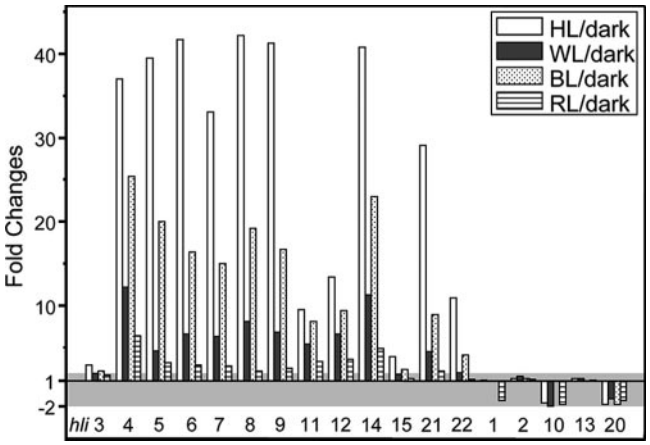

FIG. 4. Effects of spectral quality and light intensity on the expression of *hli* genes. Expression changes of *hli* genes in high, white, blue, or red light (normalized to data for treatment in darkness). *hli6* to *hli9* form an operon, which is duplicated (*hli16* to *hli19*) in this strain. Only probe sets for one *hli* operon (*hli6* to *hli9*) are present on the microarray, thus also representing expression data for *hli16* to *hli19*. Changes (*n*-fold) less than 2 are gray shaded.

High-light-inducible proteins. The most differently expressed group of genes in our experiments were the high-light-inducible genes (*hli*) (Fig. 4). Their gene products, the HLIPs, are thought to protect the photosynthetic apparatus from excess excitation energy during stressful conditions by directly or indirectly dissipating excess absorbed light energy (14, 15, 34). It has also been proposed that the HLIPs could serve as transient carriers of chlorophyll (12) and that they may play a role in the regulation of tetrapyrrole biosynthesis (54). *Prochlorococcus* MED4 carries as many as 22 copies of this gene (4, 45). Among these, *hli6*, *hli7*, *hli8*, and *hli9* form an operon, which has been duplicated (*hli16*, *hli17*, *hli18*, and *hli19*) in this strain (4). Both copies of the operon were significantly upregulated under all light conditions except green light, though the effect was most dramatic in high light (Fig. 4). The same expression pattern was apparent for *hli4*, *hli5*, *hli14*, and *hli21*, whereas only modest to minor changes were detectable for *hli11*, *hli12*, *hli22*, *hli3*, and *hli15* (Fig. 4). Genes for five additional HLIPS, *hli1*, *hli2*, *hli10*, *hli13*, and *hli20*, were constitutively expressed under all experimental conditions (Fig. 4), showing array fluorescence values above the noise threshold (noise threshold is $\leq$16), between 27 and 475, after robust multiarray average normalization (data not shown). Since these five HLIP genes (*hli1*, *hli2*, *hli10*, *hli13*, and *hli20*) were expressed but did not alter transcript levels upon transfer to light, their function must differ from that of the others.

Response to light intensity. Not surprisingly, high-light treatment had the most dramatic effect on gene expression in our experiments. The most highly upregulated gene (61.2-fold upregulated upon darkness-to-high-light transition) was PMM1359, which codes for a protein of unknown function. Yet, PMM1359 possesses similarities to the COG2259 protein domain (a predicted membrane protein of unknown function) and to the *doxD*-like domain (a subunit of the terminal quinol oxidase), with E values of $2e^{-13}$ and $2e^{-12}$, respectively. The most highly downregulated gene (11.3-fold reduced in high light) was PMM1148, again a gene of unknown function. There are no orthologs for PMM1148 outside the marine *Synechococcus*/*Prochlorococcus* lineage, but the gene is very highly conserved within the eight available genomes within this group (>60% identity; >80% similarity).

Response to changes in the redox state of the photosynthetic electron transport chain. DCMU, a specific inhibitor of PSII electron transport, blocks the Q_B site of the D1 protein and thus leads (upon illumination) to net oxidation of the plastoquinone pool. By treating the cells with DCMU, we attempted to differentiate between redox state-dependent and merely photoreceptor-dependent (not related to photosynthesis) changes in gene expression brought about in blue-light- or red-light-exposed cells. The expression of only four genes, PMM0336, PMM0356, PMM1359, and PMM1462, was influenced by DCMU (in white light) treatment relative to only white-light treatment (see Table 4). Since only a limited number of genes responded to DCMU, and moreover, PMM0356 was upregulated, we exclude the possibility that photosynthesis-dependent ATP and NADPH biosynthesis in cells ceased, which would arrest overall transcription and ultimately lead to a drop-off in transcript abundance.

None of the four DCMU-influenced genes described above were assigned a function in the published *Prochlorococcus* MED4 genome (45). However, recent studies assigned the product of PMM0336 to the group of plastoquinol terminal oxidases (PTOX) (11, 32). Several functions have been reported for PTOX, which have a modulation of the plastoquinone redox state in common. PTOX has also been shown to play an important role in carotenoid biosynthesis (5) and to catalyze electron transfer to O_2 (the latter phenomenon is known in chloroplasts as chlororespiration) when the plastoquinone pool is highly reduced, thereby preventing its overreduction (8). Though we do not know the exact function of the four genes that responded to DCMU treatment, it is very likely that some of them play an important role in the adjustment of the redox state of the electron transport chain. We suspect that biochemical characterization of these genes would reveal that their activity is intimately linked to the specific adaptation to high light in *Prochlorococcus* MED4.

Specific response to light quality. (i) Blue-light response. Of all the tested light colors, blue light caused the most changes in gene expression (Fig. 2 and 3). Sixty-one genes were downregulated and 97 genes were upregulated by blue light (Fig. 3; also see Table S1 in the supplemental material). These genes can be classified into three different groups based on their expression under other light treatments (Table 3; also see Table S1 in the supplemental material). Group 1 encompasses genes that respond to blue and high light but not to red light; group 2 contains genes that respond to blue, red, and high light; and group 3 contains genes antagonistically regulated in blue and red light. We suspect that different signal cascades concur in these three groups. As the majority of the changes are common to blue light as well as high light (Fig. 3; also see Table S1 in the supplemental material), we postulate that the high-light response is triggered by a blue-light-absorbing photoreceptor.

There has been no clear evidence that cryptochromes are present in MED4. However, it is known that cryptochromes and DNA photolyases share exceptionally high sequence similarity (55). Our observations of differential gene expression in blue light provide the first functional suggestion that at least one of the genes annotated as photolyase (45) or photolyase related (10) in MED4 (PMM0285, PMM0425, and PMM1360) might indeed serve as a blue-light-sensing cryptochrome instead of a photolyase.

Genes in group 2 (responding in blue, red, and high light) (Table 3) are presumably controlled by a chlorophyll-mediated signaling pathway, as chlorophyll has absorption maxima in the blue and red spectral regions. The absorption of blue-light and/or red-light photons by chlorophylls may ultimately induce alterations in the redox state of the photosynthetic electron transport chain, thus indicating a mere photoreceptor-independent, redox-mediated signaling pathway. Among the red-light- and blue-light-responsive genes are, e.g., the HLIPs (Fig. 4). We hypothesize two different mechanisms for their control. First, a high-light-triggered response is detectable (apparently mediated by a blue-light receptor). Second, a redox-mediated regulation is observed, as determined by the response to blue light and also to red light (Tables 3 and 4). Table 3 summarizes the differential expression levels for blue light and red light. Although the *hli*s are induced by red light as well as blue light, the response in blue light is much more pronounced. Presumably, blue light triggers a stronger response than that induced

TABLE 3. Comparison of differentially expressed genes for BL and RL treatment, with ratios for BL, RL, and HL treatment with respect to treatment in darkness also shown[a]

Gene	Description	Ratio for indicated treatments			
		BL/RL	BL/dark	RL/dark	HL/dark
PMM0861	Possible virion host shutoff protein	**2.7**	**2.2**	−1.1	1.2
Group 1					
PMM0043	Flavoprotein	**2.2**	2.8	1.4	**4.4**
PMM0286	NUDIX hydrolase	**2.2**	**2.5**	1.1	**3.0**
PMM0417	Hypothetical	**−2.3**	−1.8	1.2	**−2.9**
PMM0365	Possible DsrE-like protein	**2.7**	**2.5**	1.0	**2.5**
PMM0689 (*hli22*)	Possible high-light-inducible protein	**3.8**	**4.1**	1.2	**10.9**
PMM1001	Conserved hypothetical protein	**2.3**	**3.7**	1.6	**7.7**
PMM1168	Conserved hypothetical protein	**2.1**	**2.6**	1.3	**5.4**
PMM1361	SDR superfamily	**2.9**	**4.3**	1.5	**6.4**
PMM1422	Conserved hypothetical protein	**2.6**	**2.9**	1.0	**3.8**
Group 2					
PMM0280	Retinal pigment epithelial membrane protein	**2.8**	**5.3**	2.0	**7.9**
PMM0316	Possible ferredoxin	**3.4**	**6.6**	2.1	**12.4**
PMM0690 (*hli21*)	Possible high-light-inducible protein	**4.2**	**8.9**	2.2	**29.1**
PMM0703	Conserved hypothetical protein	**2.8**	**4.7**	1.9	**11.2**
PMM0817 (*hli17*)	Possible high-light-inducible protein	**5.5**	**15.0**	2.8	**33.1**
PMM0818 (*hli16*)	Possible high-light-inducible protein	**5.7**	**16.4**	2.9	**41.7**
PMM1135 (*hli14*)	Possible high-light-inducible protein	**4.7**	**23.0**	**4.9**	**40.8**
PMM1359	Conserved hypothetical protein	**6.6**	**37.2**	**5.9**	**61.2**
PMM1396 (*hli9*)	Possible high-light-inducible protein	**7.3**	**16.7**	2.5	**41.3**
PMM1397 (*hli8*)	Possible high-light-inducible protein	**10.0**	**19.2**	2.2	**42.2**
PMM1404 (*hli5*)	Possible high-light-inducible protein	**6.4**	**20.0**	3.2	**39.5**
Group 3					
PMM1670	Conserved hypothetical protein	**−4.0**	−1.4	2.6	**6.2**
PMM1671	Conserved hypothetical protein	**−7.7**	−1.7	**4.2**	**9.6**
PMM1672 (*des9*)	Fatty acid desaturase, type 1	**−15.4**	**−2.1**	**6.4**	**18.9**

[a] Significant changes (≥2-fold, with FDRs of ≤0.05) are shown in bold numbers. Positive values indicate induction and negative values reduction of gene expression upon transfer to the respective light treatments.

by redox signaling (induced by a blue- plus red-light response). The observed *hli* induction under high light would thus be the integration of a blue-light and a blue/red-light redox-mediated signal. However, other sensors absorbing in the blue spectral region, which still remain to be identified, cannot be excluded.

(ii) Red-light response. There is no evidence for the otherwise widely occurring phytochrome system in *Prochlorococcus.* Hence, we were surprised to find a specific response to red light. Genes PMM1670, PMM1671, and PMM1672, for example, were induced in red light and high light but became repressed in blue light, thus belonging to group 3, described above (Table 3). PMM1670 and PMM1671 are organized in an operon and code for genes of unknown function. The former belongs to the COG4100 group of cystathionine beta-lyase family proteins and is widely conserved within the eubacterial radiation, whereas PMM1671 is specific for the *Prochlorococcus* lineage. In contrast, PMM1672 is an ortholog of a gene well studied in other cyanobacteria (27) that encodes a fatty acid desaturase (*des9* and *desC*) and is located on the reverse complementary strand relative to PMM1670 and PMM1671. It is very likely that PMM1670 to PMM1672 are driven by one and the same bidirectional promoter, which would explain their similar expression patterns. The expression of fatty acid desaturases is induced during both cold acclimation and light induction in other cyanobacteria (27, 47), presumably because photosynthetic activity is strongly decreased under both conditions (27). One mechanism by which cells compensate for this reduction in photosynthetic activity is the manipulation of the glycerolipid unsaturation level of their thylakoid membranes through the activity of these desaturases (27). Since the

TABLE 4. Genes affected by the electron transport inhibitor DCMU (DCMU/WL)[a]

Gene	Description	Ratio for indicated treatments					
		DCMU/WL	HL/dark	WL/dark	BL/dark	RL/dark	GL/dark
PMM0336 (PTOX)	plastoquinol terminal oxidase	**−3.6**	**3.0**	**3.8**	**2.6**	**2.5**	1.0
PMM0356	α/β hydrolase fold domain	**2.0**	**−3.1**	−1.8	**−2.9**	**−2.0**	−1.0
PMM1359	Conserved hypothetical protein	**−4.2**	**61.2**	**17.4**	**37.2**	**5.9**	1.1
PMM1462	Conserved hypothetical protein	**−2.1**	**−3.6**	−1.3	−1.7	**−2.8**	**−2.9**

[a] Data shown are average expression values of genes that were induced (positive ratios) or reduced (negative ratios) by DCMU or light exposure. Significant changes (≥2-fold, with FDRs of ≤0.05) are shown in bold numbers. Also shown are ratios for different light treatments with respect to treatment in darkness.

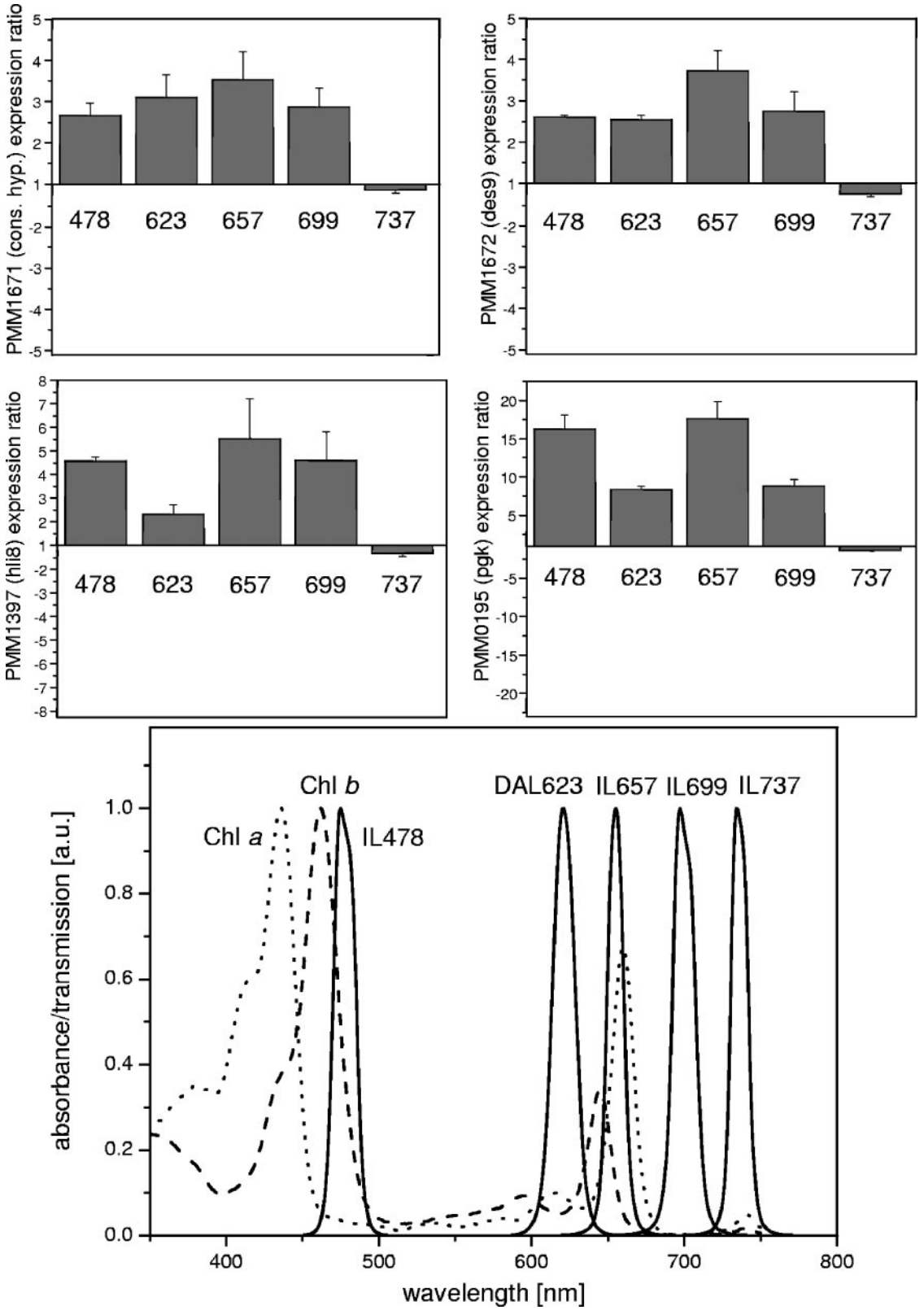

FIG. 5. Wavelength-dependent gene expression of group 3 genes and two control genes (*pgk* and *hli8*) in the red spectral region determined by quantitative real-time PCR. qPCR expression ratios (relative to the data for darkness treatment) are average values ± standard errors for four independent PCR runs, each in triplicate ($n = 12$). The greatest changes in gene expression occurred with interference filters with maximal transmission wavelengths at 659 nm. The bottom panel shows the spectral-transmission properties of the interference filters used (solid lines) and the absorption properties of isolated divinyl-chlorophylls *a* (dotted lines) and *b* (dashed lines).

light quality mediating the activation of *desABD* was not tested previously, it is difficult to compare our results directly to those in previous work. However, in contrast to what was found for *Synechocystis* PCC 6803, where transcript levels of *desA*, *desB*, and *desD* genes were strongly induced by light and *desC* did not change (27), we found that in *Prochlorococcus* MED4, only *desC* varied in transcript levels upon transfer to different light colors.

In order to obtain more-specific information about the origin of the red-light responses of genes belonging to group 3, we investigated the wavelength-dependent gene expression of PMM1671 and PMM1672 in the red spectral region. For this experiment, we used narrow-bandwidth interference filters with maximal transmission wavelengths at 623 nm, 657 nm, 699 nm, and 737 nm (Fig. 5). As a control, we included one blue-light filter with a transmission maximum at 478 nm (Fig. 5). Furthermore, the expression levels of *hli8* and *pgk*, which responded in blue and red light (probably regulated by redox signaling), were measured simultaneously. Compared to what was found for darkness, all four genes were upregulated in red light, with maximal transmission wavelengths at 623 nm, 657 nm, and 699 nm, showing the strongest change at 657 nm (Fig. 5). A maximal expression at 657 nm concurs well with the absorption maxima of divinyl-chlorophylls *a* and *b*, the major light-harvesting pigments of *Prochlorococcus*. However, in case of PMM1671 and PMM1672, a different behavior in blue light, compared to microarray results, was detected. The most likely explanation for this discrepancy might be the different wavelength ranges of transmitted light for the filters used. It is very well possible that the broader range of the blue-light filter (used in the microarray experiment) activated a specific blue-light receptor response that absorbs at wavelengths other than 478 nm. It is noteworthy that the blue-light interference filter (maximal transmission at 478 nm) overlaps with the absorption of divinyl-chlorophyll *b*. Thus, the observed changes in gene expression could reflect a change in the redox state of the photosynthetic electron transport, resulting in a response similar to that observed with the 657-nm-interference filter, directly supporting the view that PMM1671 and PMM1672 are under redox signaling through chlorophylls. Interestingly, exposing cells to far-red light (737 nm) did not induce changes in expression in any of these four genes. Since the greatest changes in gene expression in the red spectral region (657-nm interference filter) occur in the absorption range of the divinyl-chlorophylls, we conclude that the color-dependent responses of PMM1670 to PMM1672 might be a combination of gene activation by photosynthetic electron transport in red and blue light and a specific blue-light repression (occurring only with broad-range blue-light filters) by blue light at wavelengths other than 478 nm.

Collectively, these data argue against the presence of a red-light sensor, which is consistent with our inability to find genes with homologies to phytochromes in the genome.

(iii) Green-light response. There is only weak evidence for a specific green-light response. We found a single gene, PMM1462, that was significantly downregulated in green light (Table 4), but since this gene also responded in red light and blue light, it is hard to determine whether regulation is coordinated by a general light receptor or by several color-specific receptors. While green-light photoreceptors have not been established thus far for *Prochlorococcus*, there are at least two candidates with absorption maxima in the green spectral region: a specific form of β phycoerythrin (*cpeB*, PMM0305) (48) and retinal, which is formed by a carotenoid oxygenase (PMM0280, Diox1) (46).

Summary. Clearly, two general regulatory responses to light can be distinguished in *Prochlorococcus* MED4: one induced by light-dependent modulation of the redox state of the electron transport chain and a photoreceptor-mediated blue-light response. Our results indicate that the function of the specific blue-light receptor (e.g., cryptochrome) (3) could actually be to sense the blue part of the white light associated with high light. Except for the possible cryptochrome homologs PMM0425 and PMM1360, the small and compact genome of *Prochlorococcus* MED4 does not contain any further known candidate genes for photoreceptors.

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