Near-UV cyanobacteriochrome signaling system elicits negative phototaxis in the cyanobacterium *Synechocystis* sp. PCC 6803

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Positive phototaxis systems have been well studied in bacteria; however, the photoreceptor(s) and their downstream signaling components that are responsible for negative phototaxis are poorly understood. Negative phototaxis sensory systems are important for cyanobacteria, oxygenic photosynthetic organisms that must contend with reactive oxygen species generated by an abundance of pigment photosensitizers. The unicellular cyanobacterium *Synechocystis* sp. PCC6803 exhibits type IV pilus-dependent negative phototaxis in response to unidirectional UV-A illumination. Using a reverse genetic approach, together with biochemical, molecular genetic, and RNA expression profiling analyses, we show that the cyanobacteriochrome locus (*slr1212/uirS*) of *Synechocystis* and two adjacent response regulator loci (*slr1213/uirR* and the PatA-type regulator *slr1214/lsiR*) encode a UV-A-activated signaling system that is required for negative phototaxis. We propose that UirS, which is membrane-associated via its ETR1 domain, functions as a UV-A photosensor directing expression of *lsiR* via release of bound UirR, which targets the *lsiR* promoter. Constitutive expression of *lsiR* induces negative phototaxis under conditions that normally promote positive phototaxis. Also induced by other stresses, *lsiR* thus integrates light inputs from multiple photosensors to determine the direction of movement.

phytochrome | transcription regulator | two-component signaling | UV-A sensor

Cyanobacteria rely on light for photosynthesis, and therefore possess many photoreceptor systems for adaptation to limiting or excess light. Although this typically entails regulation of genes involved in light-regulated metabolism, critical signaling pathways regulate movement toward or away from weak or strong light, instead using light to regulate a behavioral cue. Phototactic motility of the cyanobacterium *Synechocystis* sp. PCC6803 (hereafter referred to as *Synechocystis*) is based on the synthesis and retraction of type IV pili (1). Light wavelengths ranging from the green (560 nm) to the red (720 nm) region mediate positive phototaxis of *Synechocystis*. Negative phototaxis is observed in response to UV-A light (360 nm; hereafter abbreviated as UV) as well as in response to blue (470 nm) and red (600–700 nm) light at high intensity (2, 3). Although light avoidance is regulated by sensory rhodopsins in Archaea and in a few proteobacterial and algal species (4), no such proteins are encoded within the *Synechocystis* genome. Known photosensors that play key roles in *Synechocystis* phototaxis include the phytochrome-related cyanobacteriochromes (CBCRs) PixJ1/TaxD1 (5, 6) and Cph2 (7), the BLUF photosensor PixD (8) and the CRY-related cyanopterin Sll1629 (9). Despite new insight into the regulatory roles of these proteins in UV-mediated phototaxis in *Synechocystis* (10), photoreceptors that directly sense unidirectional UV are unknown.

One potential candidate for a negative UV phototaxis sensor in *Synechocystis* is encoded by the *slr1212* locus (hereafter des-

ignated *uirS* for UV intensity response Sensor based on this work). A member of the CBCR photoreceptor family (11), UirS possesses three N-terminal transmembrane (TM) helices similar to those of the ethylene-binding domain of the *Arabidopsis* ethylene receptor *ETR1* (12), along with two PAS domains, a CBCR GAF domain, and an H-box containing histidine kinase (HiK) domain at the C-terminal end (13). UirS belongs to a CBCR subfamily with two conserved Cys residues within its phycocyanobilin (PCB)-binding GAF domain (14), with other notable examples being *Synechocystis* SyPixJ/TaxD1/Sll0041 (5, 15) and TePixJ/Tll0569 and Tlr0924 from *Thermosynechococcus elongatus* (14, 16, 17). Previous studies have shown that the region containing TM helices is responsible for ethylene binding in UirS (12), whereas its GAF domain can bind to the linear tetrapyrrole PCB to yield a violet/green light-switchable CBCR (18).

UirS is encoded in 1 of 16 two-component signaling gene clusters in *Synechocystis* (19). This cluster also harbors two response regulator genes, *slr1213* (hereafter designated *uirR* for UV intensity response Regulator) and *slr1214* (hereafter designated *lsiR* for light and stress integrating response Regulator) (Fig. 1A). An AraC family transcription regulator (20), UirR possesses an N-terminal receiver domain and a C-terminal DNA-binding domain (Fig. 1B). Because AraC subfamily members often act as activators of stress responses, such as MarA, SoxS, and Rob in *Escherichia coli* (21), we reasoned that UirR may function to transmit a UV light signal perceived by UirS. The present studies were undertaken to address the hypothesis that UirS functions as a negative UV phototaxis sensor. Our studies establish that UirR is indeed a transcriptional activator of the *lsiR* gene, which encodes one of six PatA-related regulators found in the *Synechocystis* genome (5, 22) previously shown to be induced by varied stresses, such as inorganic carbon and iron limitation (23, 24), exposure to H₂O₂ (25), and high light (26). Using a combination of biochemistry and forward/reverse genetics, we establish that the *uirS*, *uirR*, and *lsiR* loci encode key components of a UV photosensory signaling system that supports negative phototaxis.

Results

UirS Is a Dual-Cys UV/Green CBCR. Expression of CBCR GAF domains in *E. coli* strains engineered for PCB production has been frequently used to examine the photosensory specificity of these phytochrome-related photoswitches (27–29). In our hands,

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Fig. 1. *uirS/uirR/lsiR* locus and domain organization. (A) Arrangement of *cobN* (*slr1211*), *uirS* (*slr1212*), *uirR* (*slr1213*), *lsiR* (*slr1214*), and *slr1215* on the chromosome of *Synechocystis*. (B) Predicted domain architecture of UirS (S), UirR (R1), and LsiR (R2) proteins. PVB is predicted to covalently bind to Cys533 (C533) and Cys561 (C561) of UirS. His658 (H658) of UirS, Asp54 (D54) of UirR, and Asp297 (D297) of LsiR are conserved residues of a putative His-Asp phosphorelay system. DB, DNA-binding domain; GAF, GAF domain; HiK, HiK domain; PAS, PAS domain; REC, receiver domain.

UirS-GAF adopted two photointerconvertible forms that maximally absorb in the UV-violet (Puv) and UV-green (Pg) spectral regions (Fig. 2A and Fig. S14). Irradiation of Puv with UV (UV light source emission is shown in Fig. S2) yielded Pg, whose absorption maximum in the green region (534 nm) is accompanied by two smaller peaks in the UV region (325 nm and 382 nm) and a blue-absorbing (414 nm) shoulder (Fig. 2A). Irradiation of Pg with green light restored the Puv spectrum with a broad absorption maximum at 382 nm, results similar to those previously reported for recombinant UirS/Slr1212-GAF (18). Absorption and difference spectra of UirS-GAF expressed in *Synechocystis* cells were nearly identical to those of the *E. coli*-expressed version (Fig. 2B). We conclude that these spectral properties accurately reflect the Pg photosensory specificity of UirS.

Mutagenesis studies have established two conserved Cys residues to be required for the blue/green photocycles of related CBCRs (14, 18). To test the importance of both Cys residues for the light-sensing activity of UirS, we expressed single (Cys561Ala,

Fig. 2. Spectrophotometric properties of recombinant *Synechocystis* UirS-GAF holoprotein absorption (A and C) and difference (B and D) spectra of purified recombinant UirS-GAF isolated from PCB-producing *E. coli* (A–D) and from *Synechocystis* cells (B). (A) UV-absorbing form (Puv, blue trace) and green-absorbing form (Pg, green trace) formed after saturating green and UV illumination, respectively. Difference spectra of native UirS-GAF isolated from PCB-producing *E. coli* (green trace) and from *Synechocystis* cells for green minus UV light illumination (Puv-Pg) (B) and dark minus green illumination of denatured Puv (black trace) or green minus dark of denatured Pg (green trace) (D) are shown. (C) Recombinant UirS-GAF from PCB-producing *E. coli* was denatured by acidic urea (8 M, pH 2.0) in the dark (black) and then illuminated with green light (green).

C561A; Cys533Gly, C533G) and double (C533G/C561A, CGA) mutants of UirS-GAF in PCB-producing *E. coli* cells. Mutagenesis of Cys561, conserved in plant and cyanobacterial phytochromes and in all CBCRs, resulted in the loss of PCB binding, as shown by a lack of near-UV or visible absorbance (Fig. S1D and E) and an absence of Zn^{2+} -dependent fluorescence in SDS gels, a hallmark of covalently linked biliproteins (Fig. S1B). By comparison, substitution of the blue/green CBCR subfamily-specific Cys533 ablated normal photochemistry and reduced but did not abolish chromophore binding: the C533G mutant retained visible light absorption and measurable, albeit reduced, Zn^{2+} -dependent fluorescence in SDS gels (Fig. S1B and C). These studies corroborate studies on blue/green CBCRs that implicate thioether linkages between both Cys residues and the bilin chromophore.

Denaturation studies were performed to assess the structure of the bilin chromophore bound to recombinant UirS-GAF. When denatured with acidic urea [8 M urea/HCl (pH 2.0)] in the dark, Puv was transformed to a green-absorbing (Pg) species with a double peak at 564 and 603 nm (Fig. S1F, black trace). This dramatic spectral shift was similar to that seen on acid denaturation of the blue-absorbing (Pb) state of blue/green CBCRs (16, 17, 30). Denaturation of the Pg state of UirS-GAF in the dark yielded a distinct green-absorbing species with a single major peak at 550 nm (Fig. 2C, black trace). Irradiation of both denatured samples with green light revealed that the denatured Pg state was photoactive, whereas the denatured Puv was not (Fig. 2C and Fig. S1F, green traces). The green-minus-dark difference spectrum of the denatured Pg form (Fig. 2D) of recombinant UirS-GAF was indistinguishable from that of acid-denatured TePixJ-GAF (30), showing a pair of peaks corresponding to a majority population of phycoviolobin (PVB) and a minority population of PCB. Taken together, these data indicate that UirS possesses a dual thioether linkage and a mixed population of 15Z PCB and PVB chromophores, whose photoisomerization to the respective 15E states initiates cleavage of the second Cys thioether linkage.

UirS-UirR Two-Component Signal Transduction System and Its Target Response Regulator LsiR Comprise a UV-Specific Photosensory Signaling System. To examine the function of the *Synechocystis* *uirS*, *uirR*, and *lsiR* loci, *uirS⁻*, *uirR⁻*, and *lsiR⁻* lines were constructed. Mutant lines defective in the positive phototaxis sensor (*pixJ1*) and the adenylate/guanylate cyclase (*cya1/2*) genes were used as control lines; *pixJ1⁻* cells exhibit negative phototaxis (5, 6), whereas *cya1⁻* (and *cya1/2⁻*) cells are nonmotile under unidirectional blue and red light (31). The use of the *cya1/2⁻* mutant as a control allows us to distinguish between directional cell movement (taxis) and preferential growth, because our assay does not assess individual cell movement per se. We also examined *cph2⁻* mutants, previously reported to exhibit positive (10) and negative (32) phototaxis under UV light. Phototactic motility was compared on 1% soft agar with unidirectional illumination of different spectral quality (light spectra are shown in Fig. S2). As has been observed under considerably higher fluence rates of visible light (3), WT *Synechocystis* cells exhibited positive ($<5 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), nonmotile ($25 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), and negative ($\geq 50 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) phototaxis as UV fluence rates are increased (Fig. 3A). This fluence rate dependency, as well as strain differences, likely accounts for distinct UV phototaxis behaviors reported for *Synechocystis* WT between laboratories (3, 7, 9, 10, 32).

By contrast with WT, *uirS⁻*, *uirR⁻*, and *lsiR⁻* mutants exhibited positive UV phototaxis at all fluence rates of UV except under the highest light intensity tested ($200 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), where negative phototaxis was observed (Fig. 3A). Consistent with previous reports, the *cya1/2⁻* mutant proved nonmotile under all fluence rates of UV, whereas *pixJ1⁻* mutants showed nonmotile ($\leq 50 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) and negative ($\geq 100 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) photo-

Fig. 3. Phototaxis and *uirS/uirR/lsiR* gene expression of WT and phototaxis mutant strains of *Synechocystis*. Phototactic motility of *Synechocystis* WT and *uirS*[−], *uirR*[−], *lsiR*[−], *cya1*^{−2}, *pixJ1*[−], and *cph2*[−] mutant exposed to unidirectional UV illumination (A, <5, 25, 50, 100, and 200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$; B, 85 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) or 16 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of blue, green, or red light for 2 d. B, blue, G, green, R, red. (C) Transcript levels of *uirS* (black), *uirR* (red), and *lsiR* (blue) from *Synechocystis* WT and mutants. Total RNAs were isolated from cells incubated for 2 d under unidirectional UV (85 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) or 16 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of blue, green, or red light. (A and B) Arrows indicate the direction of light, and the dotted lines represent the initial position of cells before illumination. (C) Data are mean $\pm$ SE ($n = 3$ –5). Asterisks denote differences between WT and mutants or between UV and visible light (blue, green, and red), with a statistical significance set at $P < 0.05$ (t test). $\Delta\Delta\text{Ct}$, threshold cycle value.

taxis. Interestingly, *cph2*[−] mutants behaved similar to the *uirS*[−], *uirR*[−], and *lsiR*[−] mutants; however, in this case, reversal from positive to negative phototaxis occurred at lower fluence rates (~ 50 – $100 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ vs. 100 – $200 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for *uirS*[−]). These experiments establish that although UirS, UirR, LsiR, and Cph2 all influence negative UV phototaxis, another UV sensing system(s) is responsible for the very high-fluence UV response.

Phototactic behaviors of WT and the three mutants were next compared under unidirectional visible light. In agreement with previous studies (2, 3), WT moved toward blue (464 nm), green (527 nm), and red (650 nm) sources but away from UV light (355 nm) at the fluence rates specified (Fig. 3B). In contrast, the *uirS*[−], *uirR*[−], and *lsiR*[−] mutants all displayed wavelength-independent positive phototaxis. Control *pixJ1*[−] cells exhibited negative phototaxis under all light conditions, whereas *cya1*^{−2} cells proved nonmotile as expected (5, 10, 31). These studies indicate that only UV-specific phototactic behavior is altered by the loss of the *uirSR* two-component module and that this module and *lsiR* are both required for the negative UV phototaxis response.

UirSR Module Is Required for UV-Dependent *lsiR* Induction but Not for *lsiR* Function. To identify potential targets of the negative UV phototaxis signaling pathway, transcript profiles of *uirS*[−], *uirR*[−], and *lsiR*[−] mutants exposed to unidirectional UV were compared under fluence rates that trigger negative phototaxis in WT. High-density *Synechocystis* DNA microarray analyses detected only two significantly misregulated genes common to both *uirS*[−] and *uirR*[−] mutant lines: the two adjacent genes *lsiR/slr1214*, whose transcript level was <10% that of the WT, and the conserved hypothetical protein *slr1215* (Table S1). Because the response regulator *lsiR* might function as the bona fide target of the UirSR two-component system, we chose the former for further study.

The transcription profiling results were confirmed by quantitative RT-PCR analysis, which revealed that *lsiR* transcript levels were 14- to 25-fold lower than WT for all three mutants (Fig. 3C). *lsiR* transcription was also specific for UV because its induction was not seen in blue-, green-, or red-grown WT (Fig. 3C).

No other gene implicated in phototactic motility was jointly misregulated in the three mutants (Dataset S1), including genes for the photoreceptors *pixJ1*, *cph2*, *pixD*, and *slr1629*; the adenylate cyclase *cya1*; pili biosynthesis/assembly *pilA1*–*A4*; the pili motor *pilT1*; and the twitching motility protein *pilT2*. Neither was the UV-dependent expression of *lsiR* altered in *cya1*^{−2}, *pixJ*[−], or *cph2*[−] mutant backgrounds (Fig. 3C). We also observed that *uirS* and *uirR* transcript abundance was unaffected by light quality or by the loss of functional *cya1*^{−2}, *pixJ*, or *cph2* genes (Fig. 3C). Taken together, these results implicate *lsiR* as the only transcriptional target of the UirSR two-component signaling system.

Complementation and overexpression studies were next undertaken. Expression of *uirS* or *uirR* in the respective mutants fully complemented negative UV phototaxis (Fig. 4A, *UirS* and *UirR*), while also restoring *lsiR* transcript levels to those seen in UV-treated WT (Fig. 4B). Similarly, WT *lsiR* (Fig. 4A, *LsiR*) fully complemented the *lsiR*[−] mutant. To test whether the conserved Asp residues in the receiver domains of UirR and LsiR were required for complementation, site-directed mutant alleles

Fig. 4. UV phototactic motility and transcript levels of *uirS/uirR/lsiR* genes in WT, *uirS* locus mutants, and complemented cell lines of *Synechocystis*. (A) UV-dependent phototaxis of *Synechocystis* WT; *uirS*[−], *uirR*[−], and *lsiR*[−] mutants; *sigE* promoter-driven *uirS* overexpressor (*UirS*) in *uirS*[−] mutant backgrounds; *trc* promoter-driven overexpressors of *uirR* (*UirR*) or site-directed mutant D54G (*D54G*) in *uirR*[−] mutant backgrounds; *lsiR* promoter-driven complementary strains of *lsiR* (*LsiR*) or site-directed mutant D297A (*D297A*) in *lsiR*[−] mutant backgrounds; *pIL1* vector controls expressed in *uirS*[−] (*pIL1*) or *lsiR*[−] (*pIL2*) backgrounds; and *pSL1211* in *uirR*[−] (*pSL1*) backgrounds. (B) Transcript levels of *uirS* (black), *uirR* (red), and *lsiR* (blue) from *Synechocystis* WT, overexpressors, and complementary strains in respective mutant backgrounds. (C and D) UV-dependent (85 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) and red-dependent (16 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) phototaxis of WT, *uirS*[−], and *uirR*[−] mutants and *psbA2* promoter-driven *lsiR* overexpressors in *uirS*[−] (*LsiRs*) and *uirR*[−] (*LsiRr*) mutant backgrounds and *pIL1* vector controls (*pIL3* and *pIL4*). R, red. (A, C, and D) Arrows indicate the direction of light, and the dotted lines represent the initial position of cells before illumination. (B) Total RNAs were isolated from cells incubated for 2 d under unidirectional UV (85 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). Data are mean $\pm$ SE ($n = 3$ –5). Asterisks denote differences between WT and overexpressors or complementary strains, with a statistical significance set at $P < 0.05$ (t test).

of UirR (D54G) or LsiR (D297A) were expressed in the respective mutants. Both mutant alleles failed to rescue negative UV phototaxis (Fig. 4A), revealing that the Asp residues of both response regulators are required for signal transduction. Unexpectedly, WT *lsiR* complemented both *uirS*[−] and *uirR*[−] mutants (Fig. 4C, labeled *LsiRs* and *LsiRs*). These results indicate that negative phototaxis under unidirectional UV can be achieved by constitutive expression of LsiR even in the absence of UirS and UirR proteins. Constitutive *lsiR* expression was also sufficient to reverse the positive phototactic response to red light (Fig. 4D). Thus, despite their close proximity in the *Synechocystis* genome, the *uirSR* module and *lsiR* are functionally distinct: *uirSR* is a UV-specific activator of *lsiR* expression, whereas *lsiR* is instead able to leverage UirS and an unknown red light photo-receptor(s) for avoidance behavior.

UirR Directly Targets the *lsiR* Promoter. To address the hypothesis that UirS regulates *lsiR* expression by modulating the binding of UirR to the *lsiR* promoter, we screened for UV-responsive *cis*-acting elements in the *lsiR* promoter region. A nested series of DNA fragments (Fig. 5A, P1–P5) were cloned upstream of a LUX reporter cassette (33). Only the reporter strain harboring the full 534-bp intergenic region from the *uirR* stop codon to the translation initiation start site of *lsiR* (L1) showed an approximately sixfold greater bioluminescence level relative to the control strain. Moreover, the entire intergenic region was also required to rescue negative phototaxis by *lsiR* expression under control of its own promoter (Fig. 5B). Thus, we conclude that the

Fig. 5. UirR binding to the *lsiR* promoter region. (A) Luminescence from *lsiR* promoter fragment (P1, −534- to −1-bp region; P2, −438- to −1-bp region; P3, −337- to −1-bp region; P4, −228- to −1-bp region; and P5, −112- to −1-bp region) and promoterless control-driven *luxAB* reporter strains (L1–L5, CO). Exponential phase cells grown under white light were exposed to unidirectional UV ($85 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) for 16 h. Numbers refer to the nucleotide positions of *lsiR* promoter fragments relative to the translation +1 start site. CO, control; PL, promoterless. (B) Phototactic motility of *Synechocystis* WT, *lsiR*[−] mutant, and complemented *lsiR*[−] mutant lines (P1–P5, PL) expressing *lsiR* promoter fragment-driven *lsiR* (P1–P5) and promoterless *lsiR* (PL) under unidirectional UV illumination for 2 d. (C) Electrophoretic mobility shift assay of *lsiR* promoter fragments (E1–E2) coincubated with purified GST-UirR protein. Thirty femtomoles of the biotin-labeled DNA segments E1 (−534- to −452-bp region, lanes 1–8) and E2 (−462- to −347-bp region, lanes 9 and 10) was incubated for 30 min at 22 °C with increasing concentrations of purified GST-UirR (0 ng for lanes 1 and 9, 20 ng for lane 2, 50 ng for lane 3, 100 ng for lane 4, and 250 ng for lanes 5–7 and lane 10). Specific (200-fold molar excess of the nonlabeled E1 segment, lane 6) and nonspecific (200-fold molar excess of *lsiR* ORF fragment, +361- to +457-bp region, lane 7) inhibitors were included. In control (lane 8), GST (250 ng) was included instead of GST-UirR.

−534- to −439-bp region of the *lsiR* promoter unique to the P1 construct is vital for UV-activated transcription.

We performed electrophoretic mobility shift assays using recombinant GST-UirR protein and biotin-labeled *lsiR* promoter fragments to test whether UirR could recognize this region. A distinct DNA–protein complex was observed with DNA fragment E1, corresponding to the −534- to −462-bp region of the *lsiR* promoter (Fig. 5C, lanes 1–7). No such complex was observed with fragment E2 (−462- to −347-bp region, lanes 9 and 10) or with GST alone (lane 8). The amount of E1–UirR complex increased with increasing UirR, with depletion of the band corresponding to the free probe (lanes 1–5). Binding could be specifically inhibited by excess unlabeled E1 DNA (lanes 6 and 7). These results support the presence of a UirR recognition sequence within the −534- to −462-bp region upstream of the *lsiR* ORF.

UirS Physically Interacts with UirR. Should UirS and UirR function as a canonical two-component signaling system, protein–protein interactions and/or phosphotransfer would be expected to occur between the UirS HiK domain and the receiver domain of UirR. In contrast to *Arabidopsis* ETR1 (34), His kinase autophosphorylation activity for TM-deleted UirS (UirSΔ) was not observed, and we could not detect UirSΔ-phosphotransfer to UirR with or without UV illumination. Because HiK activity is not required for ETR1 signaling (35), we examined whether UirS could physically interact with UirR using yeast split-ubiquitin and two-hybrid assays. Using full-length UirS and truncated UirSΔ (Fig. S3A), yeast split-ubiquitin assays showed that UirS (and UirSΔ) can self-interact based on histidine auxotrophy, red/white, and β-galactosidase analyses (Fig. S3B), a result consistent with HiK dimerization (36). Yeast two-hybrid assays showed that UirS can also interact with full-length UirR polypeptide via the expected interaction between the UirS HiK domain and the UirR receiver domain (Fig. S3C). Interaction between UirS and UirR was further supported by in vitro pull-down assays (Fig. S3D), which showed copurification of GST-UirR and His-tagged UirSΔ.

Discussion

Our studies establish that the membrane-bound CBCR HiK UirS and its cognate AraC-family response regulator UirR function as a UV-sensing two-component signaling system in *Synechocystis*. Interestingly, AraC subfamily members in *E. coli* often act as activators of stress responses (21). UirR binds to the

Fig. 6. Proposed model for the UirS/UirR/LsiR-based negative UV phototaxis signaling pathway in *Synechocystis*.

promoter of the adjacent gene *slr1214* that encodes the PatA-family response regulator LsiR. Among other PatA-related proteins encoded by the *Synechocystis* genome are TaxP1 (PixG/Sll0038) and PixE (Slr1693), both of which are located within phototaxis operons (5, 6), and thus may share the same regulatory target(s) as LsiR. Similar to the CBCRs SyPixJ1, TePixJ, and Tlr0924 (15–18), UirS possesses a dual-Cys-linked PVB chromophore whose photoactivation triggers conversion between UV (Puv) and green (Pg) light-absorbing states. Physiological analyses indicate that LsiR functions as a signal output regulator of avoidance movement of *Synechocystis* cells under unidirectional UV illumination.

Our studies also show that constitutive *lsiR* expression is sufficient for phototaxis reversal under unidirectional light. Because LsiR is induced by multiple cellular stresses (23–26), this response regulator would appear to integrate unidirectional light stress with general stress signaling systems. Based on our interaction data, we propose a model in which UV activation of UirS induces *lsiR* expression via Pg-dependent release of UirS-bound UirR (Fig. 6). This model is based on the observation that UirS and UirR are constitutively expressed, regardless of light quality, and the knowledge that UirS is an integral plasma membrane protein (37). UirR thus functions as a mobile signal carrier that ultimately must locate the *lsiR* promoter (*plsiR*). Although we have been unable to detect phosphotransfer from UirS to UirR, we show that the conserved Asp residue of UirR is required for function. It is thus possible that phosphorylation is required for UirR to function as an activator of *lsiR* transcription, possibly by promoting UirR homodimerization (20). The identity of the phosphorylating agent(s) responsible, if any, remains unknown at present. The regulatory activity of LsiR also requires the equivalent Asp; however, UirS is clearly not required for LsiR function.

How might LsiR reverse the direction of *Synechocystis* cell movement? Phototactic motility of *Synechocystis* uses a type IV pilus motor to drive pilus extension, tethering to the surface or to neighboring cells and then retracting to move toward the direction of extension (1). To achieve negative phototaxis, differential LsiR activation on the irradiated “proximal” and shaded “distal” sides of the cell could be used either to activate the distal motor or to inactivate the proximal motor. This could be accomplished by differential activation of a kinase or inactivation of a phosphatase (or vice versa), which alters LsiR stability and/or localization. Alternatively, a second-messenger gradient could be used to alter the activity of LsiR differentially. Both hypotheses require that the light gradient sensed by a photoreceptor trigger a differential response on the proximal and distal sides of the cell. In a second-messenger model, it is interesting to note that *cph2*[−] mutants exhibit a phenotype very similar to loss-of-function mutants in the UirS signaling system shown here (Fig. 3A). Cph2 possesses GGDEF and EAL regulatory output domains, which could generate a UV-dependent gradient of di-cGMP to affect the activities of LsiR and/or the pilus motor differentially. However, expression of LsiR results in negative phototaxis under red light, indicating that multiple photoreceptor pathways support negative phototaxis when the LsiR protein is present.

It is well established that the blue/green CBCR SyPixJ/TaxD1/Sll0041, which possesses a methyl-accepting chemotaxis signaling domain, functions to promote positive phototaxis under visible light (1). For this reason, SyPixJ is probably antagonized by LsiR

action, particularly under unidirectional blue or green light. Because *SypixJ*[−] mutants exhibit negative phototaxis under all wavelengths of visible light, *Synechocystis* cells must contain other visible light sensors responsible for perceiving visible light gradients, and these may also involve a role for LsiR. Like those present in other cyanobacteria, multiple photosensors that could serve this function are representatives of the cyanobacterial phytochrome, CBCR, BLUF, and CRY families. Of particular note, the blue-absorbing CRY-related cyanopterin Sll1629 (9), the BLUF protein SyPixD/Slr1694 (8), the red/green CBCR Slr1393 (38), and the red/far-red light sensor Cph1 (39) all could contribute to sustaining the signaling gradient necessary for LsiR-dependent (and potentially LsiR-independent) negative phototaxis at elevated fluence rates of visible light. This work also illuminates the exquisite complexity with which cyanobacteria tune not only their metabolism but their behavior in response to light and other environmental cues.

Materials and Methods

Strains and Culture. The motile *Synechocystis* sp. PCC 6803 WT was used to generate mutants and transformants that contained genes conferring spectinomycin, chloramphenicol, kanamycin, and gentamicin resistance. All strains were grown on 1% BG-11 agar plates buffered with 10 mM N-Tris (hydroxymethyl) methyl-2-aminoethanesulfonic acid (TES)-KOH (pH 8.0) at 28 °C under the white light (10 μmol·m^{−2}·s^{−1}) with or without 10–20 μg·mL^{−1} appropriate antibiotic(s).

Motility Assays. Exponentially growing *Synechocystis* cells were streaked onto 1% BG-11 agar trays containing 10 mM glucose and then incubated under unidirectional illuminations of UV, blue, green, and red light sources (Fig. S2) for 2 d.

***Synechocystis* High-Density 134K Gene Chip Analysis.** Gene expression profiling was conducted with the *Synechocystis* high-density 134K NimbleGen microarray designed to accommodate 3,673 transcripts (Roche NimbleGen Inc., Madison, WI).

Protein-Protein Interaction Assay in Yeast. Protein interactions among UirS and UirR were examined by the yeast mating-based split-ubiquitin system and the yeast Matchmaker GAL4 Two-Hybrid system (Clontech).

In Vitro GST Pull-Down Assays. His₆-UirSΔ and GST-tagged UirR proteins were purified by nickel affinity chromatography (Qiagen) and glutathione Sepharose 4B (GE). Purified His₆-UirSΔ protein was immobilized by nickel affinity chromatography on nickel-nitrilotriacetic acid (Ni-NTA) metal-affinity resin (Qiagen) and then incubated with purified GST or GST-UirR. GST fusion proteins bound to His₆-UirSΔ were detected by Western blotting on a PVDF membrane (Millipore) using a rabbit anti-GST antibody (Santa Cruz).

Gel Mobility Shift Assay for DNA-Protein Complexes. Biotin-labeled DNA fragments containing the −534- to −452-bp (E1) and −462- to −367-bp (E2) regions in the *lsiR* promoter region, specific (nonlabeled E1 fragment) and nonspecific (+361- to +457-bp region within *lsiR* ORF) competitors of E1, purified GST-UirR, and GST proteins were used in gel mobility shift assays (Chemiluminescent Light Shift EMSA kit; Pierce Biotechnology).

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